

EFFECT OF THREE LEVELS OF CAROTENE
SUPPLEMENTATION DURING LACTATION
ON THE PERFORMANCE OF BEEF
COWS AND THEIR CALVES

By

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INTRODUCTION

The importance of vitamin A in beef cattle nutrition has been recognized for more than 25 years. Among the more common vitamin A deficiency symptoms often observed in cattle are: temporary night blindness which will become permanent if not corrected, unthriftiness, muscular incoordination and convulsive seizures, reduced resistance to infection due to keratinization of epithelial tissue, severe diarrhea in young stock, a decline in sexual activity in males of breeding age, and dead or weak newborn from deficient females. Periodic droughts and poor feed supplies have caused much concern among cattle producers as to the possibility of vitamin A deficiencies.

In years of adequate rainfall, the vitamin A requirement of mature beef cattle may be met by the provitamin, beta-carotene, which is present in large amounts in young, growing pasture grasses. Further, the ability of mature cattle to store large amounts of vitamin A in their bodies may tide them over deficiency periods, such as may occur each winter in this area. However, during periods of prolonged drought the vitamin A problem may become acute, particularly for young cattle whose body reserves are usually low. Changes in production systems to include more feeding of young cattle, greater use of low quality roughages, and fall calving have caused increased attention to be given to the possibility of vitamin A

deficiencies in beef cattle. Calves dropped in the late fall when there is very little, if any, green feed available and nursing dams on vitamin A deficient rations may suffer from vitamin A deficiency.

Due to the low milk output of beef cows, the suckling calf receives a relatively low intake of vitamin A after the period of colostrum, and this further complicates the problem.

Previous work at this station has shown that carotene supplementation of the dams during gestation to protect her against a deficiency, providing she enters the gestation period with ample liver stores, is not necessary. However, vitamin A deficiency symptoms can occur in young, suckling calves, even though the dam exhibits no outward symptoms.

Studies of a long-time nature on the importance of vitamin A in beef cattle nutrition have been underway at this station since 1946. From these studies, it appears that in cow-calf operations, an adequate supply of carotene for the cow during lactation in order to protect her calf may be of the utmost importance. Therefore, this experiment was conducted to study the effect of three levels of carotene supplementation during lactation on the vitamin A nutrition of the beef cow and her calf.

REVIEW OF LITERATURE

Hart and Guilbert (1933) reported the occurrence of vitamin A deficiencies and heavy calf losses in beef cattle on rations devoid of green feed for several months. Young cattle developed the vitamin A deficiency symptoms more quickly than mature cattle. Calves from cows on vitamin A deficient rations developed clinical symptoms of avitaminosis A. The most common cause of death was pneumonia. After green feed was available for a short time, the losses ceased and the remaining cows calved normally. The symptoms were later confirmed by Guilbert and Hart (1934), Moore and Sykes (1940), Schmidt (1941), Ritzman et al. (1945), and Helmboldt et al. (1953).

Using the onset of night blindness as the criterion for estimating carotene requirements, Guilbert and Hart (1935) concluded that cows could maintain their liver stores and produce normal calves if they were given the equivalent of 1.0 to 1.5 mg. per 100 lb. of body weight during gestation, and 4.0 mg. of carotene per 100 lb. body weight per day during lactation. They also demonstrated that temporary night blindness caused by a vitamin A deficiency would be followed by permanent night blindness if the animal were not given carotene or vitamin A soon after the onset of the disorder.

Davis and Madsen (1941) reported that an intake of 2.7 mg. of carotene per 100 lb. body weight per day was not sufficient to allow young Hereford or Shorthorn cows to produce normal calves.

Wheeler et al. (1957) fed mature Hereford cows rations supplying 1.5, 5, 15, and 25 mg. of carotene per 100 lb. of body weight daily. Plasma and liver carotene showed a direct relationship to carotene intake. None of the levels of carotene intake were sufficient to maintain vitamin A liver stores. Night blindness was produced in the calves within one month of age when their dams received only 1.5 or 5 mg. of carotene per 100 lb. of body weight. Roberts et al. (1956) fed three levels of carotene (0, 30, and 140 mg. per head daily) to three groups of bred Hereford heifers. The liver vitamin A values varied directly with the amount of carotene fed. There was no significant difference in liver or plasma levels of vitamin A of calves at birth or at 56 days of age, regardless of the level of carotene intake of their dams during gestation.

Guilbert et al. (1940) reported that cows on a minimum vitamin A ration were able to produce live young, but that the calves were weak and soon died. Vitamin A supplementation of the cows at three to four times the minimum requirement (18-24 mg. per 100 lb. body weight) beginning the last month of pregnancy resulted in normal growth of the calves for at least three months following parturition.

Watkins and Knox (1954) fed range breeding cows supplemental carotene from alfalfa at levels of 8.8 to 27 mg. per

cow daily for one month before calving and at levels of 19.6 to 51.7 mg. per cow after calving. The supplemental carotene had no beneficial results in terms of percentage calf crop and produced no significant differences in blood plasma carotene or vitamin A.

The recommended carotene allowance for wintering pregnant cows according to the National Research Council (1950) is 6.0 mg. per 100 lb. of body weight. This recommendation is based on the work reported by Davis and Madsen (1941) and Madsen and Davis (1949). The National Research Council recommends 6 mg. per 100 lb. "to provide for normal growth and reproduction and to build up and maintain a moderate storage of vitamin A". The National Research Council recommends that lactating cows receive 300 mg. daily. Morrison's (1956) standards for gestation and lactation are 5.5 and 9.0 mg. per 100 lb. of body weight, respectively.

Daniel (1957) states that beef cows may use body stores of carotene to maintain their health for long periods, but must receive relatively large amounts in the feed in order to protect their calves against a deficiency. Carotene requirements for lactation are much greater than for gestation. Under normal range conditions, it may be more economical to give calves vitamin A directly, such as in creep feed, than to feed high levels to their dams.

Mohler (1939) reported that when plasma carotene decreased to 25 mcg. percent and blood vitamin A to 16 mcg. percent or less, most animals begin to manifest early symptoms of avitaminosis A.

In further studies of the carotene and vitamin A nutrition of beef cattle, Guilbert and Hart (1935) found that 67 to 93 percent of the body stores of carotene and vitamin A are found in the liver. The body stores appear to increase as the animal grows older as is evidenced by the fact that aged cows had six times greater total liver stores than younger animals under comparable conditions.

Church et al. (1956) reported that cows on a high plane of nutrition depleted their vitamin A liver stores faster than cows on a low plane of nutrition. It was concluded that for the production of healthy calves from cows on low carotene rations, the carotene intake during subsequent lactation should be 20 mg. or more per 100 lb. of body weight, regardless of plane of nutrition.

Van Arsdell et al. (1950) fed Hereford cows carotene at levels of 38, 75, and 106 mg. per day during the last 45 days of gestation and the first 30 days of lactation, and noted no difference in health or vigor of the calves, although blood carotene levels varied directly with carotene intake. Baker et al. (1954) reported no reproductive failure in Hereford cows given carotene-low rations alone or supplemented with 60 mg. of carotene per day during gestation. Further work by Church et al. (1954) showed that reproductive failure resulted in Hereford cows only after severe depletion of liver vitamin A stores, which included 3 gestation periods.

Madsen and Davis (1949) found over a period involving several generations that reproduction was satisfactory when

beef cows were given 90 mcg. per kg. (4 mg. per 100 lb. body weight). They concluded that when the vitamin A plasma level dropped below 18 mcg. per 100 ml., the possibility of a cow producing a normal calf was poor.

Payne and Kingman (1947) found that blood carotene levels of 82 mcg. per cent were sufficient for aged cows, but that heifers need more than 97 mcg. percent. A blood level of 117 mcg. percent was adequate for heifers. Watkins and Knox (1950) found less carotene than this in the blood of range cows during winter months in New Mexico, but noted no difficulty with reproduction. Similarly, much lower figures are given by Sutton and Soldner (1945), Baker et al. (1954), and Church et al. (1956).

Studies by Davis and Madsen (1941), Madsen and Davis (1949), Baker (1953), and Church et al. (1954) have indicated no appreciable decline in conception rate or breeding efficiency in beef cattle on deficient rations.

Sutton and Soldner (1945), Long et al. (1952) and others have noted marked seasonal variations in blood carotene. Braun (1945) reported that lower blood vitamin A resulted from parturition, abortion, or acute infections, although these changes appeared to be independent of changes in blood carotene. Kuhlman and Gallup (1941, 1944), Van Arsdell et al. (1950), Sutton and Soldner (1943), Sutton et al. (1945) reported a drop in carotene and vitamin A blood levels associated with parturition and beginning lactation.

Sutton et al. (1945) found that the maximum decrease in blood plasma carotene of lactating cows occurred one week following parturition. The maximum decrease in blood plasma vitamin A was reached three days after parturition. Sutton et al. (1947) found a rapid decline of carotene and vitamin A in colostrum and milk with each successive milking. Colostrum was approximately ten times as potent in carotene and six times as potent in vitamin A as normal milk. Levels closely approaching normal milk were reached at the end of the third day.

Data reported by Guilbert and Hart (1934) indicate that lactation results in a more rapid depletion of liver reserves than gestation. Baker et al. (1954) and Church et al. (1954) found that liver stores were depleted rapidly during gestation and lactation on low-carotene rations. Baker (1953) reported that carotene supplied at a level of 60 mg. per day was not sufficient to prevent depletion of liver reserves during gestation. When supplied at levels of 300 mg. per day during the first three months of lactation, a slight increase in liver vitamin A occurred in one trial, but further depletion was observed during a second experiment.

Working with beef calves, Braun (1945) reported a significant correlation between vitamin A liver stores and blood vitamin A only when the liver vitamin A fell below "normal" levels. Similarly, Baker et al. (1954), Hjarde et al. (1954) and Jones et al. (1955) noted only small differences in the liver vitamin A contents of cows given different amounts of

carotene. However, in dairy calves, Rousseau et al. (1954) reported that when plasma and liver vitamin A were expressed as logarithms, a positive linear relationship existed. It should be noted that the range in which he studied the relationship was rather low for liver stores of vitamin A. Thomas and Moore (1952) likewise reported that on a ration of natural feedstuffs the level of carotene intake was linearly related to liver storage in calves.

Riggs (1940) and Jones et al. (1943) summarized the vitamin A depletion studies conducted at the Texas station over a seven-year period and involving more than 300 steers and heifers. They observed that the depletion time of cattle varied with age and year. The time required for the depletion of liver reserves of vitamin A in cattle 3 to 16 months of age, as indicated by night blindness, ranged from 45 to 268 days. Young animals become depleted in less time than older animals. A carotene level of 0.45 mg. per 100 lb. of body weight was not enough to maintain life during extended periods of feeding. Steers supplied this level after depletion fattened, but enough generalized edema was present to cause their carcasses to be condemned as food. A carotene level of 1.25 mg. per 100 lb. of body weight was not high enough to prevent night blindness. Slight liver storage of carotene and vitamin A occurred when the cattle received 2.5 to 3.0 mg. of carotene daily per 100 lb. of body weight.

The rate of depletion of hepatic stores of vitamin A and carotene by beef steers decreases as the liver reserves are

reduced, according to the reports of Frey et al. (1946, 1947). These workers suggest that the rate of depletion of vitamin A stores of beef cattle is proportional to the total liver reserve.

Braun and Carle (1943) observed that the vitamin A content of the bovine fetal liver, although low, was in direct relationship to the mother's diet. Baker et al. (1954) found that the liver stores of vitamin A and carotene of newborn calves from cows receiving 0 and 60 mg. of carotene per head daily did not appear to be related to the treatment of the dam during gestation or her liver stores at parturition.

Fountain et al. (1948) observed that plasma vitamin A levels of newborn calves produced by cows on green pasture during the non-lactating period was higher than those of calves produced by cows receiving grain, hay and green pasture. Plasma vitamin A levels for calves produced by cows supplemented with large doses of vitamin A ester or alcohol during the non-lactating period were comparable to those of the calves whose dams received only pasture.

Kuhlman and Gallup (1940) reported that Jersey cows could reproduce normally when maintained for long periods of time on a carotene intake equivalent to 4.5 mg. per 100 lb. of body weight. Cows fed rations containing lower amounts of carotene produced a smaller percentage of normal calves and had more trouble with retained placentas after calving. This report includes 31 gestation periods recorded from a herd of 17 Jersey cows. Kuhlman and Gallup (1941) noted that the carotene requirement of Jersey cows was higher during

the early phase of lactation than during the latter phase. The same authors (Kuhlman and Gallup, 1942) concluded that the requirement for normal conception was apparently the same as the requirement for normal calving performance. In 21 cases where cows received the equivalent of 2.0 to 3.9 mg. of carotene daily per 100 lb. of body weight during the 90-day period preceding service, 1.99 services per conception were required. When the daily carotene intake was equivalent to 4.0 to 5.9 mg., 6.0 to 9.9 mg. or 10.0 to 35.3 mg. per 100 lb. of body weight, the services per conception were 1.35, 1.15, and 1.23, respectively.

Spielman et al. (1946) using plasma vitamin A of newborn calves as the criterion, reported that supplementing with 1 million I.U. of vitamin A produced twice the response of 1 million I.U. of carotene.

Hilton et al. (1944) concluded that 7500 I.U. of vitamin A daily was enough to promote growth in dairy heifers but that the level should be increased to 30,000 I.U. to insure normal reproduction.

Ronning et al. (1953) summarized the results of an eight-year study on the carotene requirements of Guernsey cattle for reproduction. The four levels of carotene intake studied were: 3.0-5.9 mg., 6.0-8.9 mg., 9.0-11.9 mg. and 12.0-15.0 mg. per 100 lb. of body weight. The results of 72 gestations indicate that an intake of 9 mg. of carotene per 100 lb. of body weight is necessary for successful reproduction. When the carotene intake was reduced below this level, a relatively

high incidence of retained placentas, abortions, blind calves and weak calves resulted. Since most of the abortions occurred prior to 180 days of gestation, these workers suggest that the critical period of vitamin A nutrition of the pregnant cow may be earlier than the last 90 days of gestation. It was observed that the gestation periods of the cows receiving the lower carotene intakes were a few days shorter. There was no apparent relationship between conception and carotene intake during the service period in this experiment.

Converse and Meigs (1938) state that for normal gestation dairy cows should receive 80 to 100 mg. of carotene daily during the last three months of gestation. When the daily carotene intake was as low as 60 mg. per cow, there was a large percentage of dead calves dropped.

Ross et al. (1948) studying Holstein heifers, found blood plasma vitamin A levels of 6 to 8 mcg. per 100 ml. plasma to be the critical level for maintenance when gain in body weight was used as the criterion.

Jones et al. (1955) reported that carotene fed to dairy cows at levels of 130 and 390 mcg. per kg. was sufficient to maintain liver vitamin A at levels of 12.4 and 18.7 mcg. per gm., respectively. These are very low liver reserves in light of the data reported on beef cows. No information was given on stage of lactation when the liver samples were obtained.

Semb et al. (1934) observed that the output of carotene in the milk of dairy cows was poor even when the plasma carotene was high. In Holstein cows, only 0.8 percent of the plasma carotene was converted to milk carotene daily.

Hansen et al. (1946) found that the vitamin A of colostrum from barn-fed dairy heifers in the first lactation was more than twice the vitamin A content of the colostrum from the same cows in the second lactation. Seven-fold variation in the colostrum vitamin A potency occurred in these cows. The cows were fed identical rations and maintained under uniform conditions during the two lactating periods. An increase in the blood plasma vitamin A concentration of the newborn calf was observed following the ingestion of colostrum and the percentage increase tended to reflect the concentration of vitamin A present in the colostrum of their dams.

Sutton and Soldner (1945) made monthly determinations of carotene and vitamin A in the blood plasma of six mature cows and sixteen bulls of each of the four major dairy breeds. The data showed a wide fluctuation in blood plasma carotene when the cattle were grazing green pasture. The blood plasma vitamin A varied over rather narrow limits in comparison to blood plasma carotene. Changes in blood plasma vitamin A did not closely follow blood plasma carotene, but tended to lag behind by about a month.

Lewis and Wilson (1945) in an experiment with dairy calves, obtained results which indicated that 32 U.S.P. units of vitamin A per kg. of body weight was the minimum requirement for growth. The level required for optimum growth was found to be 64 U.S.P. units of vitamin A per kg. of body weight. When both growth and liver storage were taken into consideration, the recommended daily intake of vitamin A for young calves was found to be about 250 U.S.P. units per kg. of body weight.

Ward et al. (1938) found that 12 to 14 mcg. of carotene per lb. of body weight per day was sufficient to prevent vitamin A deficiency symptoms in dairy calves. They also found that dairy heifers fed a carotene deficient ration during the winter months showed no deficiency symptoms if they had been on good pasture during the summer months.

Dolge et al. (1956) supplemented four groups of eight 63-day-old Holstein calves with either 20, 50, 125 or 312.5 mcg. carotene from artificially dehydrated alfalfa hay or 4, 10, 25, or 62.5 mcg. vitamin A, from a dry carrier, per lb. liveweight daily. Growth as measured by increases in liveweight, height at withers, heart girth or paunch girth was essentially unrelated to carotene or vitamin A intake. The response to carotene and vitamin A intake was additive and simultaneous feeding did not adversely effect the utilization of either the carotene or vitamin A.

The effect of vitamin A and carotene intake during the first 90 days of life of dairy calves and the vitamin A depletion time thereafter was studied by Jacobson et al. (1949). Calves permitted to nurse their dams on pasture for 90 days could be depleted in 113 to 120 days. Calves on the limited whole milk ration without vitamin A supplementation could be depleted in 2 to 4 weeks.

Hibbs and Krauss (1946) reported that regardless of the amount of vitamin A fed to dairy calves, the blood level seldom exceeded 25 mcg. per 100 ml. and that the decrease of blood vitamin A during the first weeks after birth could largely be offset by feeding additional vitamin A.

All workers are not in agreement on liver storage in the newborn calf as affected by the carotene intake of the dam. Wise et al. (1946) observed that massive doses of vitamin A administered to dairy cows during late pregnancy caused an increase in liver stores and plasma levels of vitamin A in newborn calves. Although the vitamin A content of the colostrum varied widely in this study, it was likewise increased by prepartum supplementation.

Stewart and McCollum (1942), studying the effect of vitamin A enriched diets on the vitamin A content of the colostrum of dairy cows, failed to find a difference in the milk of control cows as compared to those fed the vitamin A rich concentrate.

Byers et al. (1955) compared blood plasma, liver, and milk fat values for carotene and vitamin A in Jersey and Holstein cows on adequate and suboptimal carotene rations. Suboptimal rations fed during gestation and for long periods of time after birth resulted in low liver vitamin A and carotene values which failed to increase when supplements of carotene as high as 15 mg. per 100 lb. of body weight were added to the ration. Repeated injections of 250,000 or 1,250,000 I.U.* of vitamin A ester failed to result in any appreciable increase in liver vitamin A or carotene in cows on suboptimal carotene rations. However, availability of the injected vitamin A used in this study is questionable.

*One I.U. of vitamin A is equivalent to .6 mcg. pure beta carotene.

Rousseau et al. (1954) fed a vitamin A depletion ration at two nutritional levels to one-day-old dairy calves until the blood plasma vitamin A values decreased to less than 4 mcg. per 100 ml. Calves in the high and low nutritional levels gained an average of 1.5 and 1.0 lb. per day, respectively. Over-all plasma carotene and vitamin A were found to decrease but were not affected by the level of intake of the depletion ration.

Wise et al. (1948) found that feeding colostrum to one-day-old dairy calves at the rate of 1 lb. per 10 lb. of body weight daily caused a large increase in carotene and vitamin A in the blood of the newborn calf. Whole milk was fed to the calves at the same rate as the colostrum. However, even when produced by cows receiving high quality roughage, whole milk did not provide sufficient vitamin A activity to prevent a continuous decline in the concentration of carotene and vitamin A in the blood to levels within or approaching the deficiency range.

Boyer et al. (1942) in studies to determine blood plasma concentration and intake of carotene and vitamin A necessary for the growing dairy calf, noted that plasma vitamin A was a more delicate measure of the state of nutrition than growth or plasma carotene. A blood plasma vitamin A level of 10 mcg. per 100 ml. was found to be necessary for adequate vitamin A nutrition of the calf, 7 to 8 mcg. was borderline, and amounts less than this were considered to be inadequate.

Byers et al. (1956) studied the performance of dairy cattle on vitamin A deficient rations for three generations. They found that calves from vitamin A deficient dams showed severe damage of the pituitary, adrenal and sex gland at birth.

Spielman et al. (1946), studying carotene utilization by the newborn dairy calf, found that intestinal infection and scours resulted in reduced absorption and utilization of carotene.

From the literature it is apparent that much knowledge has accumulated concerning vitamin A nutrition. We now know the symptoms of avitaminosis A and that there are many variables that affect the vitamin A requirement such as age, plane of nutrition, etc. There is a question as to the availability of different vitamin A preparations and the effect of different methods of administration. There appears to be a species and breed difference in requirements. There is little agreement in the literature in respect to the vitamin A requirement of the beef cow. It appears that the first few months after birth is the most critical period in the life of the beef animal. Studies are therefore necessary to determine what levels are required by the beef cow in order to protect her calf.

EXPERIMENTAL

Sixteen, bred, Hereford heifers, approximately $2\frac{1}{2}$ years of age, were selected from the experiment station herd for this study in the fall of 1956. The heifers were wintered at the Lake Carl Blackwell range on dry, weathered grass, with $2\frac{1}{2}$ lb. of cottonseed cake per head daily and minerals free choice. The winter of 1956-57 proved unusually mild and there was some green feed from winter annuals available in the native grass pastures.

On February 15, the heifers were placed in dry lot at the experimental feeding shed and fed a ration consisting of weathered native grass hay, cut in December and devoid of carotene, plus five pounds of ground milo and three pounds of cottonseed meal per head daily. A mineral mixture of 2 parts salt and 1 part steamed bone meal was available, ad lib. Upon calving, the cows were continued on the same ration and assigned to one of three levels of carotene supplementation.

During the first three months of lactation, the cows were divided into three lots and supplemented with 0, 5, or 10 mg. of carotene per 100 lb. body weight per day. The 5 and 10 mg. levels of supplementation gave the cows approximately 50 and 100% of Morrisons' (1956) standard for beef cows during lactation. The cows were assigned to treatment according to calving order; the first cow to calve was assigned

to the 0 level, the second to the 5 mg., the third to 10 mg., etc. This procedure was followed in allotting all cows.

The cows and their calves were removed from the experiment when the calves reached 3 months of age. Data were obtained on body weights of cows and calves at parturition and at monthly intervals thereafter. The cows and calves were closely observed throughout the experiment for symptoms of vitamin A deficiency. A record on the incidence of scours and other abnormalities was maintained throughout the course of the study. The calves had access to a creep feed mixture of 2 parts rolled oats, 1 part milo, and 1 part cottonseed meal.

The carotene used in this experiment was a carrot oil concentrate in soybean oil.* It was mixed with cottonseed meal and fed individually every third day. The carotene content of the oil was determined by chemical analysis twice during the trial and the amount fed was adjusted to provide the desired intake. Daily allowances of carotene were also adjusted at monthly intervals according to the individual weight of the cows. The carrot oil concentrate used in this experiment was stored in an airtight metal drum in the basement of the Animal Husbandry building.

Blood samples were taken from the cows prepartum (March 22), at parturition, and at 1, 2, and 3 months after calving.

*The carrot oil concentrate was generously supplied by Nutritional Research Associates, Inc., South Whitley, Ind.

The calves were bled as soon as possible after birth and at 1, 2, and 3 months of age.

Liver samples were taken from the cows at parturition and at 3 months following parturition. Liver samples were taken from the calves at 3 months of age.

Blood samples were not obtained from the animals which died on experiment, although liver samples were taken after an autopsy was performed by the Pathology Department of the School of Veterinary Medicine.

The blood sample, approximately 20 ml., was collected in a prepared lithium citrate tube to prevent clotting. The sample was then centrifuged and the plasma was removed and stored in a frozen state until analyzed for carotene and vitamin A according to the method of Kimble (1939).

The liver samples were obtained from the cows while restraining them in a squeeze chute by means of a head gate. The liver biopsy technique as described by Van Arsdell (1952) and Whitehair et al. (1952) was followed. An area, approximately 6 inches square, was clipped on the right side of the cow over the 12th and 13th rib, about 10 inches from the midline of the backbone. This area was washed with a 50 percent alcohol solution and then anesthetized with 30 ml. of 4 percent procaine hydrochloride solution. After about 20 minutes an incision approximately 3 inches long was made in the area between the 12th and 13th rib. A trocar and canula, 1 inch in diameter and 6 inches long, was inserted into the incision and forced through the peritoneum. The liver surface

was located with the aid of a flashlight, and a core of liver tissue weighing 1 to 3 gm. was removed with a special instrument. Four sulfa tablets were placed in the incision prior to suturing it with catgut. A smear was placed on the area of the incision following the operation and for several days thereafter to keep flies away from the wound. If a sizeable knot developed in the area of the incision, 3 ml. of streptomycin and 5 ml. of penicillin were administered daily until the swelling subsided. As soon as the liver sample was obtained, it was wrapped in tinfoil, placed in a gelatin capsule, and stored in a deepfreeze until analyzed.

The liver samples were obtained from the calves while restraining them in a portable squeeze chute by means of a head gate. The side panels of the chute were removed so as to make the right side of the calf more accessible. An area, approximately 6 inches square, was clipped over the last rib on the right side, about 4 inches from the midline of the backbone. This area was cleansed with a disinfectant soap and a 50 percent alcohol solution. The area was then anesthetized with 10 ml. of 4 percent procaine hydrochloride solution. After 15 minutes, an incision approximately 4 inches long was made immediately behind the last rib. The incision continued through the peritoneum into the abdominal cavity, making the liver very accessible. While holding the liver with a pair of forceps, a sample approximately 3 gm. in weight was removed and the incision was then sutured. Immediately following the operation and for the following three days, 1 ml. of

streptomycin and 2 ml. of penicillin were administered. The suturing material was removed from the calves after approximately a week. The liver samples obtained were stored for future analysis in the manner described above. Liver samples were analyzed according to the method of Gallup and Hoefer (1946).

Milk samples were taken from the cows following the completion of the three month lactation period. The cows were restrained in a close pen with their hind legs tied firmly. Each quarter was milked as thoroughly as possible. No cow gave more than one quart even though the calf had been taken away from her at least twelve hours prior to the milking. The milk obtained was mixed thoroughly and composite samples were then taken and stored in a frozen state until analyzed according to the method of Leshner et al. (1945).

At the end of the trial, all data were subjected to analysis of variance according to Snedecor (1956). Since the treatments imposed were at three levels and equally spaced, a test for linearity of response was made to determine the extent that treatment means were linear. If a high proportion of the treatment sum of squares is removed by the linear test, it is indicated that non-linear variation is negligible. It was found upon analysis (see Appendix Table V) that the linear effect accounted for from 86 to 99 percent of the treatment sum of squares whenever significance was found at the 5 percent or less probability level.

RESULTS AND DISCUSSION

The weight changes of the cows, birth weights, and gains by monthly periods for the calves are shown in Table I. Individual weights of the cows and calves are given in Appendix Table I. There were no consistent differences in gain or loss of weight of the cows while on test. No recognizable symptoms of vitamin A deficiency were observed in any of the cows during the course of the experiment. However, no real attempt was made to check for night blindness, reported to be one of the first symptoms of avitaminosis A. No statistically significant differences were found to exist among cows weights. One cow in Lot II died of a lung infection of unknown origin during the third month of lactation.

There was no apparent influence of carotene supplementation of the dams on weight gains of the calves to three months of age. All calf weights were lower than might be expected under good range conditions, possibly due to the drylot conditions under which the experiment was conducted. Two of the lightest calves were lost in Lot I (0 level of carotene supplementation) which tended to increase the mean weight of this lot at the end of the experiment. The results obtained in this trial are similar to those reported by Daniel (1957). The calves which survived appeared to have ample vitamin A for growth. This would agree with the work of

Jones et al. (1943) who found that beef steers continued to gain even after first symptoms of vitamin A deficiency were observed.

TABLE I

Weight Changes of Beef
Cows and Their Calves

Lot number and mg. carotene fed cows per 100 lb. body weight daily during lactation	Lot I 0	Lot II 5	Lot III 10
Mean cow weights (lb.)			
Parturition	849 ± 64 ^a	738 ± 85	843 ± 89
At 1st month lactation	851 ± 32	770 ± 54	845 ± 101
At 2nd month lactation	857 ± 23	799 ± 41	812 ± 84
At 3rd month lactation	840 ± 34	823 ± 60	818 ± 60
Weight change, parturition to 3rd month	-9	-85 ^b	-25
Mean calf weights (lb.)			
Birth	73 ± 8	69 ± 7	58 ± 15
At 1 month	106 ± 18	105 ± 10	95 ± 15
At 2 months	135 ± 26	132 ± 13	121 ± 20
At 3 months	173 ± 24	167 ± 23	153 ± 27
Total gain	100 ^c	94	95

^a Refers to the standard deviation.

^b The lightest cow in Lot II died of unknown cause during the third month of the experiment and is not included in the terminal weight data.

^c The two lightest calves in Lot I died during third month of test and are not included in terminal weight data.

All of the calves in this experiment appeared normal at birth. In many of the calves, scouring was apparent a few days after birth and continued intermittently throughout the study. Seventeen individual cases of scours were treated with antibiotic in calves of Lot I, 15 in Lot II, and 22 in

Lot III. Thus, diarrhea was noticeable in a number of calves throughout the trial, regardless of the level of carotene supplementation of their dams. This is in agreement with the observations reported by Church (1956) and Daniel (1957).

Severe diarrhea was noted prior to the death of two calves in Lot I. One calf contracted a navel infection following birth, and an autopsy revealed severe damage to the kidneys. Extensive blood was observed in the feces of the other calf prior to his death. From the post-mortem findings, it was not possible to definitely establish the cause of death in these two calves, but it was believed due to a complication of malnutrition and secondary infection. The role of vitamin A as a cause of these death losses is uncertain, although the liver vitamin A levels of the calves that died were the lowest of all the calves on test.

Mean plasma carotene and vitamin A values are given in Table II for the cows and calves. Individual plasma carotene and vitamin A data is given in Appendix Tables II and III. The parturition values for plasma carotene and vitamin A of 46.6 mcg. and 15.3 mcg. per 100 ml., respectively, for the cows in this study would be considered below normal. These values were comparable to those reported by Church (1956), but were considerably higher than observed by Daniel (1957), who reported average values of 28.7 mcg. and 8.9 mcg. per 100 ml. for plasma carotene and vitamin A, respectively. Payne and Kingman (1947) found that blood carotene levels of approximately 118 mcg. per 100 ml. were necessary for normal

TABLE II

Mean Vitamin A and Carotene Contents of Plasma of
Beef Cows and Their Calves (mcg. per 100 ml.)

	Lot No.	Prepartum ^a	Parturition	Lactation		
				1 mo.	2 mo.	3 mo.
COWS						
Plasma						
Carotene	I	80.15 ± 18 ^c	37.93 ± 33 ^b	40.81 ± 17	25.82 ± 6	23.85 ± 3
	II	91.07 ± 33	46.71 ± 35	104.77 ± 39	111.54 ± 37	114.04 ± 16 ^d
	III	85.75 ± 38	55.13 ± 44	192.75 ± 68	258.23 ± 80	275.40 ± 214
Plasma						
Vitamin A	I	4.96 ± 7	15.39 ± 3	15.65 ± 8	7.63 ± 4	10.02 ± 5
	II	19.04 ± 7	12.13 ± 6	15.99 ± 6	16.97 ± 7	14.34 ± 3
	III	22.52 ± 6	18.48 ± 2	18.12 ± 4	19.02 ± 5	16.16 ± 5
CALVES						
Plasma						
Carotene	I		15.30 ± 7	16.84 ± 20	10.39 ± 2	12.11 ± 8 ^d
	II		9.97 ± 5	12.78 ± 6	18.36 ± 9	29.07 ± 14
	III		12.09 ± 11	11.87 ± 6	18.20 ± 9	41.28 ± 13
Plasma						
Vitamin A	I		17.44 ± 3	11.99 ± 7	7.11 ± 4	4.55 ± 4
	II		16.34 ± 7	7.32 ± 3	7.21 ± 4	4.75 ± 1
	III		14.47 ± 6	15.71 ± 9	8.26 ± 6	6.29 ± 3

^a All prepartum blood samples were taken on the same day, March 22, 1957. One sample was lost from Lots I and II.

^b Refer to Appendix Table V for analysis of variance and linear effect.

^c Refers to the standard deviation.

^d Blood samples not obtained from one cow in Lot II and two calves in Lot I that died.

reproduction and lactation in beef cows. Madsen and Davis (1949) reported that reproduction in beef cows was poor when plasma vitamin A dropped below 18 mcg. per 100 ml. at or near parturition.

A tremendous amount of variation was found in the plasma vitamin A of blood samples taken from cows prior to parturition, even though they had been exposed to the same environmental and feeding conditions. At the end of the three month lactation period, the plasma carotene and vitamin A values of the cows directly reflected the level of carotene supplementation. This is in agreement with Wheeler et al. (1957), who found that plasma and liver carotene showed a direct relationship to carotene intake. A drop in plasma carotene and vitamin A at parturition was observed in most of the cows. This phenomenon has been observed by many workers (Kuhlman and Gallup, 1941; Van Arsdell et al., 1950, and Braun, 1945).

Carotene supplementation of cows of Lots II and III after parturition resulted in a rapid increase in plasma carotene, while plasma vitamin A increased at a much slower rate. This trend has also been observed by Sutton and Soldner (1945) who state that changes in plasma vitamin A tend to lag about a month behind changes in plasma carotene. The cows' plasma carotene values were found to be significantly different at one month ($P < .005$), two months ($P < .005$), and three months ($P < .05$). A significant difference ($P < .025$) in plasma vitamin A was observed in the cows two months after parturition, but not at one or three months (see Appendix Table V for analysis of variance).

The mean plasma vitamin A value of all calves (16.08 mcg. per 100 ml.) was adequate at birth. This is approximately four times the values reported by Daniel (1957) and may have reflected the better vitamin A nutrition of the cows used in this study. At every bleeding the plasma vitamin A values of the three lots of calves showed no significant difference. The plasma carotene of the calves at three months of age was significantly different ($P < .05$). The plasma carotene and vitamin A levels of the calves tended to reflect the supplemental carotene fed to their dams after one month of age. This is not in agreement with the work of Baker (1953), Church (1956), or Daniel (1957) who found no consistent effect of supplemental carotene fed to the dams on the plasma carotene or vitamin A of their calves. At three months of age, the calves' plasma vitamin A values were very low and might be considered in the deficiency range. Ross et al. (1948) reported that 6 to 8 mcg. of vitamin A per 100 ml. plasma in dairy calves appeared to be the critical level for maintenance when gain in body weight was used at the criterion.

The carotene and vitamin A contents of the livers of the cows and calves are given in Table III. Individual liver data are reported in Appendix Table IV. At parturition, liver vitamin A levels were three times those reported by Daniel (1957). The tremendous variation in liver carotene and vitamin A within lots is apparent from the standard deviations shown in Table III. Neither the 5 nor 10 mg. per 100 lb. body weight levels of carotene supplementation were adequate

TABLE III

Mean Vitamin A and Carotene Contents of Livers of
Beef Cows and Their Calves
(mcg./gm. dry matter)^a

	Lot No.	Parturition	3 mo. Postpartum
COWS			
Liver Carotene	1	11.00 \pm 8	14.09 \pm 7 ^b
	2	16.30 \pm 16	5.49 \pm 3
	3	10.83 \pm 9	4.16 \pm 3
Liver Vitamin A	1	63.95 \pm 57	23.77 \pm 24
	2	80.72 \pm 111	11.37 \pm 4
	3	78.59 \pm 50	46.49 \pm 48
CALVES			
Liver Carotene	1		1.39 \pm 1
	2		2.58 \pm 1
	3		3.25 \pm 0
Liver Vitamin A	1		4.53 \pm 6
	2		5.37 \pm 4
	3		8.18 \pm 9

^a A moisture content constant of 71% was used in converting liver to a dry matter basis.

^b Refer to Appendix Table V for analysis of variance and linear effect.

to maintain liver stores of the cows over the three month lactation period. However, symptoms of vitamin A deficiency were not observed in any of the cows, regardless of carotene intake.

The rate of depletion of liver vitamin A as effected by carotene supplementation was not consistant, since Lot II lost more than Lot I. This is in agreement with the work of Church (1956) who reported no apparent trend in depletion of liver stores of lactating beef cows due to carotene supplementation. However, the highest level of carotene supplementation (Lot III) resulted in less liver depletion than for Lots I and II. The analysis of variance of the cows' liver carotene indicated a significant difference ($P < .01$) among the lots at three months postpartum.

The carotene and vitamin A liver stores of the calves tended to reflect the level of carotene supplementation given their dams. A significant difference ($P < .05$) was found to exist in the carotene content of the calves' liver at three months of age. No significance was found to exist in the vitamin A content of the liver of the calves from cows fed the three levels of carotene during lactation.

Milk samples taken at the end of the three months lactation showed a higher carotene and vitamin A content for cows of Lot III. The mean carotene content of milk samples from cows of Lots I, II, and III were 5.40, 5.10, and 6.54 mcg. per 100 ml.; mean vitamin A content of milk from cows of the three lots were 4.83, 4.03, and 7.12 mcg. per 100 ml. of milk,

respectively. A great deal of variation in the carotene (two and one-half times) and vitamin A content (five times) of the milk was observed within lots.

From the results of this study, together with those reported by Daniel (1957), it appears that the health and body weight of the beef cow is not affected by depletion prior to calving, or carotene supplementation during the first three months of lactation as practiced in this experiment. The general health of the calves that survived showed no relationship to the supplementation of their dams. In both studies, death losses occurred in calves from cows on the lowest level of supplementation. Scours and inadequate gains were observed in all three lots.

From this study it appears that the high level of carotene supplementation during the first three months of lactation (10 mg. per 100 lb. body weight) was sufficient to maintain an adequate level of plasma vitamin A in the lactating beef cow, although the calves' plasma vitamin A may have been approaching a deficiency level. This level of supplementation was not adequate to prevent depletion of the liver stores of the cows. The liver stores of all calves were very low when samples were taken at three months of age. This is in agreement with the work of Daniel (1957).

It appears that beef cows, entering lactation with low reserves of vitamin A, must receive relatively large amounts in the feed in order to protect their calves against a deficiency. In this study, all the calves appeared to have

adequate plasma vitamin A at birth, but were soon depleted to a condition which might be considered bordering on a deficiency. It would take much higher levels of carotene in the ration of the cow than practiced in this experiment to promote liver storage of vitamin A in the calf. Since there is very poor transfer of vitamin A and carotene through the milk, following the colostrum period, it appears that supplementing the calf directly would be the most efficient method.

SUMMARY

Sixteen, bred, three-year-old, Hereford heifers were selected in the fall of 1956 for this study. They were wintered on weathered native grass pasture and supplemented with $2\frac{1}{2}$ lb. of cottonseed cake per head daily. In mid-February, the heifers were placed in dry lot and fed weathered native grass hay (cut in December, and devoid of carotene), 5 lb. ground milo and 3 lb. of cottonseed meal per head daily, with a mineral mixture of 2 parts salt and 1 part steamed bone meal ad lib., for the remainder of the experiment. Following parturition, the cows were divided into three lots and individually fed supplemental carotene for the first three months of lactation. Lots I, II, and III received 0, 5, and 10 mg. per 100 lb. body weight per day, respectively. Data was collected on the blood, liver, and milk carotene and vitamin A, as well as on weight changes and any abnormalities occurring in the cows and calves during the trial. In the analysis of variance of the data, a test was made for linearity of response since the treatments imposed were at three levels and were equally spaced.

No deficiency symptoms were observed in the cows. Carotene supplementation during early lactation had no consistent effect on weight gains of the calves to three months of age. Two calves were lost in Lot I whose dams received no supplemental carotene. Plasma carotene and vitamin A levels of

cows and their calves tended to reflect the level of carotene supplementation during lactation. The cows' plasma carotene values were found to be significantly higher due to carotene supplementation at one month ($P < .005$), two months ($P < .005$), and three months ($P < .05$) postpartum. Plasma vitamin A was significantly increased ($P < .025$) by carotene supplementation of the cows two months after parturition, but not at one or three months. The plasma carotene of the calves from supplemented dams at three months of age was significantly increased ($P < .05$). Cows on the highest carotene intake showed less depletion of liver carotene and vitamin A than cows of the other lots. The analysis of variance of the cows' liver carotene at three months postpartum indicated a significant effect of supplementation ($P < .01$) among the lots. Neither the cows nor calves showed a significant difference in liver vitamin A. Liver stores of carotene and vitamin A of the calves tended to reflect the level of supplementation given their dams; liver carotene was found to be significantly different between the three lots ($P < .05$) at three months of age.

It appears that beef cows, if depleted of vitamin A during gestation, must receive relatively large amounts in the feed in order to maintain the blood levels of the calf and provide for liver storage. Calves in this study had adequate plasma vitamin A levels at birth, but were depleted to a deficient or borderline condition in three months. It appears that higher levels of supplementation than practiced

in this study would be necessary to maintain liver vitamin A storage in the cow and to protect her calf. Since there is poor transfer of vitamin A and carotene in the milk, it appears that supplying the beef calf directly would be the most efficient method.

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Appendix

TABLE I
Body Weight of Cows and Their Calves During Lactation (lb.)

Cow No.	Parturi- tion	1 mo.	2 mo.	3 mo.	Birth wt.	1 mo.	2 mo.	3 mo.
Cows					Calves			
Lot I								
8	850	872	880	900	80	120	164	190
11	745	800	855	827	75	92	126	--- ^a
41	890	843	820	838	74	102	138	146
43	850	878	868	820	76	129	150	183 ^a
44	910	864	860	816	60	88	95	---
Lot II								
33	800	820	839	868	76	110	150	195
1	685	723	740	--- ^a	60	120	130	147
5	621	705	780	784	71	98	115	145
20	755	784	800	780	63	97	136	176
19	828	820	835	860	73	100	130	153
Lot III								
16	700	710	712	730	60	120	150	170
18	800	746	722	765	61	96	128	175
23	820	830	841	844	68	89	110	149
9	940	938	926	885	71	99	121	166
32	915	953	870	865	30	74	90	108
37	885	892	800	821	60	94	125	150

^a Animals died during the course of the study.

TABLE II

Plasma Carotene and Vitamin A Content of the Blood of Cows
During Late Gestation and Lactation (mcg./100 ml.)

Cow No.	Prepartum		Parturition		1 mo.		2 mo.		3 mo.	
	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A
Lot I										
8	a	a	96.23	15.37	69.46	24.26	35.40	11.75	22.15	16.56
11	85.60	15.07	20.41	18.96	42.50	6.66	25.62	0.83	26.12	8.51
41	98.26	4.09	26.82	16.26	30.74	24.24	23.74	7.67	24.98	15.07
43	80.34	.0	33.10	15.40	28.46	14.30	19.92	10.71	23.28	5.07
44	56.41	.68	13.11	10.96	32.89	8.78	24.43	7.17	22.74	4.90
Lot II										
33	a	a	90.87	9.58	160.09	7.22	163.22	28.14	121.21	11.90
1	92.16	17.24	18.72	6.68	126.34	21.42	103.68	9.92	b	b
5	123.30	12.47	77.76	14.92	84.90	17.39	61.97	18.99	92.86	18.77
20	103.04	16.90	31.88	21.52	93.50	14.01	128.51	16.09	128.36	14.52
19	45.77	29.57	14.30	7.96	59.00	19.93	100.30	11.71	113.73	12.19
Lot III										
16	33.22	18.52	133.34	16.77	305.90	18.82	277.86	18.62	278.91	23.46
18	109.94	23.41	82.28	20.60	236.80	23.71	255.88	24.35	162.72	17.54
23	83.62	12.98	37.14	17.20	150.75	15.73	158.50	20.03	134.27	17.84
9	71.45	30.00	36.00	20.67	156.22	12.41	173.38	10.35	247.28	15.84
32	144.05	22.91	19.26	15.00	184.17	22.56	348.08	19.45	135.02	14.01
37	72.22	27.30	22.74	20.62	122.65	15.47	335.67	21.31	694.18	8.24

a Plasma samples were broken

b Animal died and sample was not taken

TABLE III

Plasma Carotene and Vitamin A Content of the Blood of Calves
at Parturition and During Lactation (mcg./100 ml.)

Calf No.	Parturition		1 mo.		2 mo.		3 mo.	
	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A	Car.	Vit. A
Lot I								
612	23.34	19.28	9.28	16.01	11.47	9.37	21.05	8.43
608	17.02	14.40	51.44	24.01	9.83	13.96	a	a
626	14.80	19.39	10.38	7.98	7.65	4.81	6.55	0.26
398	4.92	20.14	7.65	5.30	12.06	3.55	8.74	4.96
472	16.38	14.00	5.46	6.66	10.92	3.87	a	a
Lot II								
611	13.11	20.05	19.86	8.54	18.12	11.86	37.79	5.19
627	9.83	21.01	18.18	9.86	20.46	6.98	36.54	5.00
630	14.34	22.98	12.02	9.96	31.34	6.92	42.50	6.30
469	2.73	9.05	7.10	5.32	14.25	9.66	15.94	3.96
471	9.82	8.60	6.74	2.91	7.65	0.62	12.56	3.28
Lot III								
610	5.46	6.60	9.28	15.75	22.15	17.84	40.12	7.45
629	9.83	15.71	23.84	27.44	12.56	5.66	20.46	6.51
628	32.48	19.07	6.14	16.95	11.28	5.73	33.07	2.90
468	11.47	12.60	11.47	5.26	15.70	0	45.48	3.19
470	6.01	10.98	9.28	6.15	13.16	11.47	47.87	7.83
473	7.28	21.85	11.20	22.70	34.36	8.88	60.68	9.88

^a Animal died and no sample was obtained

TABLE IV

Carotene and Vitamin Concentration of the Livers of
Cows and Their Calves (mcg./gm. dry matter)^a

Cows					Calves		
Cow No.	Parturition		3 mo. Postpartum		Calves No.	3 mo. of age	
	Car.	Vit. A	Car.	Vit. A		Car.	Vit. A
Lot I							
8	24.34	160.93	8.00	51.52	612	0.00	14.41
11	10.34	36.21	4.14	2.34	608	1.10	1.07
41	8.62	66.55	.72	2.83	626	1.90	3.07
43	7.90	42.14	3.79	15.59	398	2.90	3.21
44	3.79	13.93	4.17	46.55	472	1.07	0.83
Lot II							
33	22.21	39.03	8.79	13.83	611	2.14	11.28
1	9.72	6.55	6.31	6.72	627	4.66	3.97
5	42.28	275.72	1.41	7.90	630	2.59	5.59
20	5.59	58.31	4.86	13.28	469	1.66	3.69
19	1.72	24.00	6.07	15.14	471	1.83	2.31
Lot III							
16	27.41	150.72	15.66	88.34	610	3.00	24.97
18	12.41	45.66	8.55	30.31	629	4.79	4.90
23	4.55	21.07	9.03	7.34	628	2.55	2.00
9	8.38	42.17	10.03	10.07	468	2.86	3.14
32	8.90	118.62	27.14	111.55	470	3.69	9.38
37	3.31	93.28	14.14	31.31	473	2.62	4.72

^a A moisture content constant of 71% was used in determining D.M.

TABLE V

Analysis of Variance of Plasma and Liver Carotene
and Vitamin A of Beef Cows and Their Calves

Cows plasma carotene at one month			
Source	d.f.	S.S.	M.S.
Total	15	94,303.25	
Treatment	2	64,190.23	32,095.12*
Linearity (1)		(63,681.35)	63,681.35
Non-linear (1)		(508.67)	508.67
Error	13	30,113.02	2,316.39

*($P < .005$)

Cows plasma carotene at two months			
Source	d.f.	S.S.	M.S.
Total	15	190,444.21	
Treatment	2	153,100.79	76,550.40*
Linearity (1)		(149,879.36)	149,879.36
Non-linear (1)		(3,221.43)	3,221.43
Error	13	37,343.42	2,872.57

*($P < .005$)

Cows plasma carotene at three months			
Source	d.f.	S.S.	M.S.
Total	14	408,263.61	
Treatment	2	179,075.05	89,537.52*
Linearity (1)		(175,419.83)	175,419.83
Non-linear (1)		(3,655.22)	3,655.22
Error	12	229,188.56	19,099.05

*($P < .05$)

Cows plasma vitamin A at two months			
Source	d.f.	S.S.	M.S.
Total	15	777.73	
Treatment	2	387.63	193.82*
Linearity (1)		(341.91)	341.91
Non-linear (1)		(45.72)	45.72
Error	13	390.10	30.01

*($P < .025$)

(continued)

TABLE V (continued)

Calves plasma carotene at three months			
Source	d.f.	S.S.	M.S.
Total	13	3,540.38	
Treatment	2	1,721.35	860.68*
Linearity (1)		(1,703.59)	1,703.59
Non-linear (1)		(17.76)	17.76
Error	11	1,819.03	165.37

*($P < .05$)

Cows liver carotene at three months			
Source	d.f.	S.S.	M.S.
Total	15	627.53	
Treatment	2	326.32	163.16*
Linearity (1)		(280.85)	280.85
Non-linear (1)		(45.47)	45.47
Error	13	301.21	23.17

*($P < .01$)

Calves liver carotene at three months			
Source	d.f.	S.S.	M.S.
Total	15	23.76	
Treatment	2	9.51	4.76*
Linearity (1)		(9.29)	9.29
Non-linear (1)		(0.22)	0.22
Error	13	14.25	1.10

*($P < .05$)

VITA

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