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To the Graduate Council:

I am submitting herewith a thesis written by Gerald G. Martin entitled "Effects of Vitamin A Supplement on Milk Production of Cows Fed Low-Carotene Diets." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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EFFECTS OF VITAMIN A SUPPLEMENT
ON MILK PRODUCTION OF COWS FED
LOW-CAROTENE DIETS

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Gerald G. Martin
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CHAPTER I

INTRODUCTION

Current trends in dairy cattle feeding toward higher levels of grain and corn silage signify the need for more complete information on the effects of vitamin A on lactating cows. Beef cattle feeders have confined steers and heifers in dry lots for fattening and fed high levels of concentrates with corn silage. Hay was often limited or of low vitamin A quality. These cattle have occasionally shown vitamin A deficiencies after a short time in the feedlot even though they had been raised on pasture. Feed efficiency and rates of gains have been improved after the addition of vitamin A supplements (9, 39).

The requirement of dairy cattle for vitamin A in reproduction and growth has been well demonstrated (33). The role of vitamin A in lactation has not been clearly elucidated.

If a high level of vitamin A in the diet or in the body is an important factor in lactation at above average production levels, evidence supporting the fact should be produced in modern dairies. Grains and roughages stored for a long period will provide only a fraction of the carotene usually fed to cows under grazing management.

This investigation was conducted to determine the effect on milk production of cows fed low levels of carotene and vitamin A supplementation. Calf mortality, liver vitamin A stores, and blood status of carotene and vitamin A were also observed.

CHAPTER II

REVIEW OF LITERATURE

Vitamin A and carotene in body tissues. All animals require a dietary source of vitamin A or one of its precursors. The vitamin or its precursor, carotene, must be present in the foodstuffs. Guilbert and Hart (22) using 2 to 18 year-old, grass fed cattle indicated that the total storage of vitamin A and carotene in the liver and body fat was estimated to be 0.6-0.7 g. in the younger age groups and 3.6 g. in aged cows. Liver storage accounted for 67 per cent to 93 per cent of the vitamin A reserve of the body. The liver stored most of the vitamin A while most of the carotene was found in the depot fat. These workers indicated that a daily withdrawal rate of 9-11 μ g. per kg. of live weight is normal. They reported that the daily minimum requirement of the bovine is 26-33 μ g. of vitamin A or carotene equivalent per kilogram of live weight daily. Using rats, Guilbert and Miller (23) obtained good growth on 6-8 μ g. per kg. live weight daily but the storage within the liver was meager. Ward (48) demonstrated that 24 μ g. per kg. body weight was adequate carotene for growth and prevention of deficiency symptoms. If larger amounts were

fed no marked increase in growth rate occurred. Boyer et al. (3) found 10 μ g. of vitamin A per 100 ml. of blood plasma was indicative of a properly nourished calf. Plasma vitamin A levels of 7-8 μ g. per 100 ml. of blood were borderline and levels below this were definitely inadequate. Holstein calves required 75 μ g. of carotene per kg. body weight intake daily to maintain adequate vitamin A plasma levels. Guernsey calves required 125 μ g. of carotene per kg. daily. Blood plasma carotene levels associated with adequate blood vitamin A were 50-70 μ g. of carotene per 100 ml. for Holsteins and 110-140 μ g. per 100 ml. for Guernsey calves. Moore (37) stated that Guernsey calves required 75 μ g. of carotene per kg. of body weight during the winter and Jersey calves required 70 μ g. Harris (24) found that the liver storage capacity for vitamin A of different species varied. He found 97 percent of the total body vitamin A in the liver of the rat and an average of 2,400 International Units (IU) per kg. of carcass. Similar values were 21 to 35 percent and 1,840 IU, for rabbits, 10 to 95 percent and 29,500 IU for guinea pigs, 50 percent and 4,400 IU for chickens, and 69 to 85 percent and 5,500 IU for man.

Depletion rate of vitamin A from plasma and liver on deficient diets. Guilbert, Miller, and Hughes (23) depleted cows to a low level of plasma vitamin A. When they gave 4.3 to 5.6 $\mu\text{g.}$ per kg. body weight of vitamin A as cod liver oil the cattle remained deficient and blind. When the dose was raised to 6.7 to 8.4 $\mu\text{g.}$ the cattle recovered. They also gave carotene from alfalfa hay at a rate of less than 26 $\mu\text{g.}$ per kg. body weight, and the cattle would not recover. The alfalfa was increased to give 26 to 33 $\mu\text{g.}$ per kg. body weight, and the cattle recovered, gained normally, and stored some vitamin A in the liver. Guilbert and Hart (21) observed vitamin A deficiency symptoms in two beef steers after 225 to 240 days on a ration of dried molasses, beet pulp, rolled barley, cottonseed meal, and calcium carbonate. Total reserves were practically gone in 282 days. During the period of low carotene intake the carotene in the adipose tissue was withdrawn without using the fatty tissue. Heifers were also depleted and their calves developed scours at 2 to 8 days of age. Milk levels were low in vitamin A content. The dam, whose calf showed deficiency symptoms, did not show clinical deficiency herself up to 6 months following parturition. New-born calves had low liver levels of vitamin A. The calves had cloudy eyes, nodules on the lungs, and diarrhea. The deficient steers mentioned previously had 75 to 120 $\mu\text{g.}$ per g. of liver

carotene stores at 121 days.

Rousseau (42) fed ten pairs of 36-day old calves a vitamin A depletion ration at a control and a low level. The low ration was fed until there was less than 4.0 μg . of vitamin A per 100 ml. of blood plasma for two seven-day periods. Analysis of blood plasma showed carotenoids and vitamin A decreased on the low intake level while no change occurred in the ascorbic acid level. Hemoglobin also decreased in the cattle on the low intake ration. He also conducted another trial with forty Holstein calves 63-days old. A depletion ration was fed that would give a 4.5 kg. gain in a 7-day period until the blood plasma level of vitamin A was below 10 μg . per 100 ml. The average age of depletion was 83 days and average body weight was 50.4 kg. He then fed five levels of carotene from alfalfa 12, 18, 24, 30, and 36 μg ., and five vitamin A levels, 2, 4, 6, 8, and 10 μg . from a dry carrier. He could maintain a plasma vitamin A level of 10 μg . per 100 ml. with an intake of 31 μg . of carotene or 8.0 μg . of vitamin A per kg. body weight. For a liver concentration of 1.3 μg . per g. of liver, the carotene and vitamin A intake had to be 63 μg . or 6.3 μg . per kg. body

weight, respectively. Rousseau stated that the calf requires 23 to 34 μg . per kg. body weight of carotene and 4.8 to 6.0 μg . per kg. of vitamin A to maintain body requirements.

Jacobson et al. (29) used calves that received varying amounts of vitamin A for the first 90 days. The calves were switched to a deficient ration until the plasma level was lowered to 4 μg . per 100 ml. Two calves on pasture with their dams required four months on the deficient ration to deplete below 4 μg . per 100 ml. A whole milk raising system group took 2 to 4 weeks to deplete. Feeding good hay increased the depletion time to 6 to 8 weeks. He concluded that after 90 days, the time to deplete to 4 μg . per 100 ml. blood plasma is a good estimate of vitamin A stores. Additions of vitamin A or carotene to deficient calves increased gains and decreased scours and mortality.

Eaton et al. (14) observed decreased feed consumption by deficient calves with blood levels below 4 μg . per 100 ml. of plasma. It required 147 to 182 days on a deficient diet to reduce carotene blood levels to 2 to 4 μg . per 100 ml. and vitamin A to 2.4 to 3.2 μg . per 100 ml. when the age of calves started on the project was 106 days. Squamous metaplasia of the interlobular and main ducts of the parotid

gland was observed in the deficient calves. He could use the uniform depletion rates to measure storage of the vitamin.

Goss (19) noted that in cows on depletion rations the carotene values in the plasma dropped fast. The original level was 0.90 to 2.93 μ g. per 100 ml. of plasma and it reached a constant value of 0.09 to 0.04 μ g. per 100 ml. of plasma after 70 to 90 days. Two cows which were fed 5.2 kg. of fresh hay with an intake of 800 mg. carotene showed blood levels of 500 to 1000 mg. per 100 ml. of plasma.

Smith et al. (44) feeding steers during the winter on corn silage which would supply 25 to 134 mg. of carotene daily per head found that these steers were depleted of liver vitamin A stores almost as readily as steers fed low carotene diets. Carotene intakes of 50 to 80 mg. per day were calculated as adequate to maintain liver storage of vitamin A, but even intakes as great as 134 mg. were inadequate to prevent depletion of the liver stores. Steers eating milo and corn with no silage were depleted from 35 to 7 μ g. per g. of liver vitamin A within 79 days. Steers on silage rations depleted one fourth of the liver vitamin A stores every 40 days. Conversion of dietary carotene to liver vitamin A in the animal seemed to be inhibited.

Eaton et al. (15) studied the effect of ascorbic acid levels in blood plasma of vitamin A depleted calves. When calves were depleted to 4 μ g. per 100 ml. of vitamin A there was no decrease in the ascorbic acid level and administrations of ascorbic acid did not cure the deficiency. Vitamin A additions did reduce spinal fluid pressure and increase feed consumption.

Spratling et al. (46) deprived calves of vitamin A from 44 to 142 days. The deficient calves showed nightblindness, edema, diarrhea, and unsteady gaits. There were also losses in appetite and the development of pneumonia. The calves that were normal had liver values of 19.1 to 29.1 IU of vitamin A per g. of liver. The deficient calves had values of 0.41 to 1.83 IU per g. of liver.

Dehority et al. (13) used sixteen Holstein calves raised to 63 days of age on a depletion diet until the plasma vitamin A averaged 8.3 μ g. per 100 ml. of plasma. Each calf was fed to sixteen weeks of age on one of four carotene levels. A level of 33 to 50 mg. per kg. live weight gave marked increase in cerebrospinal fluid pressure and a 67 mg. level gave only slight elevation in pressure over the control group. No total pressure increase was noticed with an 84 mg. level. The plasma levels increased both in carotene and

vitamin A content. Hazzard et al. (25) conducted another study using forty-eight Holstein calves. Two levels of vitamin A depletion rations were fed. The 100 per cent depletion and 60 per cent depletion diet were fed for 12 and 24 weeks respectively. The difference between the two levels was noticed in calves as the 100 per cent rations raised cerebrospinal fluid pressure, slowed gains, and interrupted heart action. The 60 per cent groups required only one-fourth as much carotene to maintain adequate cerebrospinal fluid pressures. Twelve weeks on the 100 per cent depletion ration was sufficient to endanger the health of the calf due to elevated brain pressures. The 60 per cent groups took 24 weeks to reach the same point.

Vitamin A and carotene efficiency for repletion of deficient cattle. Grifo et al. (20) fed thirty-two calves of a vitamin A depletion ration until they were partially depleted of vitamin A stores. The calves were fed a 26 and 105 $\mu\text{g.}$ per kg. of carotene level for four and sixteen-week periods. During the first period cerebrospinal fluid pressures increased in the calves on the 26 $\mu\text{g.}$ level. During the second period these calves utilized carotene seven-tenths to nine-tenths as effectively as the 105 $\mu\text{g.}$ per kg. calves based on carotene samples obtained terminally from

repleted calves. Utilization of carotene was unaffected after a sixteen week period of deficiency on levels above 12 μ g. of carotene per kg. of body weight. There was evidence of faulty carotene conversion to vitamin A in a test conducted by Erwin (17). Deficient steers did not increase in liver vitamin A levels as carotene was provided at levels of 33 μ g. per kg. body weight.

Riggs (40) found that if 460 μ g. per kg. live weight of vitamin A was added to a depletion diet it would prolong the time to reach night blindness by fifteen or more days.

Frey (18) used 114 Hereford steers on a vitamin A and carotene-free diet. He supplemented the ration with preformed vitamin A. Cattle getting the basal ration had lower consumption and lower average daily gains than those receiving vitamin A supplement. Dietary vitamin A did not exert a sparing action on hepatic stores of carotene. The blood storage of carotene decreased faster than hepatic stores. The hepatic stores a vitamin A increased at a linear relationship throughout the range of vitamin A supplement fed. Serum levels and hepatic stores of carotene and vitamin A appeared to be controlled by different body mechanisms. He found that 270 days would be required to deplete these steers down to 0.2 μ g. of vitamin A per g.

of liver.

Requirements of vitamin A for growth of cattle.

Perry et al. (39) stated that the rate of gain and daily feed consumption increased in steers while the blood plasma level of carotene decreased and vitamin A increased as a vitamin A supplement was provided. An intake of 10,000 IU of vitamin A per head daily gave a 20 per cent increase in gains. Levels of 30,000, 40,000, and 50,000 IU did not improve performance. Efficiency of feed conversion was increased 7 to 10 per cent.

Chapman and other workers at Florida (9) placed seventy-two, two year old steers on Rosetown-St. Augustine Pasture. The rate of gain was stimulated by 11.5 per cent when 25,000 IU of vitamin A was added. A level of 50,000 IU raised the gain 17 per cent over the control group. The normal liver levels were 261 to 557 IU per g. dry weight of vitamin A. Braun (6) using forty-nine cows on four different dietary levels of carotene and vitamin A observed changes in the blood levels of the vitamin A and carotene at parturition, following abortions, and during acute infections.

Jordon and Smith (30) found feeder steers were depleted of liver vitamin A stores when they were maintained on corn silage calculated to contain sufficient carotene.

Steers fed this corn silage through the winter and spring developed vitamin A deficiencies even though they were supplemented with 7,000 to 8,000 IU daily of preformed vitamin A. These cattle had adequate plasma levels but the liver levels remained low despite 8,000 IU each day. Cady (8) noticed that a group of eighty-nine steers were going off feed after they had been gaining 0.9 kg. per day. Examination showed that the cattle had lost hair, were foundered, had swollen legs, had scours, and exhibited an unnatural gait. Nightblindness was observed in eleven steers and three were totally blind. The ration was changed from old corn to new corn and the hay was changed to new crop alfalfa. Some of the worst cases were given cod liver oil via stomach tube. Most of the cattle cleared up in a very short period but several cattle in the nightblind group had to be sold as the eye condition persisted.

Madsen (35) observed 651 beef carcasses that were condemned on account of edema from 1941 to 1946. These cattle had eaten corn stalks, straw, and alfalfa hay that was bleached. The affected animals showed deficient blood plasma levels of both carotene and vitamin A. The United States Department of Agriculture (47) calculated some losses in carotene on three types of forages. Field cured hay lost 95 per cent of the carotene from cutting to feeding. Hay

that had been rained on lost 98 per cent of the carotene, but wilted silage only lost 55 per cent of the carotene.

Requirements of vitamin A for reproduction in cattle.

Kuhlman and Gallup (33) used prairie hay as a source of carotene on eighteen cows for thirty-four gestations. Reproduction was somewhat impaired if the average daily intake during the last ninety days before calving was below 88 μ g. per kg. of body weight. The calves were born weak and expressed deficiency symptoms. They also reported low production in late lactation after vitamin A deficiency had been encountered prior to parturition. They indicated there may be a different requirement for carotene for lactation than for gestation and reproduction. Maynard and Loosli (36) reported that 10 to 20 mg. per 100 kg. are required for lactation. Extra amounts of carotene are needed for adequate reproduction. Madsen, Hall, and Converse (34) reported cystic pituitary glands in thirteen of fifteen dairy and beef cattle that had previous vitamin A deficiency. These cattle varied in age from one day to 787 days old. The cysts occurred either in the residual humor or within the posterior lobe causing compression or glandular parenchyma damage. This high incidence suggests that this may be part of the pathology of vitamin A deficiency.

Byers (7) fed Jersey and Holstein cows on a low carotene ration during late pregnancy. The calves from these cows being fed low carotene rations were slightly lighter than were previous calves from the same cows when fed adequate carotene. This calf group was then fed a depletion diet and calved at two years of age. There were losses in reproductive efficiency and abortions. Some calves were so weak that they died soon after birth. Only two normal calves were produced from cows fed 50 μ g. of carotene per kg. of body weight over a twenty-eight month period. This suboptimum level continued for two or three generations resulted in lower reproductive efficiency and in abnormal and damaged pituitary, adrenal, and sex glands. Kane (31) used corn silage fed within the year it was stored that provided 1-40 mg. carotene per kg. of live weight. Cows getting silage that contained 7.5 μ g. carotene per kg. produced weak calves. Cows fed 13.6 μ g. carotene per kg. had marginal reproductive efficiency. He indicated that silage may not provide enough carotene for normal reproduction.

Davis et al. (12) feeding cattle 30 to 45 mg. of carotene per day found that the cows had poor reproductive efficiency and had calves deficient in vitamin A. The cattle were depleted below 14 μ g. of carotene per 100 ml. of plasma

and vitamin A levels decreased below 10 μ g. per 100 ml. of plasma. Braun (5) separated pregnant cows into groups with different vitamin A intakes. The vitamin A content in the livers of both the fetuses and their dams was in direct relation to each other. A range for the fetuses was 0.0 to 24.2 IU per g. while the cows ranged from 449 to 940 IU per g. of vitamin A.

Vitamin A effects on lactation. Copeland (11) fed Jersey cows 31.5 μ g. crude carotene per kg. of live weight. This was comparable to the intake under dry conditions in the Southwest. An adequate level of 315 μ g. per kg. live weight was fed to another group. Data from twelve cows indicated that milk yield can be reduced through an inadequate supply of carotene or vitamins over relatively short periods, especially at the peak of lactation. The cows on the adequate level produced 10 per cent more milk.

Ronning et al. (41) conducted a twenty-year study to see if carotene levels would influence production and reproduction. A total of 197 gestations and 153 lactations from Jersey, Guernsey, and Holstein cattle showed that good reproduction could be maintained with 157 to 178 μ g. of carotene per kg. live weight daily. Normal lactation occurred within this range of carotene fed. Blood plasma

carotene was not reliable in evaluating reserves needed to prevent reproduction problems. Calf mortality was the largest problem encountered in the investigation.

Hilton et al. (26) fed a vitamin A depletion diet to deplete to 12 to 15 vitamin A units per g. of butter. He gradually added back fish liver oil to provide vitamin A and found that the requirement for production of a high value vitamin A butter was 200,000 IU of vitamin A daily. He also found that vitamin A per se was about three times as effective as carotene from dehydrated alfalfa hay in restoring the vitamin A activity of the butterfat. Wilbur (49) depleted cows so that the level in the butter was 12 IU of vitamin A per g. He added alfalfa hay for 21 days to the rations and found that it required approximately 550,000 IU of vitamin A daily to restore vitamin A to the highest level.

Hodgson et al. (27) fed stored feed for three years to Holstein cows to observe the vitamin A and carotene levels produced in the butterfat. The carotene contents of hay, grass silages, and silage and pasture were 15, 57, 197 and 259 μ g. per g. dry matter respectively. The butterfat carotene values were 3.6, 6.5, 6.8, and 7.9 μ g. per gram. There was a close correlation between the color and carotene content of the butterfat. These rations furnished enough vitamin A for normal body activities and reproduction, and

the butter contained 50 IU of vitamin A per gram. The vitamin A activity in the butterfat attributable to the carotene was 11.2 per cent for hay rations, 12.7 per cent for hay and silage, and 11.3 per cent for silage and 13.7 per cent for the pasture ration.

Summary of review of literature. It definitely can be shown that vitamin A plays an important role in growth, reproduction, vision, infections, and gland functions. There are different requirement levels for growth and reproduction in cattle. Several factors may influence conversion of carotene to vitamin A both in the body and also within the liver, although there is some contradiction in the literature as to carotene storage and mobilization. The composition of diets influences depletion time and rates. Many investigators have shown responses to vitamin A addition to rations in terms of rate of gain, calf mortality, and feed consumption. Data relative to effects of vitamin A on lactation are inconclusive.

CHAPTER III

EXPERIMENTAL METHODS

General Description. This experiment was a continuous trial extending through two years at the University of Tennessee Dairy Farm. Thirty-five lactations consisting of twenty-four with identical twins and eleven individuals from the herd were used in the investigation. Feed weights, milk production, blood and liver analyses of carotene and vitamin A and general observations were recorded for complete lactations. Four of the twins were used in two lactations.

Source and selection of animals. Identical twins previously used in a growth experiment were started on the project in the fall of 1964. Non-twin pairs were selected from the University herd based on previous lactation, age, and body weights. Several pairs were selected for lactation comparisons. All animals were placed on deficient diets four months before calving. Several cows being removed from the herd were used to check a liver biopsy technique. Liver content of vitamin A was determined in a group of seven cows fed above normal rates of grain and limited roughage along with a control group of seven cows on normal diets.

Feeding schedule. All of the cattle had been receiving silage, hay, and a grain mix. Some pasture or direct cut

grass had been fed to the non-twin cows. Each cow was placed on a depletion diet, Table I, four months before the expected calving date. On this diet the cows ate approximately equal parts of beet pulp and grain mixture. In addition, all cows received 2.2 kg. of two year old hay daily. Corn silage was added to the ration of 4.5 to 6.8 kg. daily at two to four weeks before calving. Some cows calved early or aborted before silage feeding started. At the time of starting silage feeding, one member of each twin pair also received 50,000 IU of a stabilized vitamin A mixed in the grain feed each day. The supplement was prepared in a premix each month to assure that 50,000 IU per day was provided in a 20 g. feeding. All animals received 2.3 kg. daily of the two-year old hay throughout the experiment. Several different lots of hay were used and these varied in carotene content. Grain feedings were regulated according to body condition but the cows were always fed high grain and low roughage diets.

Feed and growth data. All feeds were weighed into the manger and refusals were recorded once daily. Silage was fed in the morning and hay was fed in the afternoon. Beet pulp was fed at mid-morning over the silage feeding. The refusal was weighed each morning before feeding for that day. Refused feed could not be accurately identified by

TABLE I

AMOUNTS OF FEEDSTUFFS FOR DEPLETION AND
LACTATION FOR CONTROLLED CAROTENE INTAKES

Feedstuff	Depletion diet	Lactation diet
	------(kg. fed) ^b -----	
Grain mix ^a	5.71	11.90
Beet pulp	5.71	3.33
Alfalfa hay	2.38	2.38
Corn silage		7.14

a. See Table II for composition.

b. Fed at the rate of approximately
110 percent of estimated requirements.

origin.

Each cow was weighed at weekly intervals after starting the project. Once each month the animals were measured for height of withers and heart girth. Calves were weighed before 48 hours post partum.

Rations used. All rations were mixed at the University of Tennessee Dairy Farm. The ingredients are shown in Table II. All hay that was fed was at least two years old and was low in carotene content at date of purchase. Some oat hay was used due to a lack of alfalfa, and it was mixed with the alfalfa hay.

Analysis of feeds. Samples of the grain mix, beet pulp, hay, and silage were taken at two-month intervals and analysed for carotene and proximate chemical constituents (1). The average feed composition is summarized in Table III, page 24. Current carotene intakes were calculated at weekly intervals for all cows on the basis of the current feed carotene. The vitamin premix was analysed for vitamin A (32) at about six-month intervals.

Blood analysis. At two-month intervals and at each liver biopsy date forty milliliters of blood were taken from the juglar vein for carotene and vitamin A analysis. Calves

TABLE II
DEPLETION RATION GRAIN MIXTURE

Ingredient	Portion %
White hominy	53.0
Oats	15.0
Wheat bran	15.0
Cottonseed meal	15.0
Salt	1.0
Dical phosphate	1.0
Irraidated Yeasts ^a	0.005
Aureofac 10 (aureomycin) ^b	0.075

a. Provide 6600 I.U. per kg.vitamin D.

b. Started October 29,1965.

TABLE III

AVERAGE FEED ANALYSIS OF DEPLETION RATION ^a

Ingredient	Moisture	Protein	Fat	Crude Fiber	Ash	N.F.E.	Carotene
				(%)			mg./kg.
Grain	10.17	15.45	3.19	7.96	5.58	57.62	0.44
Beet pulp	9.85	8.89	3.58	19.32	4.88	56.68	1.98
Hay	10.67	14.06	1.47	31.15	7.58	35.04	3.16
Silage	70.20	3.10	.83	7.01	1.70	17.17	9.97

a. Feed sampled at two month intervals includes thirteen analyses.

were sampled in the same manner at forty-eight hours after birth or sooner if they were weak or morbid. The plasma was separated by centrifugation and the carotene and vitamin A extracted by ether. Duplicate samples of five milliliters of plasma were used for the determination of carotene and vitamin A (32). The values were averaged if they were in close agreement.

Liver analysis. Liver samples were obtained by a biopsy method as the cows were started on the depletion diet, again before calving, and at two-month intervals throughout lactation. Liver samples were taken only once from the cows on the high grain experiment, at the time they were finishing the experiment. Each sample was frozen as collected and brought to the laboratory for immediate analysis. The liver sample was then weighed and saponified using aqueous potassium hydroxide. An ether extract was made after cooling of the samples according to procedure of Bunnell et al. (4). Color reactions of the samples were read in duplicate.

Liver biopsies. Liver sampling was done by a biopsy technique that was an adaption of the methods described by Chapman et al. (10), Erwin (16), Hughes (28), and Bone et al. (2) using an aspiration device to withdraw the sample. They

used a special fitted syringe with a serrated edge cannula and a trocar that was 22.5 cm. in length. A 1 to 3 g. sample could be obtained if sampled on the right side of the animal in the eleventh intercostal space. Bone (2) using this method of aspiration reported a 0.5 per cent death loss in making 1200 liver biopsies. Hughes (28) reported three deaths in 500 biopsies due to a rupture of large blood vessels and possibly an infection from penetration of an abscess. The average weight of his specimens was 3.8 to 5.1 g. Chapman (10) placed his thumb over the cannula to retain the sample in the cannula, and reported on death losses over a two year period sampling a large number of cattle every twentieth-eight days.

The instrument used in this study was a stainless steel cannula and trocar. The cannula was 22.5 cm. long, 6.3 mm. in diameter, and was fitted with a star pointed trocar measuring 25 cm. A flat flange was placed on top of the cannula so the thumb could fit over the end for a tight seal.

During the biopsy the animal was placed in a squeeze chute to control its movements. The right side over the rib cage was washed with a soapy solution. A 10 cm. square was shaved over the eleventh intercostal space at about the same height as the hip joint. A few long bodied cattle had to be

sampled through the tenth intercostal space to reach the liver. The shaved surface was scrubbed with alcohol until clean. A small stab wound was made with a scapel with the hole being just large enough to make easy entry for the cannula. The trocar and cannula which had been sterilized in a germicide solution, were passed aseptically through the skin, muscle wall, and diaphragm. The instrument was pointed ventrally at an angle of forty-five degrees with the horizontal plane and at a slight angle with the sagittal plane. As soon as the tip passed through the diaphragm and entered the liver, the trocar was withdrawn from the cannula. The cannula was pushed into contact with the liver capsule. There was a characteristic feeling upon penetration of the liver tissue. The cannula was rotated and pushed until it reached through the liver or until the flange was next to the skin. The cannula was then moved slightly from side to side to free the liver core, the thumb was placed over the flange to form a seal, and the cannula was carefully withdrawn. The sample was extruded onto absorbent paper and the liver tissue and blood were separated. The liver sample was transferred to a test tube and frozen in dry ice. The usual weight of the liver core obtained was 1 to 2 grams.

Over 150 biopsies were performed on the cattle with no adverse effects noticed. Milk weights on the biopsy date

did not differ from the normal. Most of the cows were biopsied six times during the experiment. The animals all healed with only very small skin scars. The liver of one cow was examined when slaughtered one day after biopsy, and only a small hair line scar could be seen on the liver surface.

Milk sampling and analysis. Weekly milk samples were drawn from the weigh jars from two milkings on each cow. Milk weights were recorded at each milking. The colostrum milk at the first milking following calving was analyzed for carotene and vitamin A. Fourteen cows were divided into two groups according to their preliminary feeding as it affected depletion rates. A comparison was made of the production of the cows on the depletion diet with their previous lactation during which adequate intake of carotene from silage, hay, and grain occurred. Records in the two years were equalized for days in milk and corrected for age to mature equivalents.

Fourteen cows that were finishing a lactation comparing normal with high grain feeding were sampled for liver and blood comparisons. Milk production figures were also noted. One group of seven cows had received normal grain allowance with roughage fed free choice, and the other seven cows had received high levels of grain with low roughage. Those comparisons would be indicative of vitamin A status of cows under common type of commercial feeding and management.

The identical twins were placed on a depletion diet and allowed to deplete blood and liver storage. One number was selected to receive vitamin A supplement throughout the lactation while the mate received a low level of carotene from silage, hay, and grain. The production of the pairs was compared for difference due to the vitamin A supplementation. Several pairs of non-twin cows were depleted for four months prepartum, but since their liver levels of vitamin A were still high at parturition they were not used for paired lactation comparisons but were left on the depletion diet until vitamin A deficient liver levels were reached. They were then fed supplemental vitamin A to see if the lactation curve could be changed due to the addition of extra vitamin A. Analysis of variance was used on the comparison data according to Snedecor (45).

CHAPTER IV

RESULTS

Effects of depletion diet. Thirty-five animals were started on the vitamin A depletion diet at four months prepartum. They were all fed the depletion diet listed in Table II, page 23, until two weeks prepartum. The effects of the depletion diet on blood plasma vitamin A and carotene for two groups of cows are shown in Tables IV, V, VI, VII, and VIII.

One group of eleven cows, Table VII, that had been fed green forage before depletion had preliminary blood plasma levels of vitamin A averaging 37.9 $\mu\text{g.}$ per 100 ml. This group dropped to 25.7 $\mu\text{g.}$ after 90 days of depletion feeding and at calving averaged 10.1 $\mu\text{g.}$ per 100 ml. Plasma carotene at the same three stages were 727, 42, and 24 $\mu\text{g.}$ per 100 ml. respectively. Another group, Table VIII, that had been barn fed the previous lactation started the preliminary period with 33.8 $\mu\text{g.}$ of vitamin A per 100 ml. decreased to 8.8 $\mu\text{g.}$ after 90 days, and reached 7.4 $\mu\text{g.}$ per 100 ml. at calving. The same group had 311 $\mu\text{g.}$ of carotene per 100 ml. of blood which was about half as much as did the green forage group at the preliminary phase. They dropped to 43 $\mu\text{g.}$ of carotene at 90 days and were back up to 55 $\mu\text{g.}$ per 100 ml. of plasma at calving. Some of this

CAROTENE LEVELS IN LIVER AND BLOOD OF BARN FED COWS AND GREEN FORAGE FED COWS DURING DEPLETION AND LACTATION

a. 50,000 IU per day vitamin A.

VITAMIN A LEVELS IN LIVER AND BLOOD OF COWS FED GREEN FORAGES DURING DEPLETION AND LACTATION

a. Supplementation vitamin A. 50,000 IU per day.

TABLE VI

VITAMIN A AND CAROTENE LEVELS IN LIVER AND BLOOD OF A GROUP OF NONSUPPLEMENTED TWINS AND A GROUP OF SUPPLEMENTED TWINS THROUGH DEPLETION AND LACTATION

Cow	3 Mo.			2 Mo.			4 Mo.			6 Mo.			8 Mo.		
	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B
----- <u>gug. per g. liver</u> ----- <u>gug. per 100ml. blood</u> -----															
Carotene															
70	1.3	65	2.7	27	.9	18	2.1	90	2.0	145	1.2	10			
75	2.1	180	0.6	21	1.4	81	1.6	118	1.4	69	1.2	84			
79	7.0	324	1.5	38	1.9	115	1.6	251	1.5	101	1.3	50			
Average	3.4	189	1.6	28.6	1.4	71	1.8	153	1.6	105	1.2	78			
Plus A															
72	---	36	2.2	10	1.0	8	1.1	108	1.5	67	1.2	74			
76	2.5	218	.5	32	1.0	24	.5	134	1.6	81	---	56			
80	7.4	297	.9	23	1.5	96	1.6	231	1.5	69	1.4	41			
83	4.2	242	4.0	63	1.4	135	1.2	207	1.7	126	.9	76			
Average	4.7	198	1.9	32	1.2	66	1.1	170	1.6	86	1.2	62			
Vitamin A															
70	14.2	26.2	9.3	5.6	.9	8.8	3.7	18.4	3.2	15	4.3	23.4			
75	18.2	26.5	2.1	4.5	.9	18.4	1.9	14.1	2.2	17	1.7	17.0			
79	32.2	25.7	9.6	6.9	4.1	15.1	4.7	24.7	6.7	18	1.0	7.9			
Average	22.2	26.1	7.0	5.7	2.0	14.1	3.4	19.1	4.0	16.8	2.3	16.1			
Plus A															
72	---	21.8	5.4	10.3	1.5	10.7	9.0	23	9.9	13	16.0	29.1			
76	19.5	25.8	1.0	2.4	3.4	19.0	6.1	35	11.1	22	---	3.8			
80	32.1	25.8	5.6	6.0	6.7	16.5	10.8	27	20.9	17	8.9	16.5			
83	11.9	29.3	8.8	7.8	4.1	34.6	2.5	30	8.2	29	7.2	23.0			
Average	21.1	25.6	5.2	6.6	3.9	20.2	7.1	29	12.5	21	10.7	18.2			

TABLE VII

CHANGES IN BLOOD PLASMA VITAMIN A AND
CAROTENE FROM PRELIMINARY THROUGH PARTURITION
STAGES OF COWS FED GREEN FEEDS BEFORE DEPLETION

Cow	Vitamin A			Carotene		
	Prelim.	3-mo.	Calving	Prelim.	3-mo.	Calving
----- (ug./100ml.) -----						
68	23.5	24.8		632	47	
T175	64.6	18.0	6.4	296	49	23
T204	39.0	23.0	5.1	280	52	37
T201	25.4	27.2	7.7	1045	40	31
T200	34.7	21.0	13.0	620	33	27
T193	68.7	30.6	7.7	1797	45	27
44	43.8	43.2	12.5	976	43	13
121	26.9	25.8	12.1	321	53	25
64	27.0	18.8	13.0	850	35	12
59	29.5	25.0	15.2	374	27	24
872	34.8	25.3	8.0	811	36	11
Av.	37.9	25.7	10.1	727	42	24

TABLE VIII

CHANGES IN BLOOD PLASMA VITAMIN A AND CAROTENE
FROM PRELIMINARY THROUGH PARTURITION STAGES OF
COWS FED ONLY STORED FEEDS BEFORE DEPLETION

	Vitamin A			Carotene		
	Prelim.	3-mo.	Calving	Prelim.	3-mo.	Calving
------(μ g./100ml.)-----						
67	19.0	6.9	12.4	316	42	45
69-1	57.1	10.6	5.8	498	12	66
70-1-A	53.2	9.5	20.4	464	4	67
69-2	14.5	5.4	3.4	70	34	30
70-2	26.2	8.5	2.0	65	27	17
71-1	43.6	11.6	6.3	388	26	26
72-1-A	55.9	15.4	7.5	312	32	40
71-2-A	12.4	4.2	0.0	39	13	4
72-2	21.9	10.4	2.4	36	15	8
73	51.7	6.6	15.1	458	56	305
74-A	62.2	16.5	18.4	488	142	147
75	26.5	6.0	3.1	180	21	31
76	25.9	2.4	6.9	218	23	24
77-A	28.3	7.3	21.3	443	31	152
78	39.3	9.5	...	537	38	...
79	26.8	7.0	4.2	324	26	38
80-A	25.7	6.0	7.1	297	23	44
83-A	29.3	7.8	...	242	63	...
84	26.9	4.4	4.3	246	18	23
85	25.7	10.3	8.2	105	27	13
86	28.5	...	0.4	252	...	21
87	39.4	19.2	...	579	154	...
88	42.2	13.4	3.3	583	133	44
113	28.1	2.5	2.3	318	19	17
av.	33.8	8.8	7.4	311	43	55

A= Cows were fed 50,000 IU vitamin A for varying periods from 1 to 3 weeks before parturition.

group had received 50,000 IU of vitamin A for varying periods from 1 to 3 weeks before parturition, and corn silage feeding was started about two weeks prepartum.

The two groups also varied in liver levels of carotene and vitamin A at three phases of the project, as shown in Table VII, page 34. The green forage group averaged 143.2 μ g. per g. of liver vitamin A compared to 21.7 μ g. per g. for the barn fed group. The green forage group dropped to 59.0 μ g. per g. while the barn group fell to 6.0 μ g. per g. after the 90-day depletion period. By the second month of lactation the two groups averaged 14.1 and 3.1 μ g. per g. of liver vitamin A, respectively, for the green forage and barn fed groups. The liver vitamin A and carotene levels, Table VIII, page 35, remained low for some time until the blood carotene was replenished by silage feeding.

Thirteen cows were eliminated before the study was finished. Nine cows were dropped due to mastitis, metritis, arthritis, or other health troubles. Five cows which died were autopsied by a veterinarian to ascertain the cause of death. The diagnoses were rumenitis, hepatitis, lipoid malignancy, abomasal torsion, and internal injuries due to dystocia. Three other cows removed from the project were observed at slaughtering for signs of vitamin A deficiency.

There were no deficiencies noticed.

During depletion one cow developed night blindness for a period of six or seven days. Swelling of the hocks and legs was noted in some cows which had low liver stores of vitamin A. The haircoat of several animals was very rough, wiry, and long. Hoof growth seemed to be increased during the confinement to tie stalls and may have caused much of the foot and leg trouble.

Calf mortality. A noticeable effect of vitamin A depletion was calf mortality and abortions. Out of thirty-eight pregnancies seventeen calves were dead at birth or aborted. Eight additional calves died before forty-eight hours postpartum and five more died due to injury accompanied by dystocia. Five of the deaths were obviously due to vitamin deficiency. Only thirteen of thirty-eight calves survived. Table IX, shows the length of gestation and condition of the calf along with a probable cause of death. The blood and liver levels of the dams of the vitamin A deficient aborted calves was below 4 μ g. per 100 ml. of blood plasma and below 1.0 μ g. per g. of liver. Other abortions and calf deaths occurred from dams with normal or high vitamin A stores. The rest of the University of Tennessee herd had a 13.5 per cent calf death loss at the same time. The difference in calf mortality (53 per cent to

TABLE IX

RECORD OF CALVING BY COWS FED VITAMIN A
DEFICIENT RATIONS FOR THREE MONTHS OR MORE PREPARTUM

Cow	Vitamin A		Gest- ation (days)	Condition of calf		Probable cause death
	Plasma $\frac{\mu\text{g.}}{100\text{ml.}}$	Liver $\frac{\mu\text{g.}}{\text{g.}}$		Birth	48 hours	
76	2.4	1.0	251	D ^a		A deficiency
113	2.5	1.5	247	D		A deficiency
69-2	3.4	0.7	245	D		A deficiency
71-2	4.2	0.6	244	D		A deficiency
84-1	4.4	4.8	280	N ^b	D	Calf infection
75	6.0	2.2	285	N	N	
80	6.0	5.7	270	D		Dystocia
73	6.6	...	264	N	N	
67	6.9	...	280	N	D	Calf infection
79	7.0	9.7	270	D		Unknown
77	7.3	...	280	N	D	Calf infection
T201	7.7	25.8	280	N	D	Calf infection
70-2	8.5	9.3	268	N	N	
78	9.5	...	273	N	N	
70-1	9.5	...	268	N	D	Calf infection
44	10.2	43.6	280	D		Dystocia
85	10.3	38.0	268	D		Dystocia
72-2	10.4	5.4	272	N	D	Calf diarrhea
69-1	10.6	...	271	N	N	
83	11.0	8.4	271	N	N	

TABLE IX (Continued)

Cow	Vitamin A 1		Gest- ation	Condition of calf		Probable cause death
	Plasma	Liver		Birth	40 hours	
	$\frac{\mu\text{g.}}{100\text{ml.}}$	$\frac{\mu\text{g.}}{\text{g.}}$	(days)			
71-1	11.6		282	D		Dystocia
86	12.2	32.5	280	D		Unknown
121	12.8	40.9	279	N	N	
88	13.4	3.7	275	N	N	
72-1	15.4		280	N	N	
74-1	16.5		277	N	N	
64	18.0	152.0	281	N	N	
T175	18.0		275	D		Unknown
T200	21.0	26.5	268	N	N	
T204	23.0	35.6	278	D		Unknown
73-2	24.7	3.6	182	D		Cow diarrhea
68	24.8	26.7	282	D		Dystocia
872	25.0	193.3	278	N	D	Cow infection
59	25.0	72.2	284	N	N	
84-2	26.5	4.6	95	D		Unknown
T193	30.6	42.0	275	N	D	Calf diarrhea
87	39.4	13.5	150	D		Unknown
74-2	64.6		121	D		Cow diarrhea

a. Dead

b. Normal

1. Vitamin A level at calving.

13.5 per cent) between cows in this experiment and the rest of the herd was highly significant ($P < 0.01$).

The calf blood at 48 hours and the colostrum were analyzed for carotene and vitamin A content. Those data are shown in Table X. Several samples of milk from the paired twins were taken during the depletion time. These averaged 142 μ g. of vitamin A per liter and 178 μ g. of carotene per liter.

High grain fed animals. Fourteen animals that had been on a high grain experiment were biopsied after finishing a full lactation. Seven cows had been fed high grain and seven others had been controls or fed normally. The high grain cattle had consumed an average of 3.2 kg. hay, 9.1 kg. silage, and 11.8 kg. of grain, which provided a total of 172.5 mg. of carotene daily. The normal ration animals ate 4.5 kg. hay, 13.4 kg. silage, and 9.1 kg. grain which provided 257.5 mg. carotene daily. Production varied from 4698 kg. to 8298 kg. of fat corrected milk. The difference in plasma and liver vitamin A and carotene between the two groups, Table XI was not significant. ($P < 0.05$)

Effect on milk production. There was no significant effect of vitamin A supplementation upon milk yield. Even cows with very low liver vitamin A post partum (2.7 to 4.8

TABLE X

VITAMIN A AND CAROTENE LEVELS IN BLOOD OF CALVES
48 HOURS POSTPARTUM AND IN COLOSTRUM MILK

Calf	Calf		Dam's	
	Vitamin A	Carotene	Vitamin A	Carotene
	----- (µg./100ml.) -----		----- (µg./l.) -----	
71	7.85	9.1	7.08	135
72	10.88	3.5		
70	8.81	3.2	1422.	410
69	7.59	6.8	166.	153
73	7.46	7.1	368.	279
74	12.80	12.1	1744.	1760
77		...	98.	167
78	1.47	5.0	364.	466
59	19.01	6.2	1460.	345
85		...	288.	390
T200	8.12	2.8		
64	21.11	7.9		
44		...	576.	334
72	5.34	2.6		
T204		...	493.	416
70	7.13	0.0		
113		...	194.	205
83	6.93	6.1	929.	922
79		...	252.	220
T193	7.92	10.4	614.	409
121	9.41	0.0	668.	286
88	6.47	5.2		
76		...	395.	322
69		...	188.	346
67		...	367.	291
av.	9.27	5.6	508.	413

TABLE XI

LIVER AND CAROTENE LEVELS OF COWS FED HIGH GRAIN
AND NORMAL RATIONS WITH LACTATION YIELDS

Cow (No.)	Lactation Yield (kg.)	Plasma		Liver	
		Carotene	Vit. A	Carotene	Vit. A.
		(μ g./100ml.)		(μ g./g.)	
High grain					
34	2296	302	24.9	7.13	19.70
T153	4698	172	24.4	3.93	59.19
126	4995	243	24.0	4.52	44.74
125	8298	219	20.9	3.65	24.04
113	5427	317	28.2	3.32	7.41
124	7204	183	19.3	3.60	34.44
93	7929	408	26.0	6.46	39.63
Average		263.4	23.96	4.66	32.74
Control					
48	4852	180	23.5	5.38	16.59
143	6312	321	25.1	3.79	21.14
136	7018	456	36.4	6.83	37.94
116	5721	193	19.3	2.94	36.91
796	7228	201	20.6	3.54	16.55
28	7235	156	24.9	3.79	63.55
49	7034	316	16.3	3.79	9.26
Average		260.4	23.73	4.29	28.85

µg. per g. of liver) produced at a higher rate than in previous lactations. The production comparisons are presented in Tables XII and XIII, pages 34 and 35

A 24-week comparison was made of the milk yield of five pairs of identical twins and one pair of added twins that were paired on the basis of similar producing ability after their own mates were eliminated for health reasons. These cows were depleted to low levels of blood and liver vitamin A before calving. Starting two or three weeks pre-partum 50,000 IU vitamin A supplement were fed one twin while the other twin received only minimal carotene from limited allowance of hay and silage, and grain. The maximum carotene intake for the depleted cows was 60 mg. per day. The supplemented twins received this amount plus 50,000 IU of vitamin A per day. Because the twins had all been barn fed they were depleted to deficiency levels of blood and liver vitamin A within sixty to ninety days after the start of the diet. After corn silage was added to the diet their vitamin A status was borderline.

The six control cows produced an average of 2730 kg. in 24 weeks compared to 2365 kg. of milk for the vitamin A supplemented group Table XII. This 365 kg. difference was not statistically significant. The average liver vitamin A content of three pairs at six months of lactation was 4.0 µg.

TABLE XII

MILK YIELD OF PAIRED COWS FOR TWENTY-FOUR-WEEK PERIOD

Control		Vit. A Suppl.		A-Suppl. Difference
Cow	Milk	Cow	Milk	Milk
(No.)	(kg.)	(No.)	(kg.)	(kg.)
69	2,913	70	1,888 ^a	-1,025
71	3,166	72	3,089	- 77
73	1,851	74	2,016	+ 165
79	2,336	80	2,100	- 236
75	4,027	76	2,985 ^b	-1,042
70	2,089	83	2,110	+ 21
Av.	2,730		2,365	- 365

a. Suspected injury to udder.

b. Metritis following abortion affected lactation.

per g. for the controls and 13.4 μ g. per g. for the supplemented. Blood plasma vitamin A at this time averaged 16.8 μ g. per 100 ml. for the controls and 22.9 for the three supplemented cows. Average blood plasma vitamin A of the six pairs at six months of lactation was 24.9 μ g. per 100 ml. for the controls and 32.9 for those supplemented with vitamin A.

Milk production of two groups of cows that had been depleted on the diet or that required more than 90 days to deplete was compared to their lactation prior to depletion, Table XIII, page 46. Several animals were controls in the twin pairing but had not received supplementation. Group A was depleted before calving and group B cattle were not depleted until after calving. The comparison was made of actual yields and mature equivalent records. Group A averaged 4165 kg. mature equivalent on the depletion diet compared to 3,902 kg. on a previous lactation under normal feeding and management. Group B produced 4,299 kg. mature equivalent compared to a previous lactation average of 4,167 kg. Group A was composed of six animals while group B had eight animals. The combined groups, group A plus B in Table XIII, page 46, averaged 4,240 kg. under depleted liver and blood stores compared to 4,054 kg. in a previous lactation. All records were adjusted for comparable length

TABLE XIII

MILK PRODUCTION OF COWS FED VITAMIN A DEPLETION
RATION COMPARED WITH PREVIOUS YEAR'S PRODUCTION
ON NORMAL RATIONS BY COWS DEPLETED OF VITAMIN A
BEFORE CALVING (GROUP A) AND BY COWS DEPLETED OF
VITAMIN A DURING LACTATION THEN SUPPLEMENTED (GROUP B)

Group and Cow	Preliminary Lactation on Normal Diets		Experimental Lactation on Depletion Diets	
	Actual Yield	Mature Yield	Actual Yield	Mature Yield
----- (kg.) -----				
Group A				
69	3,010	3,762	4,072	4,560
70	2,321	2,577	2,322	2,415
71	4,082	5,307	4,552	5,281
73	2,967	3,649	3,142	3,519
75	4,095	5,159	5,344	5,879
79	2,347	2,958	2,952	3,335
Average	3,137	3,902	3,731	4,165
Group B				
72	3,599	4,175	4,228	4,482
76	3,365	4,038	3,982	4,340
59	6,382	6,892	6,945	7,083
64	5,632	5,914	7,166	7,309
T200	2,802	3,418	1,613	1,774
T204	2,113	2,473	2,193	2,346
T193	2,891	3,440	3,020	3,231
T201	2,430	2,989	3,432	3,809
Average	3,652	4,167	4,072	4,297
Average Groups A and B	3,431	4,054	3,926	4,240

of lactation. These differences were not statistically significant.

Five cows which required several additional months to deplete due to large reserves were placed on supplementation in late lactation after they were depleted as shown by liver analysis. The lactation curve was plotted for eight weeks before supplementation and eight weeks following the addition of 50,000 IU of vitamin A to the diet. The average lactation curve for the five cows during these 16 weeks was essentially a straight line and did not change as a result of supplementation as is shown in the graph in Figure 1.

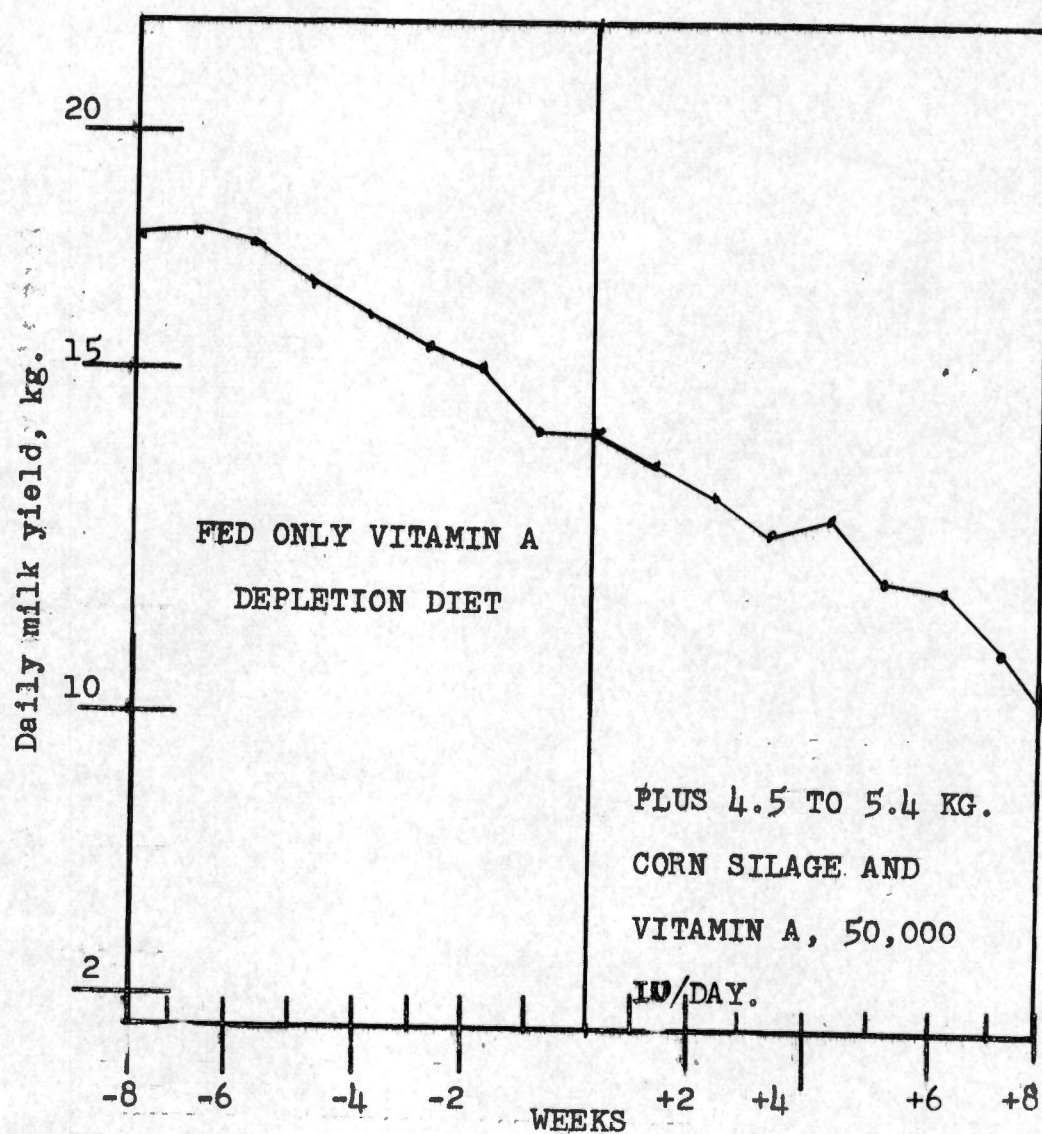


Figure 1. Average lactation curves of 5 cows before and after supplementary vitamin A depletion diets with carotene and vitamin A.

CHAPTER V

DISCUSSION

The preliminary period of this project was studied for the effect on plasma vitamin A and carotene of the depletion diet. The twins had been eating stored feed, and all but one of the other cows had had access to green forage. The depletion ration provided 7 to 8 mg. per day of carotene which was considerably lower than the N.R.C. (38) recommendation.

Effects of the depletion diet. The preliminary blood levels of vitamin A of 33.8 μ g. per 100 ml. for the barn fed twins and 37.9 μ g. per 100 ml. for the green forage group were within normal range for cattle on similar diets. The green forage group had twice the plasma concentrations of carotene as did the barn fed twins. The extra green forage (millet and orchard grass) accounted for the wide differences. To further illustrate the reserves in the green forage group, after 90 days of the depletion ration, vitamin A levels fell to 8.8 μ g. per 100 ml. in the barn fed group whereas the green forage group decreased only to 25.7 μ g. per 100 ml. Vitamin A in both groups fell rapidly at calving time as a result of the cows making colostrum and a changing of the blood proteins prior to parturition. The absence of carotene

in the diet produced a rapid depletion of carotene plasma levels in both groups. Carotene in the barn fed group was increased at calving because of the corn silage added to the ration two weeks before calving. It took twice as long to deplete the green forage group to levels below 6.0 μ g. of vitamin A per g. of liver as it did for the barn fed group.

A cow was considered depleted when the blood plasma level of vitamin A was below 5.0 μ g. per 100 ml. of plasma. The liver levels were more accurate in evaluating vitamin A status than were the blood levels. Several cows would show normal blood levels yet the liver levels would be quite low. If calving or infection altered the liver reserves after depletion the blood levels might not indicate deficiency. The cow could be borderline, just getting enough carotene for blood levels to be adequate, and at the same time the animal would become deficient quickly due to a lack of storage if an infection or calving lowered the level below body needs.

Effect on body functions. Prior to some of the early calvings and abortions there was an attack of scours in the cattle. The stools were black and viscous. This may have been a result of vitamin A deficiency. However, the diarrhea was observed in a number of animals which were

not depleted; so it may have been due to the character of the ration or to some infection in the barn. Another characteristic of the depleted cows was an edematous swelling in the legs that occurred on several animals that were low in liver and blood reserves. The hocks, legs, and feet were swollen most noticeably before calving when they were on the depletion diet. One cow was observed with typical nightblindness. Several tests were made of other depleted cows which did not show nightblindness. This symptom may not be common enough to be a good indicator of vitamin A deficiency. Hair of several of the experimental animals was rough and wiry and it was noted that it improved after the animal was placed on green forage following the termination of their experimental lactations.

Effect on mortality. Calf mortality was the most outstanding treatment effect noticed. The four deaths that were attributed to a deficiency of vitamin A occurred in dams that had low liver levels of vitamin A. This rate of mortality in the experimental group was high, but the herd experienced a 13.5 per cent rate of mortality. Several calves were found dead after birth from cows that had high levels of vitamin A. Most of the cows were biopsied one month before expected calving. Some of the cows calved

early but it seems unlikely that the biopsy precipitated the calving as in other cows milk yields on the day of biopsy were unaffected and a slaughtered cow showed only hairline marks on the liver. The four deaths and abortions show that vitamin A was not sufficient for reproduction and the cow has different requirements for reproduction and production as Kullman and Gullup (33) had concluded.

Effect on milk production. In the twin comparisons lactations were summarized for only the first 24 weeks because cows suffered attacks of mastitis or other health defects after 24 weeks. The pairs which milked beyond 24 weeks in comparable health tended to continue the trend of the early part of the lactation which indicated no advantage for the cows fed vitamin A supplement. It could not be demonstrated in any of the lactation data that there was any increase due to vitamin A as suggested by Copland (11).

The supplemented cows were getting 50,000 IU per day, which was twice the recommended amount for maintenance and about one-third more than recommended for maintenance plus reproduction recommendations. The depleted cows only received about one-sixth of the requirement during depletion and they could not have received over 60 mg. of carotene per day if all of the feed had been consumed. This would

be below the minimum requirement for maintenance and reproduction. It could be enough to keep the cows in a borderline position so no difference was noticed in milk yield, as day to day requirements for production were met.

The high grain-low roughage group did not show any difference in blood or liver vitamin A and both were normal. The cows fed high grain received three times the carotene requirement for maintenance, and the normal group was considerably higher. As both groups received an excess of carotene, it was not unexpected for the blood and liver vitamin A to be comparable.

Vitamin A may not have been the most single limiting factor in the lactation comparisons. All cows ate large amounts of soil after being turned to pasture. This action may indicate other factors in the ration were deficient, and these could have inhibited a response to supplementation with vitamin A. The milk production of the twins that had been depleted averaged better than their production the previous year on normal rations. The cows which had been fed green forage and required quite a period to deplete did not produce unfavorably on the depletion diet compared to the normal previous lactation. The differences were not significant, but the combined groups produced more milk on the experimental rations than on a previous normal ration.

Those cows that did not have calving troubles started producing at high levels, and for the healthy cattle persistency was good. The lactation curve was normal even though the cows were depleted before being supplemented. These observations would minimize the importance of unknown deficiency factors.

Liver biopsy. The method of biopsy that was used was successful in getting one and two gram samples of liver for analysis. Sampling took only about five minutes per cow. No observed side effects were noticed as a result of repeated samplings up to six times per cow at two month intervals. There were no deaths out of more than 160 biopsies performed, and no morbidity which could be definitely connected with the biopsy.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Thirty-five cows including identical twins and paired cows were used at the University of Tennessee Dairy Farms to determine the effect on milk production of adding vitamin A supplement to low carotene diets, typically high grain and low roughage rations.

The first phase of the experiment was preliminary to the lactation period in which the cattle were depleted of both blood and liver carotene and vitamin A. Low levels of vitamin A (3.1 $\mu\text{g.}$ per 100 ml. of plasma and 0.91 $\mu\text{g.}$ per g. of liver) were obtained in 90 days from cows fed barn stored feeds, but not from cows which had been fed green feed. The depletion diet was successful in reducing body stores of vitamin A, and carotene intakes were kept at subminimal levels for the complete lactation on some of the cows.

Milk production was not affected significantly by additions of vitamin A supplement at the rate of 50,000 IU per day to the feed source of hay, silage, and grain which provided about 35 $\mu\text{g.}$ of carotene per day. Except for poor lactations from cows which aborted, the vitamin A

deficient diet produced no adverse effects on milk production. Identical twins which were depleted and maintained on deficient diets produced equally as well as their mates given 50,000 IU of vitamin A daily. Fourteen cows which were fed the deficient diets for eight months or more produced as much milk as they had in previous lactations on adequate diets.

Calf mortality was 45.5 per cent in the experimental group compared to 13.5 per cent in the station herd. The abortion rate in the experimental cows was significantly higher ($P < 0.05$), a rate of 19.3 per cent over 2.3 per cent in the rest of the dairy herd. This emphasizes the need of adequate levels of vitamin A and carotene in the body for normal reproduction. Also this suggests that the requirements for reproduction are greater than for milk production.

Edema, nightblindness, rough hair coat, and scours, which are typical of vitamin A deficient animals, appeared in the depleted cattle indicating that the blood and liver levels were inadequate for the animal to function properly.

The biopsy technique was successful in obtaining a sample that could be used to check the vitamin A status of the cow.

The type of high grain diets used by commercial

dairymen will not necessarily affect the liver and blood vitamin A status unless exceptionally poor quality roughage must be used. Providing supplemental vitamin A for dairy animals is not expensive insurance if there is any doubt that reproduction and body function may be impaired by the absence of the vitamin or its precursor.



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