

VITAMIN A AND CAROTENE CONTENT OF
THE BLOOD PLASMA OF DAIRY CALVES

by

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THE BLOOD PLASMA OF DAIRY CALVES

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INTRODUCTION

Knowledge of the level of certain constituents in the blood is useful in the evaluation of the nutritional status and general health of animals. Evaluation of nutritional status with respect to vitamin A has been attempted by measurement of blood carotene or vitamin A levels. There remain, however, many problems in the interpretation of these levels. The body stores of carotene and vitamin A, the current intake of different forms of this vitamin, and the presence of antagonistic materials in the diet are important factors in relating vitamin A and carotene blood levels to the true state of nutrition of the animal.

With these facts in mind, studies were made of the carotene and vitamin A content of the blood plasma of calves being raised under two systems of feeding. These studies were made in cooperation with the Department of Dairying, Oklahoma Agricultural Experiment Station.

REVIEW OF LITERATURE

Some effects of vitamin A deficiency. Vitamin A deficiency in animals shows itself in many varied forms, making it difficult to correlate apparent deficiency levels in the blood with the actual health of the animal. Eaton et al. (4) reported exophthalmos in six of eighteen vitamin A depleted calves, muscular incoordination in five, diarrhea in four, and convulsions in one. In addition, spinal fluid pressures at the termination of the experimental period were elevated in the calves receiving only the depletion ration, while in those receiving the supplemental vitamin A it remained essentially the same as at the start of the experiment. Squamous metaplasia of the interlobular and main ducts of the parotid gland was observed in eight of nine depleted calves but not in any of the six controls.

Adequate vitamin A and nicotinic acid were reported by Lundquist and Phillips (17) as important factors in the control of calf scours. This was confirmed by Spielman et al. (28) who reported that incidence of scours was significantly lower in calves from dams fed either the alcohol or ester form of vitamin A prior to parturition than in calves from control dams. Also, calves from dams fed alfalfa leaf meal, which is high in carotene, had fewer cases of scours than calves from control dams.

Sellers and Eden (27) reported no rise in plasma vitamin A following single or repeated oral doses of the vitamin to scouring calves while the vitamin A level of control calves had risen 300 to 500 percent.

Carotene and vitamin A requirements of calves. Under ordinary conditions, the calf is born with practically no vitamin A or carotene in the plasma. Phillips et al. (26) pointed out that, upon determination of the blood plasma vitamin A and carotene at intervals of 12, 24, and 48 hours, no measurable amounts of these constituents could be found before suckling. The ingestion of colostrum quickly brought the vitamin A level to a value of 10-12 mcg. per 100 ml. plasma. Similar results were also reported by Moore (18).

That the vitamin A and carotene concentration in the blood is sufficiently low in the new born calf to indicate a vitamin A deficient state, has been reported by Lundquist and Phillips (17). Also, they reported that it appeared very unlikely that the very young calf could convert carotene to vitamin A. This observation was based upon the feeding of large amounts of carotene in milk from birth, which caused an increase in plasma carotene but not plasma vitamin A. Several of the calves so treated displayed definite symptoms of vitamin A deficiency.

Wise et al. (34) found that the concentrations of carotenoids and of vitamin A in the blood serum of calves, subjected to standard herd dietary and managerial practices, from birth to ten weeks of age, were closely related to the types of feeds consumed. Although there was a marked variation in the concentrations of carotenoids and of vitamin A in the blood serum of individuals, the general trends in the levels of these constituents in each calf were similar. The values started at about 5 mcg. per 100 ml., raised to about 17 mcg. during the colostrum period, dropped to 8 mcg. at 5 weeks, and then reached a plateau of about 14 mcg. at 8 weeks. The marked reduction of these constituents in the

blood during the interim between colostrum ingestion and effective hay consumption indicated a need for vitamin A supplementation during this period and further emphasized the importance of feeding palatable hay high in carotene.

Hibbs and Pounden (9) conducted experiments to determine the effect of different rations and early rumen development on the levels of vitamin A and carotenoids in the blood of young dairy calves. Rumen inoculations, accomplished by direct transfer of cud material from cows in the herd to the calves, were supplied to about one-half the calves in order to make certain that they had access to the microorganisms present in the rumens of adult animals. Neither the inoculations nor the type of ration fed was found markedly to influence the blood plasma vitamin A. It seems reasonable to assume, therefore, that very young calves inefficiently utilize carotene in natural feeds. At four days, the plasma values averaged 14 mcg. per 100 ml. plasma, falling to a value of 9 mcg. at 42 days.

Keener et al. (13) maintained, however, that the minimum carotene requirements (as determined by condition of the eyes) for dairy calves of the Guernsey and Holstein breeds living at a temperature of 50 to 70° F. is 12 mcg. per pound (26 mcg. per kg.) of body weight per day. These results are based on calves from birth to 6 months of age. They were able to show that the environmental temperature affects the carotene minimum, since during severe winter weather, the minimum requirement to maintain adequate blood levels was at times increased 2-fold.

Normal blood levels of vitamin A and carotene in calves. Boyer et al. (1) conducted early experiments to determine the blood plasma concentrations and intakes of carotene and vitamin A necessary for the growing

calf. They reported that blood plasma vitamin A is a more delicate measure of the state of vitamin A nutrition in the calf than either growth or blood carotene. A vitamin A level of 10 mcg. or more per 100 ml. was found to correspond with adequate vitamin A nutrition, as determined by growth response. A level of 7 to 8 mcg. per 100 ml. plasma was stated as the border line level, while values below this were definitely inadequate. Daily intakes of vitamin A which would maintain deficient, borderline, and adequate concentrations of vitamin A in the blood plasma of mixed breeds from four weeks of age to one year were found to be approximately 6, 12, and 18 mcg. per kg. of body weight, respectively. The daily carotene requirement necessary to prevent deficiency symptoms (eye disorders and retarded growth) was found to be 75 mcg. per kg. of bodyweight for Holstein yearlings and 125 mcg. per kg. for Guernsey yearlings. This intake produced a blood plasma carotene level of 50 to 70 mcg. of carotene per 100 ml. of plasma for Holstein and 110 to 140 mcg. of carotene per 100 ml. for Guernseys. These values resulted in a vitamin A level of from 8 to 10 mcg. per 100 ml. plasma.

Following this work, Moore et al. (20) in a series of controlled intake experiments, established that Guernsey calves from birth to 4 months of age required an intake of 34 mcg. of carotene per pound (75 mcg. per kg.) of body weight during the winter months to maintain a normal spinal pressure. This intake maintained a vitamin A level of from 6 to 14 mcg. in the Guernsey calf.

Similar work was reported by Moore and Berry (19), who found the vitamin A content of the blood plasma of calves of the Holstein, Ayrshire, and Guernsey breeds from birth to four months of age to vary from 7.2 to 14 mcg. per 100 ml. of plasma.

Jacobson et al. (11) and Eaton et al. (4) consider that calves are depleted in vitamin A, as determined by the occurrence of deficiency symptoms (eye disorders, muscular incoordination and scours), when their plasma levels are below 4.0 mcg. per 100 ml.

Lewis and Wilson (16) state that a vitamin A intake of 8 mcg. per kg. of body weight is the minimum for growth, although low plasma levels of vitamin A were observed, of the order of 6 to 7 mcg. per 100 ml. plasma. Hibbs and Krauss (8) reported that, at the end of 21 days, the average level of plasma vitamin A was 11.1 mcg. per 100 ml.

Effect of prepartum diet. It has been shown that supplementation of the prepartum diet of heifers affects, to a large extent, the blood condition of the new born calf with respect to vitamin A and carotene. Spielman and associates (29) fed a control group of heifers during the last 60 days of gestation a normal ration while two other groups received daily one million I. U. of carotene or one million I. U. vitamin A, respectively, in addition. No significant differences were observed in the mean plasma carotene of the new born Holstein and Guernsey calves. However, plasma vitamin A of calves from the carotene supplemented cows was twice that of the normal group, (4.6 mcg. as compared to 2.1 mcg.), while the vitamin A supplemented groups showed a fourfold increase (9.3 mcg.). These values compare well with those reported by Moore and Berry (19). Wise et al. (33) found a similar increase by feeding "dry vitamin A" from Distillation Products, Inc., but were unable to confirm these results when the source of carotene was pasture grass. No reason was advanced for these divergent results.

Spielman et al. (28), upon feeding daily the equivalent of one million I. U. of vitamin A in the form of carotene in dehydrated alfalfa

leaf meal, the ester form of vitamin A, and the alcohol form of vitamin A to cows of mixed breed 30 days prepartum, found that the alcohol form caused the greatest increase in vitamin A content of the plasma of the new born calves. The ester form produced a greater increase than carotene from alfalfa leaf meal. Average values for calves according to treatment of the cows were as follows: Control, 3.5 mcg.; carotene, 3.3 mcg.; alcohol, 7.3 mcg.; and ester, 8.8 mcg. Eaton et al. (5) and Parrish et al. (23) have shown that the fetal storage of vitamin A can be utilized by the newborn calf.

Effect of colostrum. Variations in colostrum feeding has become one of the more important factors in the plasma vitamin A level as affected by the early diet of the dairy calf. Early work by Moore (18) indicated that colostrum ingestion substantially increased the plasma vitamin A level of calves which were depleted at birth.

Sutton and Kaeser (30) found the average vitamin A in the blood plasma of calves to be increased 12.4 mcg. per 100 ml. in the first three days of colostrum. This increase resulted from an average intake of 99,614 I. U. of vitamin A ingested from colostrum during the three-day period. When colostrum feedings were extended to seven days, the blood level continued to rise, reaching a peak of 18.9 mcg. on the seventh day; this was 6.8 mcg. above that of seven-day-old calves which had received colostrum for only three days. Later studies (12) have shown that the feeding of colostrum causes more rapid weight gains and a superior physical appearance of calves.

Nezvesky et al. (22) observed the effect on new born calves of feeding colostrum from dams whose basal ration had been supplemented with one million I. U. of vitamin A daily for 30 days prior to the calculated date

of parturition. Their results showed that the fortified colostrum significantly increased the plasma vitamin A from birth to 5 days of age, as compared with normal colostrum.

In studies by Jacobson et al., (10) calves fed colostrum milk until 60 days of age had much higher vitamin A and carotene blood levels than calves fed whole milk. The extra antibodies consumed by colostrum-fed calves did not prevent the occurrence of scours but seemed to aid in the prevention of fatal infections.

Effect of supplementation. Realizing the necessity for supplementation to maintain healthy calves, Lewis and Wilson (16) set up six groups of 4 Holstein calves, 2 to 9 days old, which they fed 32 to 1024 U. S. P. units of vitamin A (8 to 256 mcg.) per kilogram body weight per day over a period of nine months. Their results indicate that 32 units per kg. (8 mcg. per kg.) body weight is about the minimum for growth, and is also adequate to prevent night blindness. At this intake, however, low plasma levels of vitamin A were observed, of the order of 24-27 U. S. P. units (6-7 mcg.) per 100 ml. blood plasma. The calves showed a maximum growth at 64 U. S. P. units (16 mcg.) per kilogram of body weight.

Hibbs and Krauss (8) further illustrated the effect of feeding vitamin A supplement to a group of Holstein and Jersey calves. At the end of 21 days the plasma levels of the control group was 11.1 mcg. per 100 ml., while the supplemented calves showed a level of 14.8 mcg. per 100 ml. The higher level in the experimental group was attributed to the feeding of 10,000 U. S. P. units (2,500 mcg.) of vitamin A per day. Also, at that time the liver stores of the controls averaged 36,000 U. S. P. units (9,000 mcg.) of vitamin A while the supplemented group had stored approximately 62,000 U. S. P. units (15,500 mcg.). No

difference between groups was noted for plasma carotene.

Forms of vitamin A. Some question has been raised as to the form of the vitamin present in the animal body and the form most useful as a supplement. Parrish *et al.* (22) found that vitamin A in the blood serum of dairy cows was present largely in the alcohol form. Before supplementation of the prepartum ration with either the alcohol or ester form of vitamin A, the esterified form averaged 10 percent of the total vitamin A of the blood serum, but during daily oral administration of one million I. U. of vitamin A primarily as the ester form, the esterified form increased to 24 percent of the total vitamin A. Most of the increase of total vitamin A of the blood serum occurred in the ester fraction. The vitamin A of blood serum of their calves, at birth and at 4 days of age, was found to be predominately of the alcohol form, while the vitamin A of the livers was mostly in the ester form. Alcohol and esterified forms of vitamin A given orally to the dam during the terminal stages of gestation seemed to have a similar effect in fortifying the vitamin A reserves of the new born calf.

The relation of the different forms. The interrelationship between carotene and vitamin A in the plasma, as affected by the stores of these two constituents in the liver, has been the subject of much speculation. Braun (2) surmised from his observations made on the livers from vitamin A-starved animals, and on samples obtained by partial hepatectomy, that utilization of stored vitamin A first forces available carotenoid stores to be converted into vitamin A, thus decreasing the plasma carotenoid level without decreasing the vitamin A level. There appears to exist in the liver a relationship between carotenoid levels and corresponding vitamin A levels similar to the vitamin A:carotene relationship observed

in the blood. Changes in the ratio with changing carotenoid levels are probably caused by the tendency of the organism to maintain a constant vitamin A store.

Eaton et al. (6), while studying the effect of feeding a massive dose of vitamins A and D to Holstein calves at birth, obtained the following results: A significant depression in the levels of carotene in the blood plasma occurred in those calves which received the vitamin supplement. This depression was apparent 2 weeks after the administration of the massive dose of vitamins and continued until the end of the 6-week experimental period. The levels of both vitamin A and D in the blood plasma of the vitamin supplemented calves was about ten times higher than that found in the control calves. No appreciable differences in liver carotene storage were observed, but the vitamin A level in the liver was raised from 4.3 mcg. per gram to 62.0 mcg. per gram.

Eaton et al. (5) obtained a similar effect on calves from dams which had received large doses of vitamin A prior to parturition.

EXPERIMENTAL

The increasing use of dry calf starters and other milk replacements in the rations of dairy calves is progressively changing the early diet of the calf from that of milk to one of primarily hay and grain. In view of this fact, two 16-week feeding trials were undertaken to answer the following questions confronting Oklahoma dairymen:

1. What is the comparative feeding value of alfalfa and prairie hay for young dairy calves as measured by skeletal growth and body weight gain when fed to young dairy calves?
2. What is the effect of deferred feeding of hay until calves are two months of age?
3. What is the effect of the total ration on blood plasma carotene and vitamin A values?

PROCEDURE

Animals: The animals for each of two trials, consisting of twenty Holstein and Jersey calves, obtained locally, were assigned by random selection to the following groups:

Group I - Received alfalfa hay from birth

Group II - Received alfalfa hay beginning at two months of age

Group III - Received prairie hay from birth

Group IV - Received prairie hay beginning at two months of age

Shortly after birth, the calves were separated from their dams, and transferred to individual pens. Water was readily accessible, and, to be certain of accurate feed intakes, the calves were bedded with dry sawdust or shavings.

The calves were fed an average of 375.0 pounds of whole milk according to the schedule for regular herd calves, as shown in Appendix Table 4. Dry calf starter, mixed as shown in Appendix Table 5, was offered on the third day and fed ad lib. thereafter, but limited to 4 pounds daily. The amounts of hays and starter eaten were determined by daily weighings of refused feed.

Blood samples were taken at birth and at two week intervals thereafter for the determination of vitamin A and carotene in the blood plasma.

Samples and analysis. The hays were sampled in the barn by withdrawing a portion from each bale fed, and storing it in a closed container until it could be brought to the laboratory for analysis. A composite sample of this hay was ground in a Wiley mill, and stored under refrigeration until carotene could be determined. Proximate analysis was made according to the A. O. A. C. method (15).

Plant carotene. Plant carotene was determined by the Willstatter and Stoll (32) extraction method, as described by Peterson (25), with minor modifications. The procedure was followed in this manner: Duplicate two-gram samples, previously ground, were weighed on an analytical balance, and transferred to glass-stoppered 250 ml. Erlenmeyer flasks. To the sample was added 50 ml. of freshly prepared 5 percent ethanolic potassium hydroxide, and, after fitting the flasks with small reflux condensers, the solution was refluxed on a steam hotplate for 30 minutes. The level of the liquid was maintained with 95% ethanol. The contents of the flask were cooled, 25 ml. of Skellysolve F (petroleum ether) were added, and the flask shaken vigorously with a ground glass stopper in place. The material was decanted through cotton into a 250 ml. separatory funnel, care being taken to retain as much of the plant material in the flask as possible. This residue was extracted until no further color was observed by shaking with a mixture of 25 ml. Skellysolve F and 10 ml. of 95% ethanol. These extractions were also added to the separatory funnel.

About 100 ml. of distilled water was poured gently through the alcohol-Skellysolve solution in the separatory funnel, and the solution gently whirled. The alkaline alcohol-water solution was withdrawn from the bottom of the separatory funnel and discarded. To free the Skellysolve layer of alkali, it was washed with two additional 100 ml. portions of distilled water. Following this, the Skellysolve layer was washed with 20 ml. portions of 90% methanol until all flavones, alkali, and xanthophylls had been removed, as indicated by the absence of yellow color in the methanol washings. Usually about 4 washings were sufficient.

The Skellysolve layer was then washed free of methanol by two 100 ml. portions of distilled water, dried over anhydrous sodium sulfate, and filtered into a 100 ml. volumetric flask. The sodium sulfate was rinsed several times with Skellysolve F and these washings added to the volumetric flask. After making to volume, a portion of this solution was read in the Evelyn Colorimeter and compared with a standard curve prepared from a pure carotene containing 90% Beta isomer, and 10% Alpha isomer. (This standard was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio). The value obtained by this method was reported as crude carotene, which was the figure used throughout this experiment. It can be related to true carotene, or the value obtained by further purification on a magnesium oxide adsorptive column as described by Wall and Kelley (31) by the equation $Y = 0.77x - 1.4$. Y is "true" carotene and x is crude carotene, both expressed in parts per million, and based on the work of Gallup and Gibson (7).

Plasma carotene and vitamin A. The method adopted for the determination of these two constituents was that of Kimble (14). This method had been shown by many workers to be adequate, and, by virtue of its simplicity, could be readily adapted to a routine procedure. It has the added advantage that both carotene and vitamin A can be determined on the same sample, thus reducing the amount of blood necessary for analysis. The procedure employed was as follows: All blood samples were taken at the same time of day (4:00 p.m.) to minimize the effect of feeding on the blood values. After centrifuging the sample 30 to 60 minutes at 1500 r.p.m. to separate the plasma, a 10 ml. sample of the plasma was transferred to a heavy wall constricted-neck test tube of about 50 ml. capacity. An equal amount of 95% ethanol was added to precipitate the plasma

protein, followed by 15 ml. of Skellysolve F. The Skellysolve F had been previously treated with "Darco" brand activated charcoal to remove materials that give extraneous color with antimony trichloride. The test tubes containing the samples were shaken vigorously by hand for five minutes, and then centrifuged to separate the phases. A 10 ml. portion of the Skellysolve phase was transferred to colorimeter tubes, and the carotene determined at 440 millimicrons in an Evelyn Colorimeter. The Skellysolve F was then removed by evaporation under vacuum at a temperature below 60° C. The residue remaining was dissolved in 1 ml. of chloroform, and 2 drops of acetic anhydride were added to remove any moisture. With the tube in place in the colorimeter, 9 ml. of a 25% solution (w/v) antimony trichloride in chloroform were added with the aid of a rapid-delivery pipette. The blue color which developed was read at 620 millimicrons within 5 seconds of delivery. From a curve for the reaction of carotene of varying concentrations with antimony trichloride-chloroform reagent, the absorption at 620 millimicrons due to carotene was determined. Absorption due to vitamin A was determined as the difference between total absorption and that due to carotene. The method of calculation is the same as that originally reported by Dann and Evelyn (3).

The methods of analysis of these two constituents have been critically evaluated by Parrish et al. (24). They described the phasic method of analysis as employed in the present study, and the effect of saponification on carotene and vitamin A values. Low vitamin A values were found to be related to the presence of large amounts of carotene and/or color inhibitors in the plasma. Saponification was helpful in the removal of these inhibitors.

In their opinion, the Kimble method (14), the shortest of the phasic methods, is suitable for the analysis of the blood serum of calves or of cows if interferring substances are of a relatively low concentration. This condition generally exists when carotenoids are less than 350 micrograms per 100 ml. of serum. Although a saponification procedure increases the time required for analysis, it should be used in the determination of vitamin A in the blood serum of dairy cattle especially when vitamin A ester and/or color inhibitors are likely to be present in significant quantities.

Calibration of the Evelyn Colorimeter for the Determination of Vitamin A.

A standard vitamin A curve was prepared from a sample of crystalline vitamin A acetate in cottonseed oil obtained from U. S. P. Reference Standards, 46 Park Avenue, N. Y. Each gram of standard contained 3.44 mg. vitamin A acetate which is equivalent to 10,000 U. S. P. units of vitamin A. The extinction coefficient at 325 mu. = 5.37.

The standard curve was established by determining the color intensity developed by reacting the antimony trichloride-chloroform reagent with graded amounts of vitamin A acetate dissolved in chloroform. This curve is presented in the appendix together with the curve prepared by Gallup in 1944 from a vitamin A preparation obtained from Distillation Products, Inc. This preparation contained about 28 mg. vitamin A in 500 mg., with an extinction coefficient of 100.2 at 325 mu. The difference in the two curves is attributed to an improved vitamin A standard used in the preparation of the later curve.

RESULTS AND DISCUSSION

Carotene Intake. The weekly feed consumption of the calves in the different groups in trials 1 and 2 is shown in Appendix, Table 1, and the estimated carotene intake is presented in Table 1.

Table 1

Estimated Average Carotene (mg.) Consumption
per animal during two-week periods.

	AGE OF ANIMALS (WEEKS)							
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16
<u>TRIAL ONE</u>								
Group 1	40.6	92.9	116.6	178.6	279.0	422.2	562.5	757.0
Group 2	37.1	65.1	90.5	138.4	191.0	319.3	501.1	627.7
Group 3	41.2	73.5	98.3	136.7	184.9	209.4	266.6	316.2
Group 4	57.5	74.5	109.5	159.7	184.4	215.9	268.4	300.8
<u>TRIAL TWO</u>								
Group 1	34.6	115.9	172.4	355.6	526.6	691.7	689.4	767.3
Group 2	33.7	59.8	81.8	122.5	304.9	429.6	554.0	554.4
Group 3	41.9	114.8	189.6	284.2	361.6	550.0	712.0	723.5
Group 4	36.2	61.0	86.6	100.3	292.3	425.3	597.4	602.4

The average carotene intake of the calves in each group (Table 1) exceeded the minimum requirement of about 30 mcg. per kg. of body weight per day (Moore et al., 1948, Keener et al., 1942) throughout the trials. If 40 kg. is taken as the average weight during the first two weeks following birth, calves in both trials received at least 70 mcg. of carotene per kg. of body weight per day during that period. Toward the end of the trials they received from 130 to 200 mcg. of carotene per kg. of body weight. This range greatly exceeded that proposed by Boyer et al. (1) as necessary for good growth in Holstein yearlings.

Unfortunately, carotene intakes for the first trial are based on the analysis of a single sample of prairie hay. This sample contained only

6.0 mcg. of carotene per gram. It is highly probable that this value does not represent the true carotene content of all the hay consumed during this trial, since it has been shown in other studies that the carotene content of alfalfa and prairie hay may vary greatly, depending on such factors as initial content, length of time of storage, and storage conditions.

In the second trial carotene intake was calculated from the results of analyses of hay samples taken at frequent intervals during the experimental period. Both the alfalfa and prairie hays fed in this trial were of superior quality with respect to carotene content, containing 20 or more mcg. of carotene per gram. Toward the end of the experiment, the calves received freshly-cut hay, some of which contained as much as 58 mcg. of carotene per gram.

The calf starter used during the first 3 weeks of the first trial was a commercial product which proved unsatisfactory because of its low carotene content. A mixed starter containing alfalfa leaf meal was substituted at this point and its feeding was continued throughout the remainder of the experiment. Since analysis of the starter mixtures indicated that they contained from 5.0 to 8.7 mcg. of carotene per gram, an average value of 6.8 mcg. per gram was used in calculating carotene intake for the remainder of the first trial and for the entire second trial.

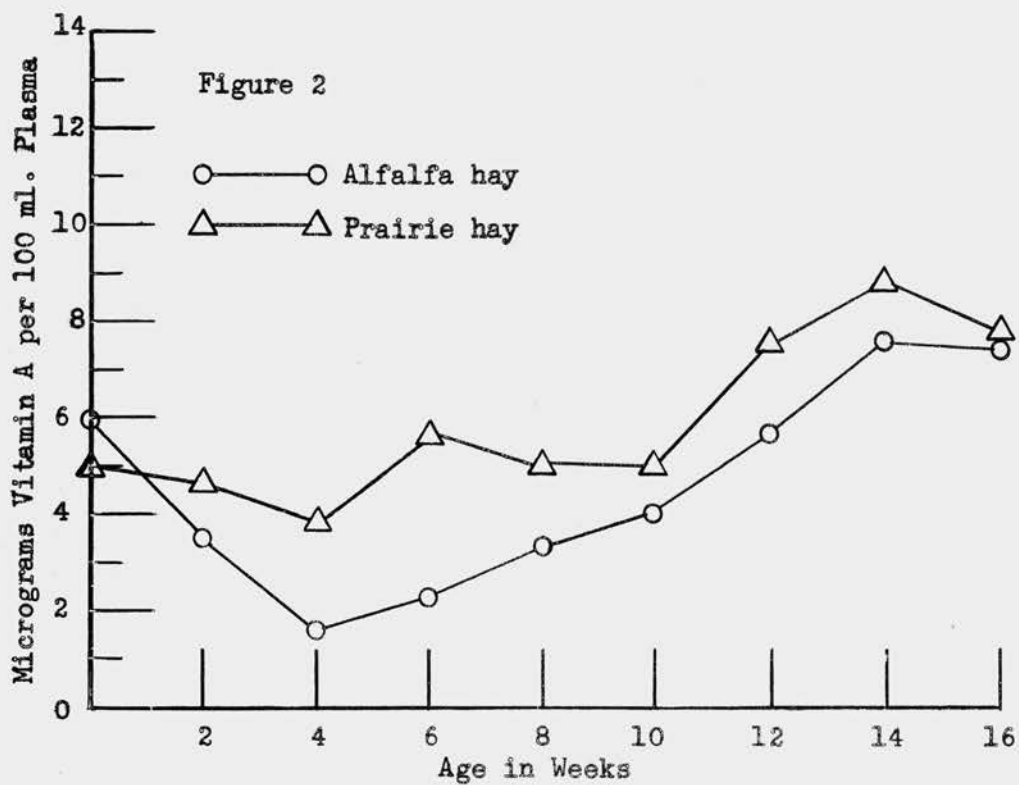
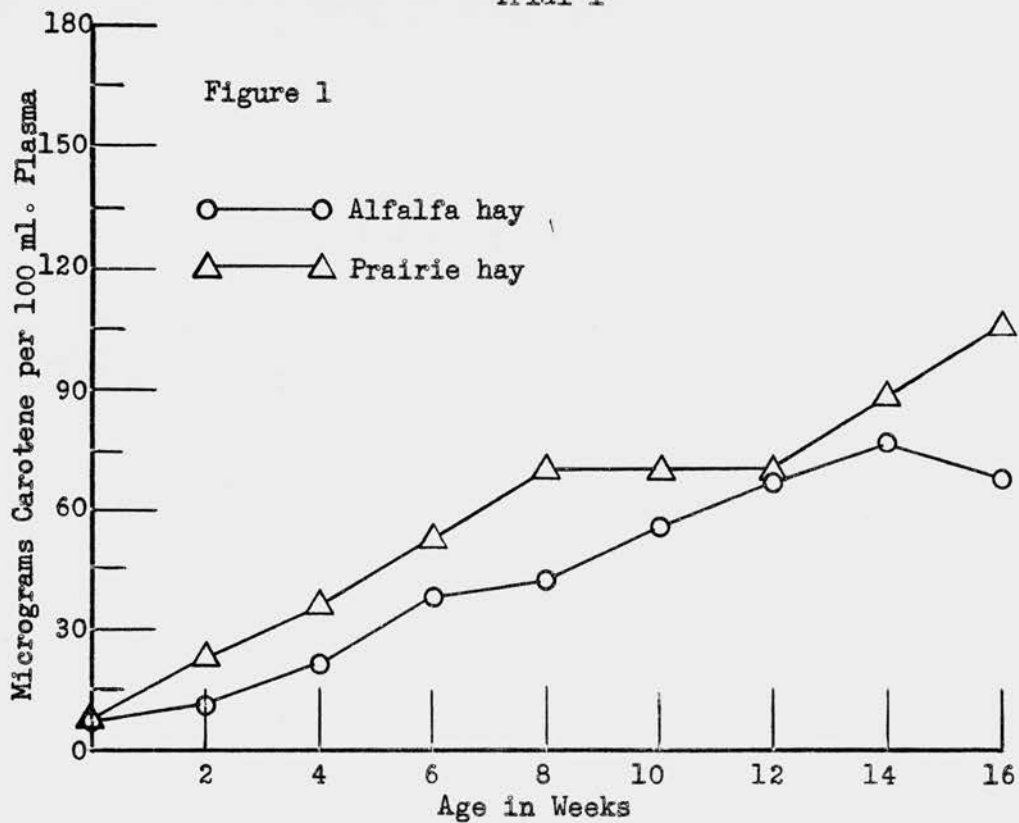
The milk consumed by the calves was not analyzed, but carotene content was assumed to be 0.44 mg. per pound, the average value for Holstein herd milk given by Morrison (21).

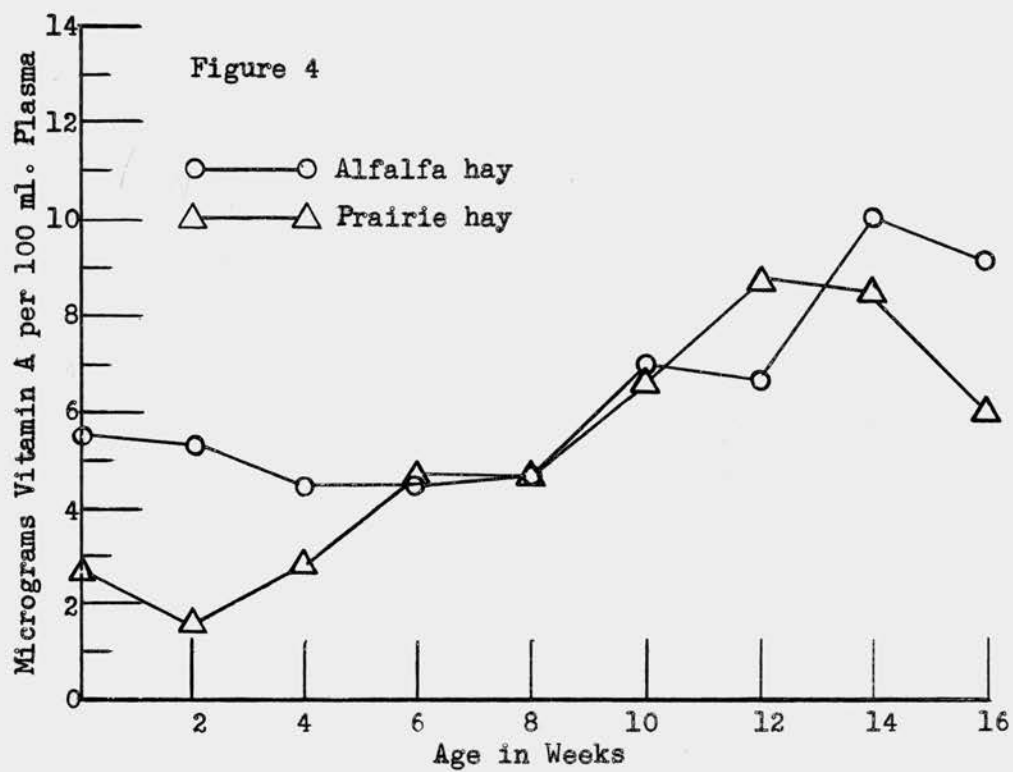
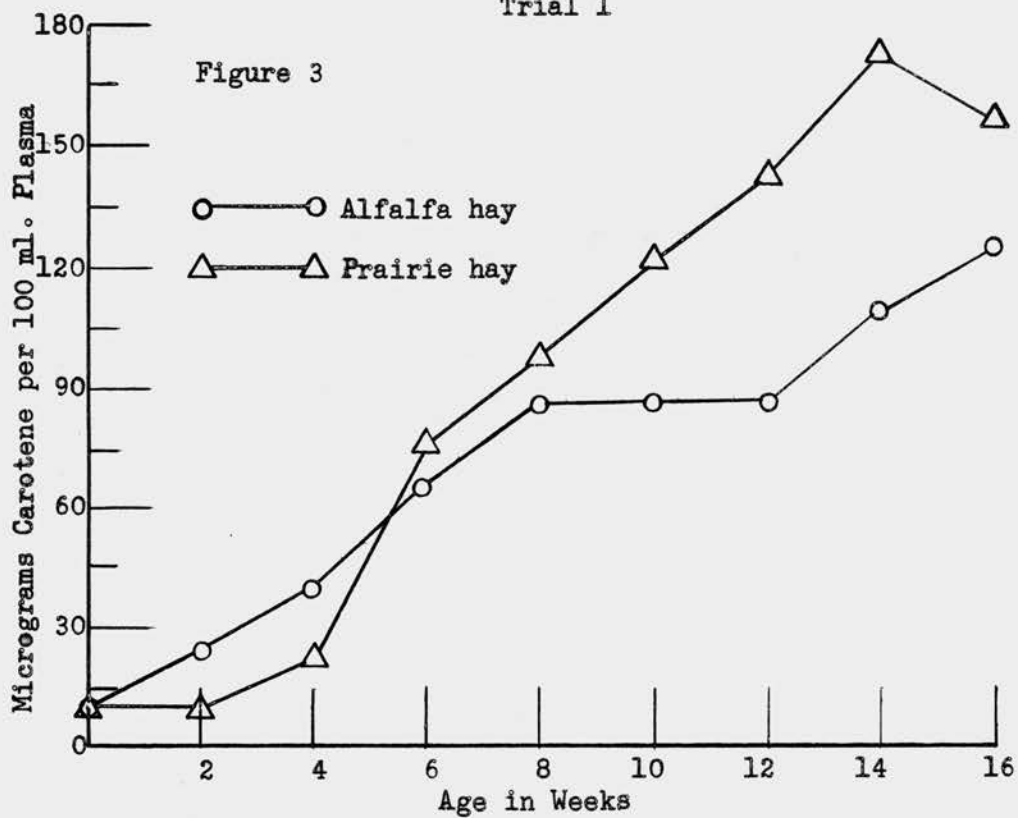
Plasma Levels of Vitamin A and Carotene. The mean amounts of vitamin A and carotene in the plasma of the calves from birth to 16 weeks of age

are shown in Figures 1-8 for each group in the two trials.

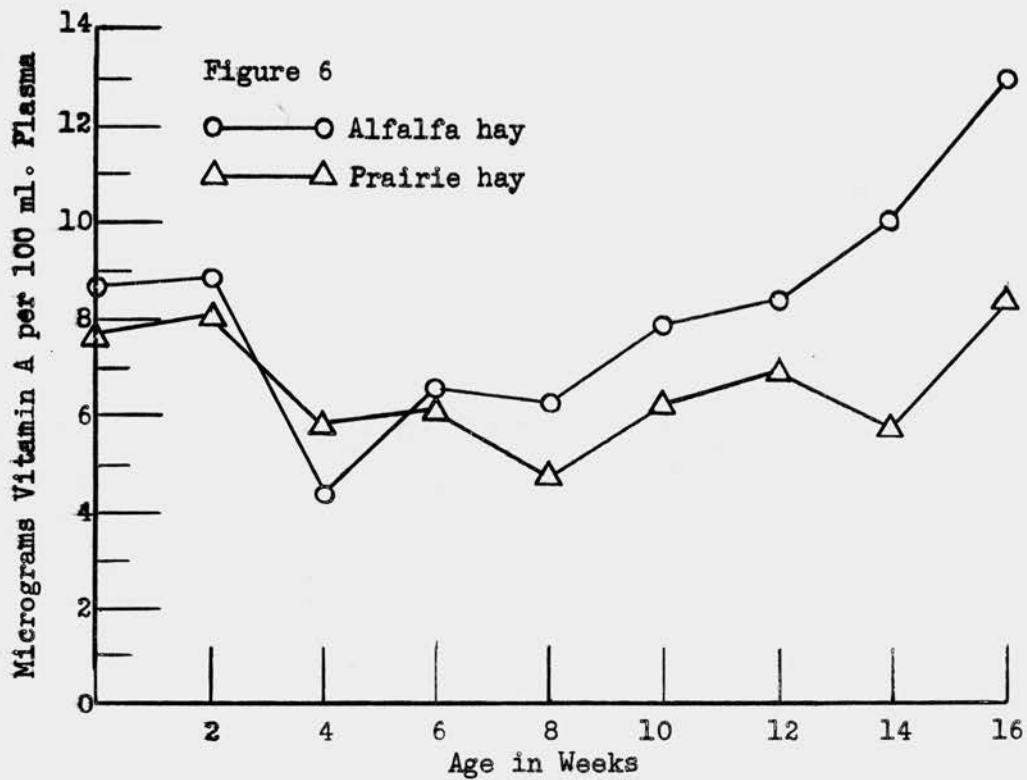
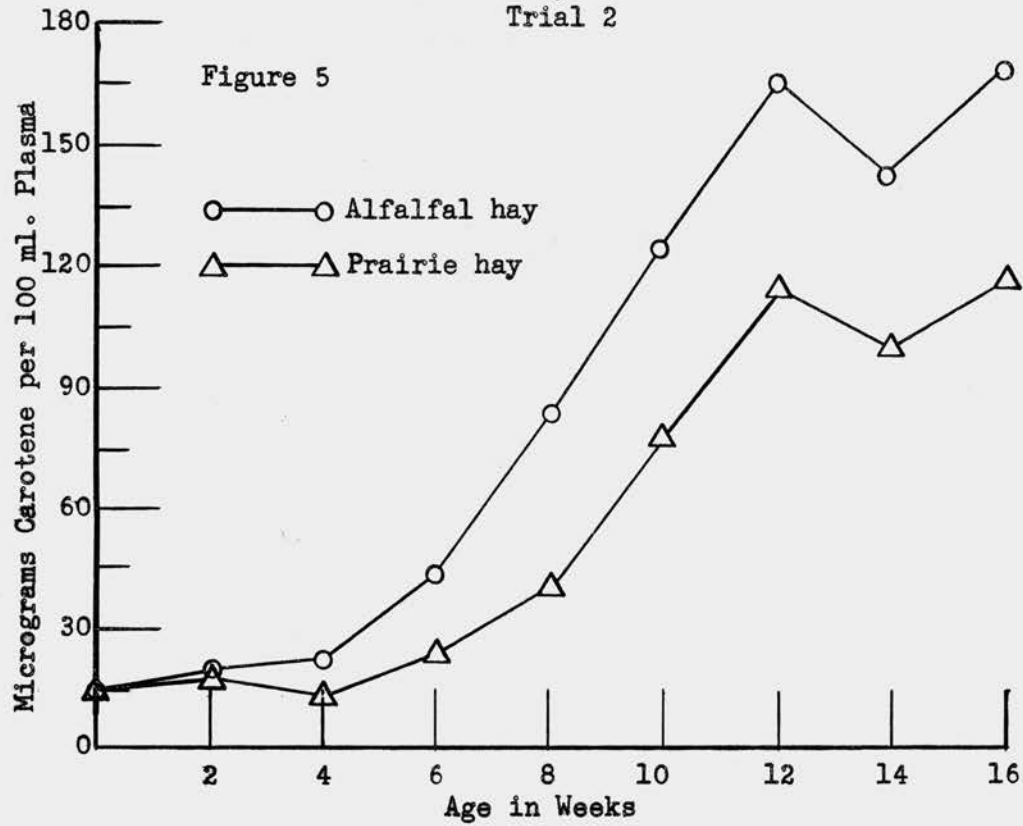
Plasma carotene levels in the first trial (Figures 1 and 3) were less than 15 mcg. per 100 ml. at birth, but increased to values of from 40 to 100 mcg. at 8 weeks and ranged from 70 to 160 mcg. at the end of the experiment. Boyer et al. (1) have reported values of from 50 to 140 mcg. of carotene for Holstein and Guernsey yearlings on a similar carotene intake. Calves receiving prairie hay generally had higher plasma carotene levels than those receiving alfalfa. This would indicate that the intake from prairie hay was possibly appreciably greater than was calculated. There was a tendency for the plasma carotene of two groups in the first trial to level off at about 10 weeks of age, when the calves were removed from milk. Calves on the delayed feeding schedule finished with higher plasma carotene levels than did those which received hay from birth. This was contrary to the expected results, and no cause for this behavior was apparent.

Plasma vitamin A levels in both trials were lower than the range of from 7.2 to 14 mcg. per 100 ml. reported by Moore and Berry (19). In view of the relatively high carotene intakes in these trials, the low vitamin A values are especially striking. In the first trial, plasma vitamin A values at 2-4 weeks fell below 4 mcg. per 100 ml. and remained there for several weeks. However, no deficiency symptoms were observed, although from previously published work they would ordinarily be expected with plasma levels this low (5). Since the analyses were conducted at two-week intervals, the effect of colostrum on the plasma vitamin A level was not detected. Wise et al. (34) have pointed out the need for vitamin A supplementation during the interim between colostrum ingestion and effective hay consumption as may be indicated here.

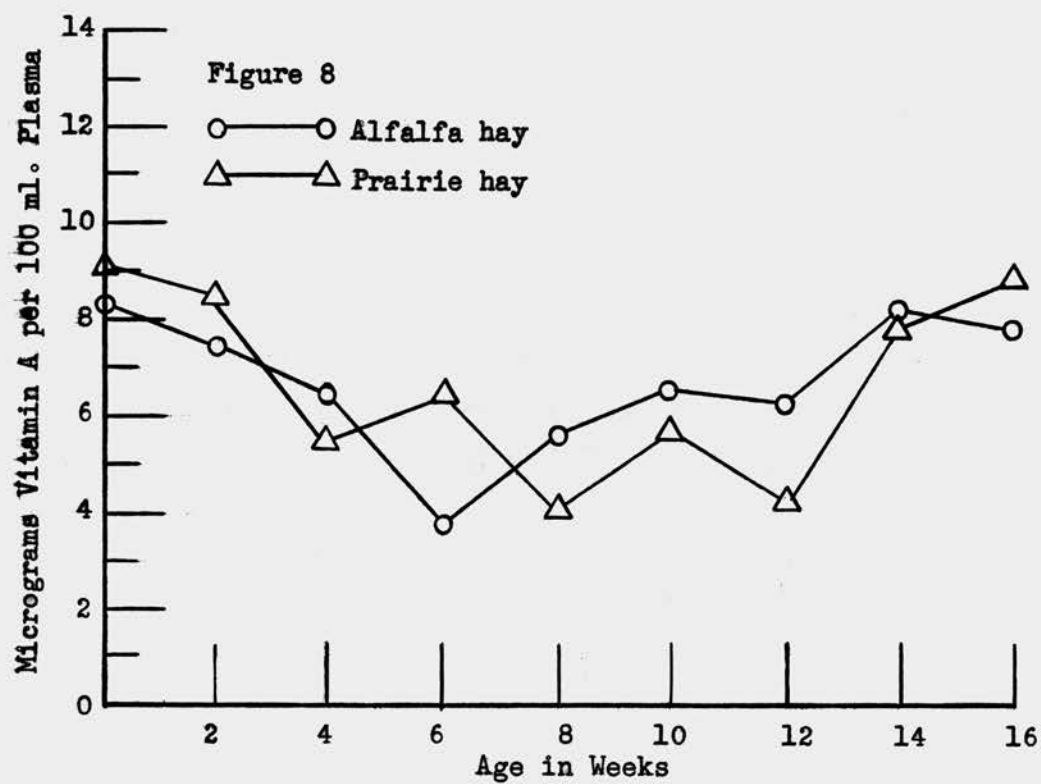
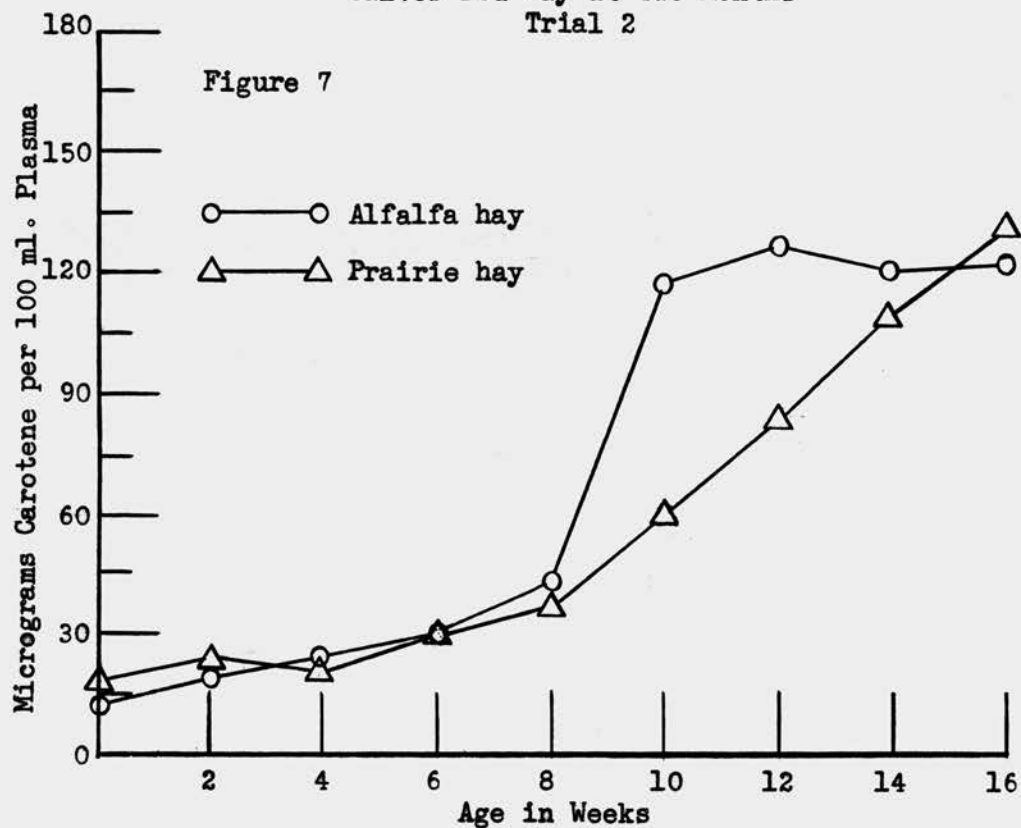
Calves Fed Hay From Birth
Trial 1

Calves Fed Hay at Two Months
Trial 1

Calves Fed Hay From Birth
Trial 2



Calves Fed Hay at Two Months
Trial 2



In the first trial, delayed feeding produced a rather striking increase in plasma vitamin A levels. During the period from 8 to 14 weeks, the average plasma levels increased from about 5 mcg. to about 9 mcg. per 100 ml. The plasma levels of vitamin A in the delayed feeding group were slightly higher at 14 weeks than were those of calves which received hay from birth. There is no explanation for the slight drop in vitamin A levels which occurred between 14 and 16 weeks in both groups.

The kind of hay fed did not affect the behavior of the plasma vitamin A levels in trial 1. Calves fed hay from birth seemed to respond somewhat better to prairie hay than to alfalfa hay (Figure 2). In view of the effect on the other two groups of this trial (Figure 4), however, this is not considered significant.

The plasma carotene levels in the second trial appear quite similar at four months to those reported by Eaton *et al.* (6). However, the levels observed in the present experiments start at a much lower value than those in the earlier experiments; 15 mcg. as compared to about 65 mcg. The extremely high plasma carotene and vitamin A levels (170 mcg. and 13 mcg., respectively) for the group receiving alfalfa hay, as shown in Figures 5 and 6, are attributed to the feeding of freshly-cut alfalfa which was very high in carotene. In most instances, the carotene levels were about 120 mcg. per 100 ml. at the termination of the trials.

Delayed feeding in the second trial exerted a marked effect on the plasma carotene level (Figure 7). There was an abrupt rise in the plasma carotene level from about 40 mcg. per 100 ml. at the eighth week to 120 mcg. at the fourteenth week. Contrary to the results obtained in trial 1, in the second trial calves fed alfalfa hay had higher plasma carotene levels than those fed prairie hay.

The vitamin A levels in trial 2 exhibited the same drop at about the fourth week as those in trial 1. The minimum reached in the second trial was not as low, however, as that reached in the first trial, 4.0 mcg. as compared to 1.8 mcg., and the calves in trial two recovered sooner from these low values.

Hay fed from birth produced an earlier increase in the plasma vitamin A of the calves than hay fed at two months; and the calves receiving hay from birth had higher levels of the vitamin at the end of the experiment than those on the delayed feeding schedule, about 10 mcg. per 100 ml. plasma in the former group as compared to about 8 mcg. in the latter. The group fed alfalfa hay from birth showed higher vitamin A levels than those fed prairie hay. This was not true in the case of delayed feeding.

Differences in the plasma content of carotene and vitamin A were related to differences in the carotene content of the two hays in trial 2 and perhaps in trial 1. When hay feeding was delayed these values generally showed little increase from birth to 6 weeks of age.

SUMMARY

An investigation consisting of two trials was conducted with dairy calves to determine the comparative effects (1) of feeding alfalfa and prairie hay, and (2) of the immediate and delayed initiation of hay feeding on the levels of carotene and vitamin A in the blood of calves from birth to 16 weeks of age.

In trial one, calves receiving prairie hay generally had higher plasma carotene levels than those fed alfalfa. Calves on the delayed feeding schedule finished the experiment with higher plasma carotene levels than did those which were offered hay from birth. Plasma vitamin A values at 2-4 weeks fell below 4 mcg. per 100 ml. and remained there for several weeks. However, no deficiency symptoms were observed.

In the second trial, delayed feeding produced a marked increase in the plasma carotene levels between the eighth and tenth week. At the termination of the trial there was no appreciable difference between the carotene levels of this group and those of the calves fed hay from birth. Changes in vitamin A levels in the second trial resembled those of trial 1, but the plasma vitamin A level dropped below 4.0 mcg. per 100 ml. for a shorter time than in the first trial.

The feeding of alfalfa hay in the second trial produced higher plasma carotene levels than did prairie hay, contrary to the results obtained in the first trial. In neither trial were the vitamin A levels affected by the type of hay fed.

Differences in the plasma content of carotene and vitamin A were related to differences in the carotene content of the two hays in trial 2

and perhaps in trial 1. When hay feeding was delayed, the levels of these two constituents generally showed little increase from birth to 6 weeks of age.

LITERATURE CITED

1. Boyer, P. D., P. H. Phillips, N. L. Lundquist, C. W. Jensen, and I. W. Rupel. Vitamin A and carotene requirements for the maintenance of adequate blood plasma vitamin A in the dairy calf. J. Dairy Sci. 25, 433-440 (1942).
2. Braun, W. Studies on the carotenoids and vitamin A levels in cattle. II. carotenoids and vitamin A in the liver, their ratio, and their relationships to blood levels. J. Nutrition 29, 73-79 (1945).
3. Dann, W. J. and K. A. Evelyn. The determination of vitamin A with the photoelectric colorimeter. Biochem. J. 32, 1008-17 (1938).
4. Eaton, H. D., C. F. Helmboldt, E. L. Jungherr, C. A. Carpenter, and L. A. Moore. Effect of vitamin A depletion on liveweight, plasma and liver levels of vitamin A and microanatomy in young dairy calves. J. Dairy Sci. 34, 386-395 (1951).
5. Eaton, H. D., A. A. Spielman, R. E. Johnson, and L. D. Matterson. The utilization of fetal stores of vitamin A by the newborn calf. J. Dairy Sci. 32, 764-768 (1949).
6. Eaton, H. D., A. A. Spielman, J. K. Loosli, J. W. Thomas, C. L. Norton, and K. L. Turk. The effect of a massive dose at birth of vitamin A and D upon blood plasma levels and liver storage in Holstein calves. J. Dairy Sci. 30, 795-802 (1947).
7. Gallup, W. D., and M. E. Gibson. The relation between so called crude and true carotene of native grass hay. Proc. of the Okla. Acad. of Sci. for 1948. 29, 84-85 (1950).
8. Hibbs, J. W., and W. E. Krauss. The effect of feeding vitamin A on the blood picture and on liver storage in calves. J. Dairy Sci. 29, 519-520 (1946).
9. Hibbs, J. W., and W. D. Pounden. The influence of the ration and early rumen development on the changes in the plasma carotenoids, vitamin A and ascorbic acid of young dairy calves. J. Dairy Sci. 31, 1055-61 (1948).
10. Jacobson, W. C., H. T. Converse, H. G. Wiseman, and L. A. Moore. Effect of substituting colostrum for whole milk in the ration of dairy calves. J. Dairy Sci. 34, 905-910 (1951).
11. Jacobson, W. C., H. T. Converse, and L. A. Moore. Effect of vitamin A and carotene intake on depletion time of young dairy calves. J. Dairy Sci. 32, 418-428 (1949).

12. Kaeser, H. E., and T. S. Sutton. Beneficial effect and economic importance of using all colostrum produced in calf raising. J. Dairy Sci. 31, 523-532 (1948).
13. Keener, H. A., S. I. Bechdel, and W. T. S. Thorp. Carotene in calf nutrition. J. Dairy Sci. 25, 571-583 (1942).
14. Kimble, M. S. The photoelectric determination of vitamin A and carotene in human plasma. J. Lab. and Clin. Med. 24, 1055-1065 (1939).
15. Lepper, H. A., Ed. Official Methods of Analysis of the Association of Official Agricultural Chemists., Seventh Edition, A. O. A. C., Washington 4, D. C. (1950).
16. Lewis, J. M., and L. T. Wilson. Vitamin A requirements in calves. J. Nutrition 30, 467-475 (1945).
17. Lundquist, N. L., and P. H. Phillips. Certain dietary factors essential for the growing calf. J. Dairy Sci. 26, 1023-1030 (1943).
18. Moore, L. A. The vitamin A and carotene content of the blood plasma of calves from birth to four months of age. J. Dairy Sci. 25, 687 (1942).
19. Moore, L. A., and M. H. Berry. Vitamin A and carotene content of the blood plasma of dairy calves from birth up to four months. J. Dairy Sci. 28, 821-826 (1945).
20. Moore, L. A., J. F. Sykes, W. C. Jacobson, and H. G. Wiseman. Carotene requirements for Guernsey and Jersey calves as measured by spinal fluid pressures. J. Dairy Sci. 31, 533-538 (1948).
21. Morrison, F. B. Feeds and Feeding, 21st edition, p. 1164, The Morrison Publishing Co., Ithaca, N. Y. (1948).
22. Nezvesky, L., H. D. Eaton, R. E. Johnson, L. D. Matterson, C. I. Bliss, and A. A. Spielman. The effect of vitamin A from pre-natal storage and from ingestion of colostrum on the neo-natal calf. J. Dairy Sci. 33, 315-323 (1950).
23. Parrish, D. B., G. H. Wise, and J. S. Hughes. Effect of vitamin A supplements on the state of vitamin A in blood serum of the dairy cow and in blood serum and liver in its neonatal calf. J. Biol. Chem. 172, 355-365 (1948).
24. Parrish, D. B., G. H. Wise, and J. S. Hughes. Vitamin A and carotenoids in the blood serum of dairy cattle. Chemical methods for determination. Anal. Chem. 20, 230-233 (1948).

- 25. Peterson, W. J. Recent developments in methods for determining carotene. Ind. Eng. Chem. Anal. Ed. 13, 212-216 (1941).
- 26. Phillips, Paul H., Norman S. Lundquist, and Paul D. Boyer. The blood plasma vitamin A content of the newborn calf and its relation to certain calfhood diseases. J. Dairy Sci. 24, 522 (1941).
- 27. Sellers, K. C. and E. Eden. The effect of white scour on the absorption of vitamin A by calves. J. Comp. Pathol. Therap. 59, 205-212 (1949). Via Chem. Abst. 44, 10117a.
- 28. Spielman, A. A., H. D. Eaton, J. K. Loosli, and K. L. Turk. The effect of prepartum vitamin A supplementation on the health and performance of the young calf. J. Dairy Sci. 32, 367-374 (1949).
29. Spielman, A. A., J. W. Thomas, J. K. Loosli, C. L. Norton and K. L. Turk. The placental and mammary transmission and fetal storage of vitamin A and carotene in the bovine. J. Dairy Sci. 29, 707-715 (1946).
- 30. Sutton, T. S., and H. E. Kaeser. Some physiological effects of extending the colostrum feeding period for dairy calves. J. Dairy Sci. 29, 13-26 (1946).
- 31. Wall, M. E., and E. G. Kelley. Determination of pure carotene in plant tissue. A rapid chromatographic method. Ind. Eng. Chem. Anal. Ed. 15, 18-20 (1943).
32. Willstatter, R. and A. Stoll. Untersuchungen Uber Chlorophyll, Berlin, Julius Springer (1913).
- 33. Wise, G. H., M. J. Caldwell, and J. S. Hughes. The effect of prepartum diet of the cow on the vitamin A reserves of her newborn offspring. Science 103, 616-618 (1946).
34. Wise, G. H., M. J. Caldwell, D. B. Parrish, F. W. Atkeson, and J. S. Hughes. Relation of typical postnatal changes in the diet of the dairy calf to the concentration of carotenoids and vitamin A in the blood serum. J. Animal Sci. 7, 70-77 (1948).

APPENDIX

TABLE 1

TRIAL 1

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods								
	1	2	3	4	5	6	7	8	9
1 Milk	38.0	35.0	35.0	36.2	36.5	32.6	38.7	42.0	42.0
Starter			0.5	2.6	5.1	6.7	8.3	11.6	11.2
Hay									3.5
2 Milk	42.0	49.0	57.3	68.2	83.0	62.0	18.0	14.0	
Starter			3.2	3.2	8.1	12.2	22.7	25.9	27.5
Hay									3.2
3 Milk	45.5	54.3	64.7	65.8	65.8	37.6	23.0	21.0	9.0
Starter		0.2	3.0	5.1	5.9	5.5	11.9	15.1	18.1
Hay									3.9
4 Milk	49.4	65.0	54.0	54.6	49.4	42.0	38.0	26.9	9.0
Starter			2.1	4.0	4.3	11.6	15.8	22.5	26.2
Hay									2.8
5 Milk	30.5	29.0	37.7	27.3	41.5	42.0	42.0	42.0	35.0
Starter		0.2	5.4	3.7	8.1	10.0	11.6	13.1	17.0
Hay									2.5
6 Milk	62.9	65.8	64.1	51.6	44.0	35.0	31.0	24.0	9.0
Starter		0.4	3.2	4.7	12.8	17.3	25.2	27.0	28.0
Hay									1.8
7 Milk	28.4	32.2	30.4	31.8	44.2	42.0	42.0	35.0	35.0
Starter		0.6	1.7	3.0	7.1	9.8	12.6	16.1	17.2
Hay									2.4
8 Milk	26.0	28.4	24.6	34.3	49.0	49.0	46.5	36.0	35.0
Starter		1.3	3.5	7.0	8.7	13.0	20.0	21.5	22.2
Hay									0.5
9 Milk	28.0	36.5	49.0	49.0	41.5	42.0	42.0	42.0	23.0
Starter			1.6	4.7	4.2	7.8	11.4	16.0	19.8
Hay									1.0
10 Milk	38.0	52.0	66.0	62.0	56.0	42.0	42.0	14.0	
Starter		1.4	3.8	6.9	12.2	19.4	25.7	28.0	28.0
Hay									2.3
11 Milk	31.0	38.0	49.0	49.0	49.0	42.0	42.0	30.0	24.0
Starter		1.4	3.5	5.3	5.8	10.3	11.5	14.6	20.2
Hay			0.7	0.7	1.0	0.8	1.0	0.7	1.4

Trial 1 (continued)

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods							Totals
	10	11	12	13	14	15	16	
1 Milk	39.0	Died. Figures not included in averages.						375.0
Starter	11.4							57.4
Hay	3.4							6.9
2 Milk								393.5
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	298.8
Hay	5.5	6.5	11.9	14.3	20.8	27.6	29.4	119.2
3 Milk								386.7
Starter	25.1	28.0	28.0	28.0	28.0	28.0	28.0	257.9
Hay	4.3	7.2	6.6	11.6	24.8	25.7	12.5	96.6
4 Milk								388.3
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	281.5
Hay	4.7	9.6	13.6	18.7	25.0	28.0	31.5	105.6
5 Milk	35.0	5.0						367.0
Starter	15.6	21.0	27.8	28.0	28.0	28.0	28.0	245.5
Hay	3.9	9.2	11.4	19.2	18.4	19.1	21.4	105.1
6 Milk								387.4
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	314.6
Hay	1.9	4.7	8.7	11.1	21.9	28.1	25.5	103.7
7 Milk	35.0	20.0						371.5
Starter	19.2	24.6	28.0	27.2	28.0	28.0	28.0	251.1
Hay	2.3	4.9	9.6	7.8	21.1	28.2	22.8	99.1
8 Milk	35.0	10.0						373.8
Starter	25.4	27.5	28.0	28.0	28.0	28.0	28.0	290.1
Hay	0.5	1.9	2.0	8.0	10.7	13.9	21.2	58.8
9 Milk	21.0							374.0
Starter	23.1	24.9	28.0	28.0	28.0	28.0	28.0	253.5
Hay	1.1	1.2	2.7	9.6	11.8	13.8	22.8	64.0
10 Milk								372.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	321.4
Hay	6.3	7.3	12.0	20.6	21.4	27.1	28.8	125.8
11 Milk	18.0							372.0
Starter	22.9	22.5	28.0	28.0	28.0	28.0	28.0	258.0
Hay	2.0	2.7	6.4	10.2	12.3	16.7	16.1	72.7

Trial 1 (continued)

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods								
	1	2	3	4	5	6	7	8	9
12 Milk	39.9	56.0	60.0	68.0	54.0	42.0	42.0	12.0	
Starter	0.9	2.1	5.3	7.4	11.2	15.4	14.7	22.2	27.0
Hay			0.9	1.1	0.6	1.0	1.4	5.0	5.9
13 Milk	38.0	46.0	66.0	58.0	44.0	42.0	42.0	33.0	3.0
Starter		0.3	3.1	2.8	6.4	12.5	17.9	22.6	27.2
Hay			1.0	0.4	0.8	1.3	2.6	4.5	7.2
14 Milk	42.0	56.0	70.0	56.0	42.0	42.0	42.0	26.0	
Starter		0.3	0.6	3.8	10.1	14.5	18.1	19.8	28.0
Hay			0.3	1.0	2.3	1.6	1.6	3.5	3.6
15 Milk	42.0	56.0	70.0	56.0	42.0	42.0	42.0	26.0	
Starter		0.3	1.1	3.9	9.1	9.9	18.5	18.9	28.0
Hay			0.7	2.0	1.9	1.9	2.1	3.3	4.3
16 Milk	42.0	41.6	2.5	Died. Figures not included in averages.					
Starter									
Hay									
17 Milk	42.0	56.0	70.0	56.0	42.0	42.0	42.0	24.0	
Starter		0.3	0.9	4.1	7.4	12.8	17.8	20.4	28.0
Hay			0.6	1.5	0.8	0.9	0.9	2.5	4.4
18 Milk	26.0	24.5	39.0	46.0	49.0	48.5	46.0	37.0	30.0
Starter		0.3	1.9	2.6	3.5	4.8	7.6	5.2	7.0
Hay			1.0	0.1	0.3	0.3	0.1	1.0	2.6
19 Milk	40.0	59.0	56.0	67.0	56.0	42.0	42.0	13.0	
Starter		1.4	2.4	3.5	6.5	9.0	13.9	21.5	22.8
Hay			0.2	0.0	0.0	0.4	0.4	1.7	2.4
20 Milk	35.5	52.0	66.0	60.0	44.0	42.0	42.0	30.0	
Starter		2.4	7.3	8.6	11.5	17.8	20.0	24.0	26.3
Hay			0.9	0.5	0.4	0.9	1.8	3.9	7.6
21 Milk	35.5	51.0	66.0	60.0	44.0	42.0	42.0	32.0	
Starter		1.9	3.1	7.7	4.9	4.9	15.9	22.2	24.1
Hay									0.5
22 Milk	27.1	39.0	48.0	49.0	42.5	42.0	35.0	35.0	33.0
Starter		2.1	4.1	4.4	6.4	5.5	6.6	14.2	17.6
Hay			0.4	0.2	0.3	0.2	1.3	4.2	7.8

Trial 1 (continued)

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Period							Totals
	10	11	12	13	14	15	16	
12 Milk								373.9
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	302.2
Hay	10.4	15.9	14.8	16.7	30.6	32.9	39.3	176.5
13 Milk								372.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	288.8
Hay	12.6	16.6	16.7	21.4	21.1	29.4	34.1	169.7
14 Milk								376.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	391.2
Hay	7.4	12.1	15.1	16.9	20.5	27.7	34.7	148.3
15 Milk								376.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	285.7
Hay	6.4	9.2	11.2	15.7	17.1	26.6	34.5	136.9
16 Milk	Died. Figures not included in averages.							86.1
Starter								
Hay								
17 Milk								374.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	287.7
Hay	7.7	11.4	15.2	19.4	21.1	27.6	38.5	152.5
18 Milk	20.0							374.0
Starter	15.9	23.0	28.0	28.0	28.0	28.0	28.0	211.8
Hay	1.0	2.5	3.6	5.2	18.7	25.2	25.9	87.5
19 Milk								375.0
Starter	27.8	28.0	28.0	28.0	28.0	28.0	28.0	276.8
Hay	4.5	7.1	13.3	23.2	26.2	24.7	33.2	137.3
20 Milk								371.5
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	313.9
Hay	10.9	18.5	28.4	34.7	32.7	36.8	42.4	220.4
21 Milk								372.5
Starter	24.7	27.7	28.0	28.0	28.0	28.0	28.0	277.1
Hay	5.7	13.8	20.6	29.0	37.0	35.7	41.3	175.5
22 Milk	21.0							371.6
Starter	18.5	16.9	18.1	25.2	21.3	26.8	27.3	215.0
Hay	10.8	9.0	11.4	16.4	25.2	25.0	27.0	139.2

TRIAL 2

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods								
	1	2	3	4	5	6	7	8	9
23 Milk	35.5	48.5	58.0	68.0	56.0	42.0	42.0	24.0	
Starter			1.2	5.8	6.2	6.8	12.3	18.8	25.8
Hay									4.4
24 Milk	26.0	29.0	35.5	49.0	44.0	42.0	42.0	34.0	28.0
Starter			3.9	6.9	9.3	8.8	6.9	9.6	9.6
Hay			0.1	0.2	1.3	1.8	3.4	6.9	10.0
25 Milk	32.5	41.0	47.0	49.0	44.0	42.0	37.0	28.0	22.0
Starter			1.0	3.5	4.6	12.0	15.8	17.3	17.3
Hay									7.8
26 Milk	39.0	48.0	55.0	68.0	58.0	44.0	30.0	21.0	12.0
Starter		0.3	5.9	10.0	10.7	5.5	8.4	12.2	19.3
Hay			2.3	3.7	2.9	5.3	10.2	10.4	12.9
27 Milk	39.0	48.0	55.0	68.0	56.0	42.0	21.0	21.0	21.0
Starter		1.1	2.7	3.8	9.1	11.7	16.4	19.6	22.3
Hay		0.8	2.3	8.0	10.4	10.4	10.6	21.5	26.2
28 Milk	26.0	28.0	29.0	39.0	46.0	49.0	43.5	42.0	42.0
Starter		1.1	2.3	4.1	4.2	9.7	11.2	15.6	16.5
Hay			0.9	3.1	2.4	2.9	5.3	10.5	12.6
29 Milk	39.0	48.0	37.0	47.0	51.0	58.0	35.0	28.0	28.0
Starter			4.1	4.9	9.8	14.1	14.8	16.9	25.4
Hay									3.0
30 Milk	26.0	28.0	33.0	38.5	42.0	46.0	49.0	45.0	36.0
Starter			0.8	2.4	6.3	10.0	14.7	17.6	18.0
Hay									9.0
31 Milk	20.5	31.5	39.0	41.5	44.5	47.0	42.0	42.0	33.0
Starter		0.6	1.9	2.1	5.1	10.4	10.8	10.5	10.8
Hay			1.5	2.6	3.1	5.0	14.1	17.1	24.8
32 Milk	28.0	40.0	48.0	49.0	55.0	64.0	44.0	36.0	6.0
Starter		0.8	2.0	4.0	3.1	11.0	17.6	20.7	25.2
Hay									5.5
33 Milk	40.0	52.0	55.5	66.0	64.0	53.0	29.0	15.0	
Starter		0.6	1.9	7.1	13.5	18.3	19.2	27.3	26.5
Hay		0.2	2.3	5.2	9.8	10.9	10.4	18.4	10.5

Trial 2 (continued)

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods							Totals
	10	11	12	13	14	15	16	
23 Milk								374.0
Starter	27.8	25.5	26.7	27.7	28.0	28.0	28.0	268.6
Hay	11.4	11.6	23.7	25.3	30.1	33.3	40.8	180.6
24 Milk	28.0	28.0	4.0					398.5
Starter	10.4	11.6	19.2	21.9	27.5	26.7	27.5	199.8
Hay	9.5	12.7	10.3	11.7	15.1	17.5	17.2	117.7
25 Milk	21.0	12.0						375.5
Starter	16.5	19.8	20.9	28.0	28.0	28.0	28.0	240.7
Hay	18.3	17.2	18.0	17.8	21.7	26.1	26.7	153.6
26 Milk								375.0
Starter	19.0	23.0	28.0	27.6	27.6	28.0	28.0	253.5
Hay	18.7	18.7	22.8	32.1	38.3	39.9	39.2	257.4
27 Milk								371.0
Starter	27.2	28.0	27.5	28.0	28.0	28.0	28.0	281.4
Hay	29.7	34.4	40.0	37.9	39.7	43.9	46.9	362.7
28 Milk	21.0	4.5						370.0
Starter	17.5	20.2	26.7	28.0	28.0	26.9	27.8	239.8
Hay	17.2	16.3	16.1	22.9	25.9	25.6	19.6	181.3
29 Milk	4.0							375.0
Starter	28.0	26.1	28.0	28.0	28.0	28.0	28.0	284.1
Hay	3.0	10.9	16.0	22.0	17.2	18.4	18.7	109.2
30 Milk	21.0	6.0						370.5
Starter	18.2	18.1	18.6	27.8	28.0	27.2	28.0	235.7
Hay	10.3	16.0	17.2	16.0	20.8	26.3	20.8	136.4
31 Milk	21.0	16.5						378.5
Starter	19.9	27.7	28.0	28.0	20.8	21.3	28.0	225.9
Hay	24.1	24.4	26.5	24.2	17.8	18.9	26.0	230.1
32 Milk								370.0
Starter	25.7	28.0	28.0	27.2	28.0	28.0	28.0	277.3
Hay	19.5	24.7	27.3	17.7	11.3	13.3	27.3	146.6
33 Milk								374.5
Starter	26.5	28.0	28.0	28.0	28.0	28.0	28.0	308.9
Hay	6.4	12.1	17.2	18.3	19.7	25.4	26.0	192.8

Trial 2 (continued)

Schedule of Feed Consumption in Pounds

Calf	Seven-Day Periods							Totals
	10	11	12	13	14	15	16	
34 Milk	18.0							377.0
Starter	14.0	14.0	16.5	19.4	19.0	19.2	18.0	146.2
Hay	2.8	5.9	6.3	12.0	12.7	18.4	16.6	77.4
35 Milk	1.5							374.5
Starter	13.3	14.3	10.4	9.2	11.0	13.1	7.6	142.3
Hay	7.5	10.8	10.8	12.6	16.6	18.7	8.7	101.4
36 Milk	6.0							374.0
Starter	16.1	13.6	15.1	18.5	19.8	28.0	28.0	203.8
Hay	14.0	18.7	18.7	25.0	24.7	30.2	39.9	229.0
37 Milk	22.0	13.5						382.5
Starter	12.8	24.5	27.2	27.5	27.4	28.0	28.0	212.2
Hay	10.8	17.4	21.3	20.8	22.6	22.6	25.4	167.4
38 Milk	21.0	7.5						374.5
Starter	18.5	22.6	26.4	27.9	28.0	28.0	28.0	237.3
Hay	13.2	16.7	15.1	15.5	14.6	16.4	17.9	117.2
39 Milk	21.0	9.0						375.0
Starter	19.1	24.0	26.5	26.0	28.0	28.0	28.0	224.2
Hay	29.7	34.3	33.4	32.4	35.2	36.6	36.9	306.7
40 Milk								377.5
Starter	26.3	27.4	27.9	27.7	28.0	28.0	28.0	254.1
Hay	17.6	16.2	16.9	21.6	24.7	25.4	24.6	204.1
41 Milk								379.0
Starter	28.0	28.0	28.0	28.0	28.0	28.0	28.0	263.7
Hay	17.1	19.9	22.2	25.2	30.6	33.0	31.5	186.8
42 Milk	22.0	21.0	4.5					373.0
Starter	19.8	24.2	24.1	27.0	27.6	27.8	28.0	236.2
Hay	10.8	14.3	15.8	21.6	24.3	24.3	23.0	141.2

TABLE 2

Mcg. Carotene Per 100 Ml. Blood Plasma

Trial One

	Calf No.	Weeks								
		Initial	2	4	6	8	10	12	14	16
Group I.	12	20.4	3.9	5.3	36.0	38.9	100.5	101.7	92.4	87.6
	13	none	11.3	20.4	46.8	60.6	58.7	76.4	96.6	62.0
	17	>0.2	0.2	37.2	62.4	56.3	42.2	52.7	65.0	84.0
	18	----	18.5	23.4	35.0	24.0	32.3	39.3	70.2	68.9
	20	----	16.1	20.4	14.6	29.1	47.3	58.7	68.3	84.0
	Ave.	6.8	10.0	21.3	39.0	41.8	56.1	67.8	78.5	70.1
Group II.	1	none	6.0	22.5	91.2	81.8		Died		
	5	8.7	41.1	30.2	69.6	110.3	96.9	123.6	120.0	147.0
	6	18.6	34.5	92.9	119.6	129.6	108.9	91.6	114.9	148.5
	7	18.6	26.1	44.4	82.5	121.5	140.4	101.7	152.7	123.6
	9	----	16.1	35.1	24.0	52.1	57.8	80.4	106.5	121.2
	21	>0.3	28.7	18.0	11.3	24.0	33.9	40.5	56.3	86.1
	Ave.	9.2	25.4	40.5	66.4	86.6	87.6	87.6	110.1	125.2
Group III.	11	11.3	11.6	26.1	70.2	67.8	123.3	96.9	88.8	135.3
	14	12.2	23.0	67.7	76.4	65.0	76.4	96.6	136.1	155.4
	15	----	21.5	24.0	68.3	78.3	76.4	64.4	86.9	117.9
	16	8.0					Died			
	19	2.6	35.6	41.1	47.9	126.0	32.3	38.9	68.9	52.7
	22	4.4	18.9	17.0	11.7	29.1	53.9	68.3	65.7	64.4
	Ave.	5.7	22.1	35.2	54.9	73.2	72.5	73.0	89.3	105.1
Group IV.	2	8.5	14.1	10.8	59.4	81.8	102.0	170.7	163.5	162.3
	3	5.5	7.8	33.9	79.1	72.3	95.9	129.9	178.2	86.7
	4	----	15.9	37.7	91.2	126.8	173.9	181.2	186.9	165.0
	8	8.4	9.6	3.8	62.4	106.8	84.6	113.3	148.5	137.7
	10	14.6	1.7	30.0	89.1	101.4	150.4	122.4	188.7	225.3
	Ave.	9.3	9.8	23.2	76.2	97.8	121.4	143.5	173.2	155.4

Table 2 (continued)

Mcg. Carotene Per 100 Ml. Blood Plasma										
Trial Two										
	Calf No.	Weeks								
		Initial	2	4	6	8	10	12	14	16
Group I.	24	14.1	8.3	31.8	15.8	32.9	42.8	65.0	128.4	92.1
	27	17.6	6.9	17.0	41.6	41.1	60.6	135.3	169.2	179.7
	31	10.5	21.9	28.7	49.7	153.9	243.0	184.2	136.5	129.9
	36	17.6	18.0	14.6	79.1	102.0	102.9	116.1	112.5	128.7
	39	15.2	43.8	27.6	33.9	92.1	173.7	221.0	168.0	305.1
	Ave.	15.0	19.8	23.9	44.0	84.4	124.6	164.3	142.9	167.1
Group II.	23	12.4	18.2	26.8	51.5	43.4	50.3	80.4	87.6	102.9
	30	16.5	27.0	23.0	12.4	45.6	144.6	172.2	151.2	131.4
	32	7.5	20.0	32.3	47.9	37.7	117.2	92.4	78.6	69.0
	35	17.6	6.6	24.5	19.5	36.6	78.6	102.9	105.3	118.5
	42	11.3	31.8	22.5	21.2	47.3	194.2	184.2	175.2	181.2
	Ave.	13.1	20.7	25.8	30.5	42.1	117.0	126.4	119.6	120.6
Group III.	26	10.4	12.6	20.0	24.5	38.3	70.2	90.6	78.6	99.3
	28	4.8	12.2	9.9	37.7	26.1	75.0	102.9	113.7	96.9
	33	25.5	26.1	19.1	30.2	32.3	24.0	61.5	76.5	67.8
	37	21.5	18.0	11.7	20.4	61.2	91.2	207.3	113.0	137.7
	40	18.5	23.4	10.8	9.9	42.3	129.6	104.0	118.8	178.2
	Ave.	16.1	18.5	14.3	24.5	40.0	78.0	113.3	100.1	116.0
Group IV.	25	9.0	7.5	6.4	35.0	31.8	47.8	84.6	89.1	136.1
	29	11.7	7.5	5.3	29.3	31.8	45.6	47.3	62.4	46.8
	34	16.5	16.5	36.0	33.3	41.1	72.9	76.5	156.6	133.8
	38	39.3	66.3	30.2	30.2	33.9	101.7	115.0	120.0	182.4
	91	11.3	25.1	28.1	21.5	42.3	30.2	98.1	105.3	151.2
	Ave.	17.6	24.6	21.2	29.9	34.4	59.6	84.3	106.7	130.1

TABLE 3

Mcg. Vitamin A Per 100 Ml. Blood Plasma

Trial One

Calf No.	Weeks								
	Initial	2	4	6	8	10	12	14	16
Group I									
12	12.9	7.38	0.50	1.56	0.75	7.95	6.39	1.98	>0.60
13	1.18	2.45	1.14	2.37	10.5	5.72	6.39	10.4	11.3
17	3.96	>0.30	2.40	3.90	1.98	0.99	3.08	8.46	6.17
18	-----	5.56	4.26	3.44	>0.30	>0.30	2.48	8.18	10.4
20	-----	2.04	0.30	>0.30	3.29	5.45	10.5	9.02	8.61
Ave.	6.01	3.55	1.72	2.31	3.36	4.08	5.77	7.61	7.42
Group II									
1	0.50	>0.60	>0.40	1.79	4.88	Died			
5	3.60	10.3	6.42	5.37	5.76	12.6	9.21	11.3	13.1
6	6.45	4.98	3.57	6.60	7.29	7.71	6.06	6.54	7.89
7	11.6	8.28	8.91	9.45	10.0	8.31	7.65	17.6	10.6
9	-----	7.37	2.39	2.03	1.13	2.93	3.86	9.69	2.58
21	-----	>0.60	4.92	1.50	>0.30	3.48	6.78	4.91	11.5
Ave.	5.54	5.27	4.44	4.46	4.89	7.01	6.71	10.0	9.13
Group III									
11	13.7	12.8	10.4	11.4	5.56	8.16	6.18	5.31	10.6
14	3.12	2.55	3.81	8.70	4.67	6.86	9.14	14.3	9.27
15	6.65	2.72	2.21	4.83	5.45	4.98	7.07	11.7	10.3
16	4.73	Died							
19	0.63	1.82	2.85	2.46	8.49	1.37	4.55	6.08	0.62
22	1.64	4.01	4.20	>0.30	0.45	3.93	11.0	7.01	8.25
Ave.	5.08	4.78	3.91	5.54	4.92	5.06	7.59	8.88	7.81
Group IV									
2	none	>0.60	1.92	9.05	5.82	7.86	8.40	5.64	3.84
3	0.39	0.60	2.81	6.93	4.05	5.04	8.40	5.70	8.94
4	-----	none	8.10	2.25	4.02	6.83	5.10	8.43	4.14
8	2.76	4.17	>0.40	4.32	4.88	7.13	16.7	17.3	9.81
10	5.25	2.58	0.66	1.01	4.79	5.76	5.67	5.07	3.09
Ave.	2.80	1.59	2.78	4.71	4.71	6.52	8.85	8.45	5.96

Table 3 (continued)

Mcg. Vitamin A Per 100 Ml. Blood Plasma

Trial Two

Calf No.	Weeks								
	Initial	2	4	6	8	10	12	14	16
Group I									
24	13.1	5.16	2.91	20.3	3.27	7.55	5.12	8.87	12.5
27	10.6	12.9	6.21	8.37	5.78	9.69	12.3	11.8	16.1
31	6.38	9.45	2.45	8.61	7.53	4.80	11.4	11.3	10.7
36	8.76	9.20	4.77	8.16	6.87	8.55	5.13	6.66	9.39
39	5.04	7.74	4.95	7.61	7.43	8.73	7.70	11.2	16.1
Ave.	8.78	8.89	4.26	6.61	6.18	7.86	8.33	9.97	12.96
Group II									
23	5.74	4.29	5.48	3.14	3.59	8.58	6.62	8.42	10.8
30	10.5	5.90	3.66	3.00	9.56	6.18	7.44	11.9	11.6
32	5.87	10.77	9.83	6.59	6.20	6.25	9.63	4.05	4.62
35	12.4	5.70	5.12	4.28	2.66	4.62	2.31	20.6	0.78
42	7.02	10.9	8.49	2.61	6.60	7.20	4.89	15.9	11.6
Ave.	8.31	7.51	6.52	3.92	5.72	6.57	6.18	8.17	7.88
Group III									
26	11.1	4.65	6.53	9.78	3.47	5.76	10.3	3.63	6.03
28	8.04	12.2	8.46	8.58	5.72	11.7	6.75	7.44	8.43
33	5.28	7.11	1.46	3.23	2.24	1.40	1.08	0.72	1.20
37	5.72	8.27	8.16	7.17	10.2	6.93	14.0	10.1	16.9
40	8.93	8.42	2.34	2.18	2.55	5.31	2.50	7.17	9.36
Ave.	7.81	8.13	5.39	6.19	4.84	6.22	6.93	5.81	8.38
Group IV									
25	4.86	4.39	3.30	4.55	6.09	9.47	7.59	8.09	15.9
29	12.9	8.39	5.97	7.63	4.11	7.65	2.48	1.95	3.69
34	9.99	11.2	5.13	6.23	1.94	3.53	4.20	3.21	5.01
38	7.28	8.88	6.89	8.28	4.16	5.49	2.60	8.52	10.1
41	11.0	9.77	6.27	5.72	4.29	2.65	4.35	17.9	9.99
Ave.	9.21	8.53	5.51	6.48	4.12	5.76	4.24	7.93	8.94

TABLE 4

Schedule of Milk Allowance by Weeks

Pounds of milk per day by breed	1	2	3	4	5	6	7	8	9	10
Holstein	6	8	10	8	8	6	5	3	3	--
Jersey	5	6	7	7	6	6	5	4	3	3

TABLE 5

Calf Starter Formula

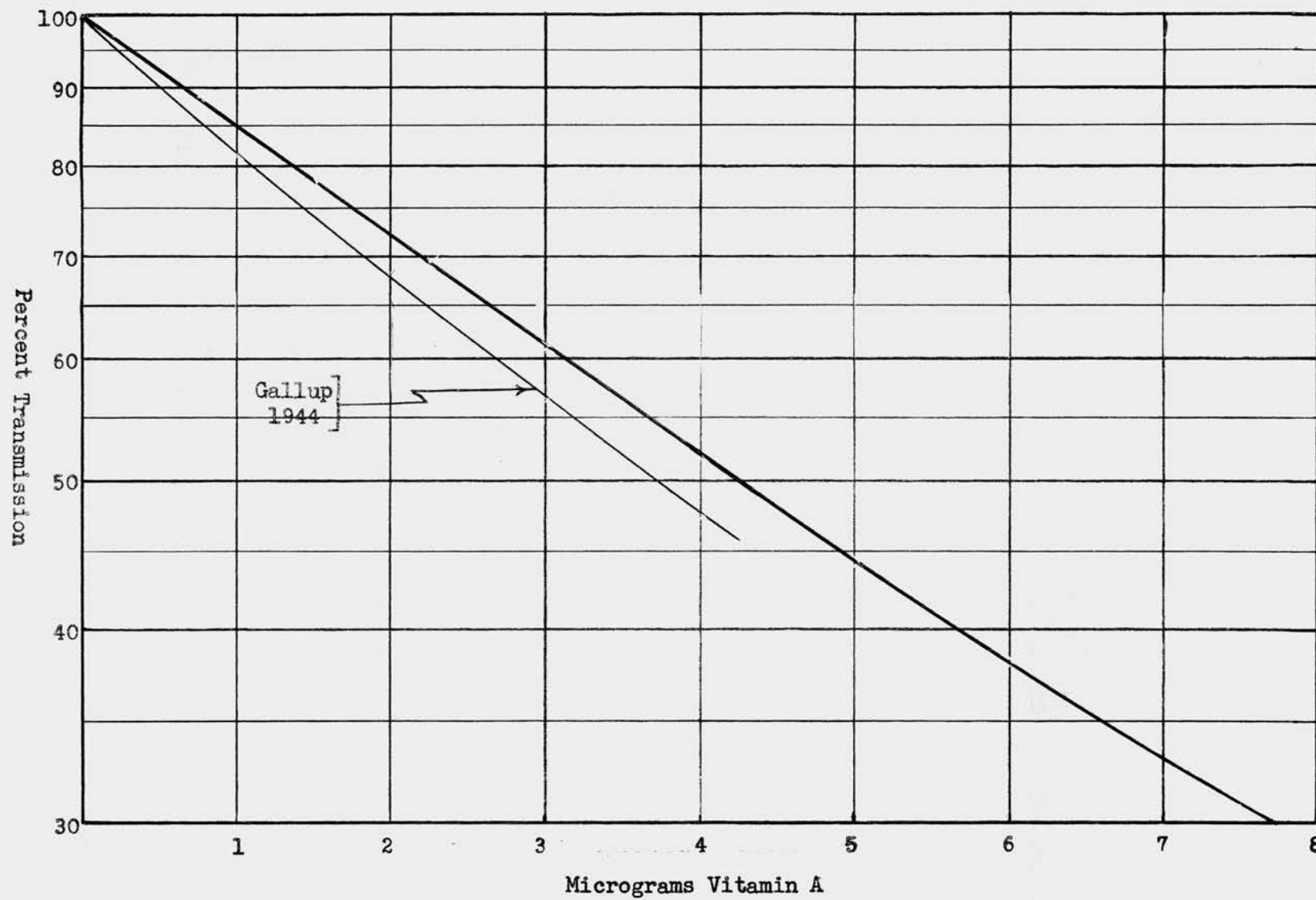
Feed Constituent	Pounds
Crimped oats	500
Crushed corn	550
Wheat bran	200
Cottonseed meal	400
Dried skimmilk or buttermilk	100
Alfalfa leaf meal	100
Molasses	150
Iodized salt	10
Steam bone meal	20
	<u>2030</u>

TABLE 6

Carotene Content of Hays

Date of Analysis	Type of Hay	Carotene Content
1-30-52	Alfalfa	19.4
	Prairie	6.0
5-6-52	Alfalfa	12.9
	Prairie	22.4
8-29-52	Alfalfa	58.7
	Prairie	28.2
10-2-52	Alfalfa	44.9
	Prairie	28.0
11-8-52	Alfalfa	26.7
	Prairie	27.3

Standard Vitamin A Calibration Curve For The Evelyn Colorimeter



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