Vitamin A Studies
With Beef Cattle

A Summary of Experimental Studies
Conducted at Oklahoma State University, 1946-1959

By

L. S. POPE, F. H. BAKER and R. W. MACVICAR
Departments of Animal Husbandry and Biochemistry

ACKNOWLEDGEMENTS

Contents

Vitamin A Studies with Beef Cattle

A Summary of Experimental Studies
Conducted at Oklahoma State University, 1946-1959.

By

L. S. POPE, F. H. BAKER and R. W. MACVICAR[1]
Departments of Animal Husbandry and Biochemistry

From a practical standpoint, vitamin A is the most important vitamin required by beef cattle. Unlike other vitamins that are synthesized within the body or digestive tract of cattle, vitamin A must be supplied in the ration. Vitamin A is formed within the body of the bovine from several plant carotenoids (particularly beta-carotene) which are found widely distributed in natural feeds, especially in young, growing plants. Mature or weathered roughages are usually devoid of vitamin A potency, as are most grains and protein supplements. Because of the peculiar structure of the vitamin A molecule, it can be easily oxidized and thus lose its potency.

Cattle raisers in the Southwest have long been concerned about vitamin A deficiencies. This area is especially subject to long periods of drought and poor feed supplies. In recent years, the problem has been accentuated by the increase in fall calving. This means that the beef cow must nurse a calf for several months while subsisting on rations low in carotene. In some herds, small calf crops, weak calves, retained placentae, and other non-specific symptoms believed to be associated with vitamin A deficiences have been observed. Often, feeding good quality alfalfa hay (which supplies many nutrients other than carotene) is recommended. Many symptoms disappear with the advent of green grass.

Recently, synthetic forms of vitamin A have been used to fortify range supplements at relatively low cost; this has been recommended as "insurance" against a deficiency. Also, there has been an increase in fattening cattle under drylot conditions in large commercial feedlots

in the Southwest. Most fattening rations are based on grain sorghum, which is devoid of carotene, and non-legume roughage such as cotton-seed hulls. Such rations are often low in carotene, and pose a potential vitamin A problem.

In many respects, the role of vitamin A in the body is still obscure. Its practical importance under a wide variety of systems of beef production is still uncertain. For example, cattle may store sufficient quantities of this vitamin in the liver during good pasture seasons, for use later when dietary intake is insufficient. Research concerning vitamin A is hampered by lack of simple and reliable analytical methods, as well as the susceptibility to destruction of vitamin A and carotene in feeds and mineral mixes. There has been much difficulty in accurately assessing the true vitamin A status of the animal without knowledge of liver stores, often necessitating slaughter of the animal. Comparatively little research has been done with beef females, particularly on the amount of carotene required during reproduction in order to protect the newborn and suckling calf.

A series of experiments were undertaken at the Oklahoma Experiment Station from 1947 through 1958 to answer certain questions associated with the vitamin A nutrition of beef cattle under range and feed-lot conditions. This bulletin briefly summarizes the results of the numerous and varied experiments which were conducted, most of which have been published elsewhere in more complete form.

REVIEW OF LITERATURE

The Role of Vitamin A

Vitamin A is necessary for the maintenance and proper function of the epitheleal tissues that cover the surface of the body and its cavities, although its exact mode of action is poorly understood. Many of the outward symptoms of vitamin A deficiency result from a malfunction in those areas of the body covered by a layer of protective epitheleal tissue. These abnormalities may occur in the membranes of the eye, lungs, digestive organs, and reproductive tract. Vitamin A is also necessary for growth. It is required for adaptation from daylight to dark vision since it is a part of rhodopsin (visual purple) in the retina of the eye. In fact, a failure to discern objects in dim light is one of the earliest symptoms of vitamin A deficiency. This has been used in certain vitamin A studies to detect the early onset of a deficiency, or to establish requirements.

Symptoms of Vitamin A Deficiency

In order of probable occurrence in beef cattle, the following symptoms have been established by many workers: night blindness, muscular incoordination and weakness, unthriftiness, rough hair, slow growth, diarrhea, respiratory infections, reproductive disorders, and edema (1). These symptoms are by no means specific for a vitamin A deficiency, however, and may be complicated by other deficiencies. With young calves, weakness at birth, susceptibility to pneumonia and digestive tract infections, watering of the eyes, cloudiness of the cornea, protrusion or "bulging" of the eye followed by permanent blindness, and death have been observed. In feedlot cattle, "fainting" or convulsions when cattle are excited or worked rapidly, excessive watering of the eyes, edema of the brisket and forelegs (anascarca), and a thin, watery diarrhea have been noted. Vitamin A deficient cattle appear especially sensitive to high solar temperatures and have peculiar panting habits. Blindness and abnormally high cerebrospinal fluid pressure have been observed in the latter stages of a deficiency. Skeletal growth may be retarded, as in the closure of the foraman through which the optic nerve passes, thus causing blindness. Urinary calculi has not been shown to be associated with vitamin A deficiency.

Vitamin A deficiency in beef cows has been reported to be the cause of poor conception rates and retained placentae, although it is difficult to ascribe many cases reported in the literature to vitamin A alone. Prolonged lack of vitamin A may lead to abortion, and to calves which may be born blind or are weak and die shortly after birth. Colostrum from deficient beef cows may be low in vitamin A potency, and later milk may be too low to protect the suckling calf. Severe incoordination (staggering gait), blindness, and unthriftiness may result in both cows and their calves in an extreme deficiency.

Factors Associated with Vitamin A Metabolism and Storage

The liver is a massive storage organ for vitamin A. As much as 67 to 93 percent of the total vitamin A in the body may be found in the liver (2). Liver reserves are built up rapidly on green pasture and slowly released as necessary to maintain blood levels during periods of dietary deficiency. Intakes of vitamin A or carotene larger than necessary to meet the daily needs of the animal are stored in the liver for later use. Most of the symptoms of a vitamin A deficiency occur only when liver stores, and therefore blood levels, become extremely low. Prompt recovery without permanent injury is generally observed when ample carotene or vitamin A is included in the diet, except for the permanent

damage to the optic nerve or changes in skeletal and ocular development. Once the minimum requirement for vitamin A is met, the further addition of vitamin A to the ration gives no beneficial response. Blood levels are governed by dietary intake and liver stores—although the exact mechanism is poorly understood (3).

The carotenoids of green plants having the proper configuration are converted to vitamin A within the body, principally at the intestinal wall. The carotene intake from lush, rapidly-growing forage may greatly exceed the daily requirements of the grazing animal. Liver stores are dissipated more rapidly, percentagewise, when the vitamin A content of the liver is high than when liver stores are relatively low (4). Certain infections, particularly those involving liver tissue, may greatly influence the storage of vitamin A and its availability to the organism.

Dietary Requirements for Carotene and Vitamin A

Dietary requirements for carotene or vitamin A depend on age, sex, body weight, and stage of milk production or reproduction of the animal. Recommended allowances given in feeding standards are only estimates and are based largely on results of feeding tests in which given levels of carotene or vitamin A were fed and no symptoms of deficiency were observed. Early studies on the vitamin A or carotene requirements of cattle may have been confounded by previous liver stores of the animal and/or the use of carotene supplements which contained nutrients other than carotene.

The task of establishing minimum requirements for vitamin A is complicated by storage of vitamin A in the liver. Also, adipose tissue contains considerable quantities of carotene, particularly if the cattle have grazed pastures containing green forage. However, as pointed out later in this publication, the nutritional value of the carotene thus stored for cattle is somewhat doubtful.

It has been established that the carotene intake of the dam during the later part of gestation may influence vitamin A blood and liver levels of the newborn calf, although the difficulty in transfer of this vitamin across the placental membrane usually results in low levels in the newborn (5). Inefficient transport of vitamin A across the placental membrane is compensated for by large quantities of carotene and vitamin A in the colostrum. This high level is observed even in dams which have been on deficient rations for relatively long periods of time. Milk secreted later in lactation may contain such small amounts of carotene and vitamin A that the nutrition of the young suckling calf may be

adversely affected unless the dietary intake of the cow is maintained at an adequate level.

Minimum carotene requirements of young dairy calves up to 12 weeks of age appears to be approximately 30 mcg. per pound of body weight daily (6). Deficiency symptoms may be associated with lower levels of carotene and vitamin A in the blood of calves than in older cattle. From studies of blood composition, it appears that levels of 10 mcg. of vitamin A per 100 ml. may be ample in the calf, and levels as low as 7 to 8 mcg. per 100 ml. are only borderline (7). In contrast, levels as low as 15 to 17 mcg. per 100 ml. in mature cattle are considered dangerously low (8). Daily intakes of 45 to 90 mcg. carotene per pound body weight for dairy cows in longtime studies at the Oklahoma station, varying with breed, has been considered adequate for reproduction (9).

For mature beef cows, requirements for carotene are by no means agreed upon. N.R.C. allowances (10) call for 60 mg. per 1000 pounds of body weight for beef cows during gestation, and this agrees closely with those recommended by Morrison (11). According to N.R.C., this is believed adequate to "provide for normal growth and reproduction, and to build up and maintain a moderate storage of vitamin A".

For lactating beef cows, N.R.C. requirements call for 100 mg. per 1000 pounds, while Morrison suggests approximately 90 mg. The basis for these requirements are principally long-term drylot tests by U.S.D.A. workers (12). However, liver storage accumulated during a previous summer grazing season can greatly modify dietary needs the following winter and should be taken into consideration. In terms of the carotene in average quality alfalfa hay, using Morrison's data, a dietary requirement of 60 to 90 mg. carotene for a 1000-pound cow would require a daily intake of 5 to 8 pounds of such hay.

Carotene levels in the blood generally reflect dietary intake, and are much higher and more variable than plasma vitamin A levels. Vitamin A levels are believed to be much more valuable as indicators of nutritional status than plasma carotene. Many workers believe that levels of vitamin A must be maintained above 16 to 18 mg. per 100 ml., to prevent deficiency symptoms in the mature cow (13). Less is known about the minimum blood levels necessary to prevent deficiencies in beef calves at birth or shortly thereafter. Blood levels of vitamin A are believed by some authorities to be a poor indication of liver reserves until liver stores have been depleted to a low level. After this point is reached, a significant correlation may be observed between blood and liver vitamin A (15). A sharp drop in plasma carotene and vitamin A

seems to be associated with parturition and early lactation, for unknown reasons (14).

With growing and fattening cattle, minimum requirements for carotene in the ration appear to be more clearly defined. Using the onset of night blindness as the criterion for estimating carotene requirements, California workers (15) suggested intakes of approximately 13 to 15 mcg. carotene per pound of body weight. At this level, however, no liver storage resulted. They reported that carotene requirements seem to be proportional to body weight rather than to energy needs, and that to compensate for lower food consumption per unit of weight, larger animals require a higher percentage of carotene in the ration than smaller animals. They also reported a higher carotene requirement during periods of active growth, and that animals on sub-maintenance rations utilize liver vitamin A less rapidly than those on a super maintenance and otherwise complete ration.

Many aspects of vitamin A nutrition with beef cattle seem to warrant further study, *i.e.*, the effect of plane of nutrition on depletion of liver reserves and dietary needs, carotene requirements of cows nursing calves, length of time required to deplete liver stores of mature beef cows, and the effect of prolonged depletion on recovery of beef cattle.

EXPERIMENTAL STUDIES

Carotene Content of Native Range Grass in Oklahoma

Except for carotene from a supplemental feed such as alfalfa, range beef cattle derive most of their carotene from native forages. During the past twenty years, chemical analyses have been made at the Oklahoma station to determine the carotene content of major species of native grass throughout the year. The relationship between the carotene content of the forage and the nutritional status of cattle subsisting upon it makes it necessary to understand yearly fluctuations in plant carotene. Seasonal variation in carotene content of four major species of "tall" grasses are shown in Figure 1, and are typical of data obtained in normal seasons in many areas of the state (16).

Four species of native grass (big and little bluestem, Indian grass and switchgrass), including 3 samples of grass from each species, were taken at intervals over a 2-year period at the Lake Carl Blackwell range, west of Stillwater.
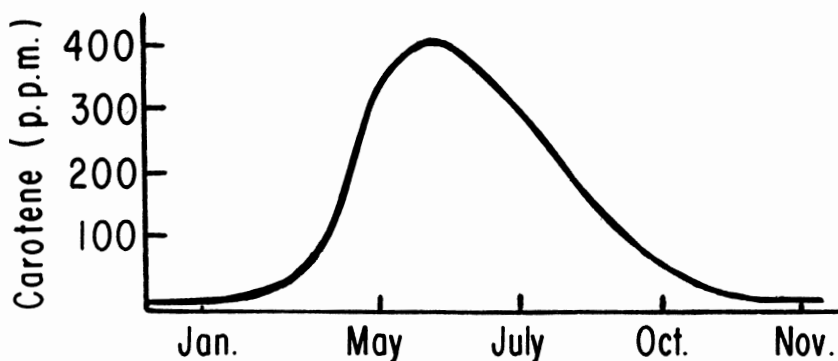
Figure 1.—Seasonal trends in the carotene content of native "tall" grass in Central Oklahoma.

Figure 1 shows a marked seasonal fluctuation, with high concentrations of carotene in the plant while rapid growth is being made. This peak in the curve is followed by a marked decline as the grass matures, followed by extremely low carotene levels in October and November and essentially no carotene in the forage during mid-winter, after weathering in the field. Similar seasonal changes have been observed in "short-grass" vegetation at the U.S. Southern Great Plains Field Station at Woodward (17). The period from October to March, therefore, is most critical to beef cattle and normally is the only season in which additional carotene or vitamin A may be needed.

Blood Levels of Carotene and Vitamin A of Cattle Under Range Conditions

Rapid increases in the carotene content of forage during the growing season are reflected in the plasma carotene and vitamin A levels of beef cows grazing native grass, as shown in Table 1. The values again represent data typical of many studies in which plasma carotene and vitamin A have been determined at this station (18).

The yearly pattern of plasma carotene corresponds directly to the carotene content of native grass (Figure 1). Plasma vitamin A increases at a slower rate, as has been observed in other studies. As native grass loses carotene in late summer and early fall, blood carotene also declines, while vitamin A remains relatively high. Plasma vitamin A reaches its lowest level in February to April of the following year. This critical period would correspond to the calving season in many herds.

**Table 1.—Plasma Carotene and Vitamin A Levels of Beef Cows Grazing
Native Grass Year-Long.**
(mcg. per 100 ml.)

Month	Plasma Carotene	Plasma Vitamin A
January	155	30.2
February	153	24.0
March	145	24.0
April	528	18.0
May	1097	29.4
June	1088	25.1
July	808	32.5
August	950	44.9
September	575	35.9
October	443	27.9
November	359	28.2
December	216	29.9

However, the carotene content of native grass is rapidly increasing by late April and May, thus the critical period may be a short one.

Since the calf is born with low stores of vitamin A at birth, the dietary carotene intake of the dam as it influences the blood picture of the calf is important. This association is shown in Table 2 (18).

The increase in daily intake of carotene for cows on native grass is reflected in increasing levels of plasma carotene, followed by vitamin A, in the calf. Again, the difference in the peaks of plasma carotene and vitamin A are apparent, with calves starting at lower levels than their dams in March and April. If a level of 16 to 20 mcg. per 100 ml. for cows and 10 to 12 mg. per 100 ml. for their calves is considered minimum, all cows and calves in these data were well above the minimum,

**Table 2.—Plasma Vitamin A Levels of Range Beef Cows and Their
Calves During the Grazing Season. Four-Year Average, 1946-1950.**
(mcg. per 100 ml.)

Month	Plasma Carotene		Plasma Vitamin A	
	Cows	Calves	Cows	Calves
March	145	16	24.0	18.1
April	528	129	18.0	14.9
May	1097	394	29.4	20.4
June	1088	543	25.1	21.2
August	950	629	44.9	26.7

or deficiency range. The greater importance of adequate carotene is obvious for fall-calving cows nursing calves on dry, weathered range grass during the winter.

Breed Differences in Carotene and Vitamin A Blood Levels

The variation in the amount of circulating carotene in the blood of different breeds of dairy cattle has long been recognized. That such a difference might occur among the three major beef breeds was invesigated at this station, using a small number of purebred females from the University herd (19). Part of the animals in each breed were maintained year-long on the Lake Carl Blackwell range area and part were taken, with their calves, to the purebred beef barn at the campus where they were fed more liberally. Plasma carotene was significantly affected by season, but was similar within age and breed. A general, though not significant, trend was noted for Shorthorns to be slightly higher than Angus and Herefords in plasma carotene and vitamin A. It was concluded that little difference exists among the three major breeds of beef cattle. Data obtained with one breed is applicable, therefore, to others without serious error.

Effect of Pasture vs. Drylot Conditions on Blood and Liver Levels of Carotene and Vitamin A

The intake of carotene from lush pasture and its effect on the vitamin A nutrition and reserves of beef cattle is further illustrated by a comparison of the plasma and liver vitamin A concentrations of two-year-old steers on pasture, in drylot, or on pasture followed by a finishing period in drylot (20). Twenty, two-year-old steers were divided into 4 lots and treated as follows, starting in late April:

Lot 1—Fed a fattening ration (shelled yellow corn, cottonseed cake and prairie hay) for 112 days in drylot.

Lot 2—On native grass pasture for 112 days.

Lot 3—In drylot on a fattening ration for 175 days.

Lot 4—On pasture for 112 days, followed by drylot for 63 days.

The results are shown in Table 3.

The data show the correlation between the carotene content of the forage and levels of plasma carotene, and to a lesser extent, vitamin A. Further, it illustrates the extent of the liver stores accumulated by steers on lush green pasture high in carotene, from April to August. Cattle

Table 3.—Effect of Drylot vs. Pasture on Terminal Plasma and Liver Vitamin A and Carotene Levels of Two-Year-Old Steers.

	Plasma (mcg./100 ml.)		Liver (mcg./gm.)	
	Carotene	Vitamin A	Carotene	Vitamin A
Lot 1—Drylot for 112 days	117.6	22.9	2.27	27.97
Lot 2—Pasture for 112 days	523.0	25.4	10.57	69.96
Lot 3—Drylot for 175 days	85.3	19.0	1.89	9.41
Lot 4—Pasture for 112 days; Drylot for 63 days	97.0	27.7	2.97	48.28

fed for 175 days in drylot continued to show a decline in plasma and liver levels of vitamin A and carotene. When removed from good pasture in late August and finished in drylot (Lot 4), previously accumulated stores were sufficient to hold plasma and liver vitamin A at a high level.

The Value of Carotene or Dehydrated Alfalfa Meal in Range Supplements

Because native grass is nearly devoid of carotene from November until growth starts again the following spring, range cattle must subsist on accumulated stores of carotene and vitamin A in their bodies, plus whatever carotene is available from annual grasses that appear in varying amounts in native range pastures nearly every winter. To study the possible benefit from adding carotene to range supplements, an experiment was conducted at the Lake Carl Blackwell range (21). Groups of yearling heifers, weaner heifer calves, and weaner steer calves were wintered on native range grass and minerals, *ad lib.*, plus cottonseed meal pellets—with or without additional carotene in the form of dehydrated alfalfa meal. Dehydrated alfalfa was added to certain supplements to supply 20 mg. of b-carotene per head daily, which is about two-thirds the recommended N.R.C. daily allowance. Weight gains during the wintering trial and plasma carotene and vitamin A levels of the cattle are shown in Table 4.

The data show an inconsistent pattern in terms of winter weight gains due to carotene supplementation. Yearling heifers of the supplemented group outgained their controls, whereas steer and heifer calves showed little difference due to supplementation. Plasma carotene and vitamin A levels were variable and may have been more influenced by pasture conditions than by supplemental carotene. Lowest levels of plasma carotene occurred during January to March, as was expected. Plasma vitamin A showed little effect from the additional carotene fed yearling heifers, and was higher only at the January and terminal

Table 4.—Effect of Supplemental Carotene on Wintering Beef Cattle.

	Yearling Heifers		Heifer Calves		Steer Calves	
	Control	Supple.*	Control	Supple.*	Control	Supple.*
Number/treatment	9	10	10	10	5	5
C. S. Pellets/Head Daily (lb.)	2.4	2.4	2.4	2.4	2.4	2.4
Avg. Winter Weight Gain (lb.)	—18	13	34	41	57	55
Plasma Carotene (mcg./100 ml.)						
11/23/48	134	129	216	209		
12/21/48	210	75	89	89		
1/22/49	71	161	40	74		
2/19/49	150	98	29	67		
3/28/49	223	596	195	230		
Plasma Vitamin A (mcg./100 ml.)						
11/23/48	14.8	17.9	19.5	16.7		
12/21/48	29.9	23.1	23.5	21.1		
1/22/49	22.7	22.1	18.4	25.7		
2/19/49	27.6	21.2	22.3	20.9		
3/28/49	17.5	16.1	9.6	19.7		

*20 mg. b-carotene per head daily in supplemented lots from dehydrated alfalfa meal.

bleedings for heifer calves. Low intakes of carotene in January and February were apparent from the drop in plasma levels in heifer calves.

In another wintering trial under range conditions, the effect of feeding cottonseed cake, soybean meal, cottonseed meal-urea pellets (25 percent of the nitrogen as urea), and cottonseed meal-milo-dehydrated alfalfa meal pellets were compared (22). Two-year-old Hereford steers (33 per lot) were wintered at the Lake Carl Blackwell range on tall native grass and fed each of the above supplements. The results are shown in Table 5.

Soybean meal alone gave greatest winter gain with these older, heavier steers. Cottonseed meal-milo-dehydrated alfalfa meal pellets improved performance over cottonseed meal alone and resulted in a marked increase in plasma carotene and vitamin A at the January, February, and March bleedings. Due to the design of the experiment, it is not possible to ascertain the effect of the carotene content of the cottonseed meal-milo-dehydrated alfalfa supplement on steer performance. Alfalfa is known to contain other nutritional factors, such as minerals favorable for rumen microorganisms in the breakdown of fibrous feeds in the paunch. However, the results do indicate an improvement in plasma carotene and vitamin A from feeding 1.0 pound of dehydrated alfalfa meal per head daily.

Table 5.—Effect of Different Range Supplements on Winter Gains and Carotene and Vitamin A Blood Levels of Two-Year-Old Steers (145 days).

	Cottonseed Meal	Soybean Meal	C. S. Meal Urea Pellets	Dehydrated Alfalfa Meal Pellets
Daily Supplement Fed (lb.)				
Cottonseed meal	3.03			1.24
Soybean meal		3.03		
Cottonseed-urea pellets			3.03	
Dehydrated alfalfa pellets				1.00
Milo				.75
Average Daily Gain (lb.)	—.22	+.27	—.11	+.07
Plasma Carotene (mcg./100 ml.)				
11/5/47	151	164	178	182
1/6/48	63	66	74	176
2/2/48	69	66	64	170
3/29/48	240	213	214	295
Plasma Vitamin A (mcg./100 ml.)				
1/6/48	24.3	21.1	24.7	32.8
2/2/48	22.4	21.5	21.7	28.8
3/29/48	21.3	23.4	23.4	27.0

Effect of Carotene Intake on Apparent Digestibility of Carotene

It has been demonstrated that carotene excretion by cattle is highly variable. Since the intake also fluctuates widely, it is of importance to know if the amount of carotene in the ration affects the amount excreted or its apparent digestibility. Two studies (23) were undertaken in which yearling steers were fed low-carotene rations and given varying amounts of carotene via gelatin capsule. Composite samples of feces were collected during a 10-day period by use of a harnass and collection bag, and were analyzed for carotene. The carotene administered daily varied from 4 to 200 times the minimum requirements proposed by Guilbert and Hart (24) of 30 mcg. per kilogram of body weight. It was felt that this range would take in most of the variations occuring in carotene intake under practical conditions.

Average data from the second trial involving 6 yearling steers, with each steer given 4, 8, 16, and 32 times minimum requirements of carotene, are shown in Table 6.

In both trials, a remarkably consistent percentage excretion of carotene was observed, despite widely varying intakes. There was a ten-

Table 6.—Carotene Balance Data from Yearling Steers Receiving Varying Carotene Intakes.

Carotene Allowance	Number of Steers	Av. Daily Carotene Intake (mg.)	Av. Daily Carotene Excreted (mg.)	Apparent Digestibility of Carotene (percent)
4 × M.R.*	6	36.0	21.5	40.5
8 × M.R.	6	70.7	43.7	38.1
16 × M.R.	5	137.2	83.7	39.5
32 × M.R.	5	282.7	171.2	39.2

*Times minimum requirement of 30 mcg./kgm. body weight.

dency for an increase in percent carotene excreted (thus a decrease in apparent digestibility) as the trial progressed. It seems possible that, to some degree, the utilization or absorption of dietary carotene may be related to total body stores of the animal at any particular time. From these limited studies, it would appear that cattle digest or absorb carotene in approximately the same amount, percentagewise, over a rather wide range of intakes. Thus, large quantities of carotene in the diet do not appear to depress availability of carotene to the animal. The data also suggest that other factors within the digestive tract may cause destruction of a certain percentage of carotene in the gut, regardless of its concentration in the ration. If so, carotene would appear to differ from many other nutrients in its pattern of availability.

The Liver Biopsy Technique and its Use in Vitamin A Studies

Blood levels of carotene and vitamin A, while relatively easy to obtain, may not reflect the true vitamin A status of the animal. The liver is known to be the chief storage organ for vitamin A in the animal body (24). However, in many vitamin A studies it was not possible to obtain data on liver levels, since this necessitated slaughter of the animal. The need for a technique which would allow routine collection of liver data from the live animal, in quantities sufficient for chemical analysis, was considered imperative.

A technique developed early in the course of these vitamin A studies permitted the collection of samples of liver tissue, 1.5 to 2.5 gm. in size (25). The liver of the adult bovine is found almost entirely on the right side, with it parietal surface convex and in contact with the right costal portion of the diaphragm. The area between the last two ribs on the right side can be opened to permit rather easy access to the surface of the organ. A point in the twelfth intercostal space, 8 to 10

inches from the midline, about one-third of the distance down from the dorsal midline to the costal arch was selected as the best site for an incision. In the calf, the liver may not have rotated into its adult position, necessitating the laparotomy technique; further, the intercostal space does not give enough room to permit an easy operation. As the calf grows older, its rumen and reticulum increase in size and weight, tending to rotate the liver to the right and into its adult position.

Cattle were successfully biopsied by restraining them in a squeeze or chute. The area of the incision was anesthetized. Special instruments consisted of a large rumen trocar with a blunt, diamond-shaped point; a cannula with beveled edge so as to fit smoothly over the hub of the trocar; and a biopsy instrument to excise a piece of tissue about one-half inch in diameter and one to two inches in depth from the liver surface, by means of a recessed knife which could be triggered to cut off the liver section. The appropriate surface of the liver was located with the aid of a specially constructed flashlight (see Figures 2 and 3).

Figure 2.—Biopsy equipment and sample of liver.
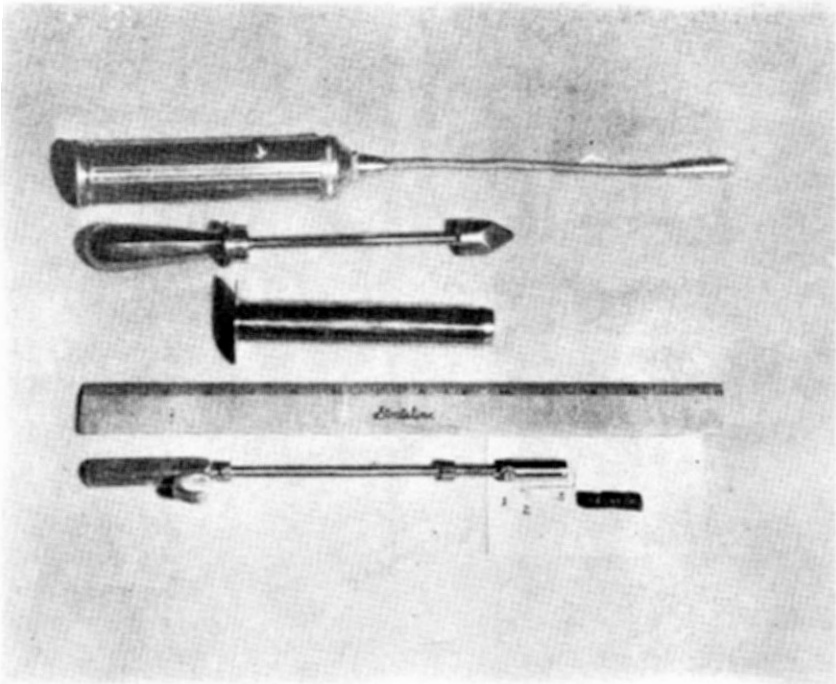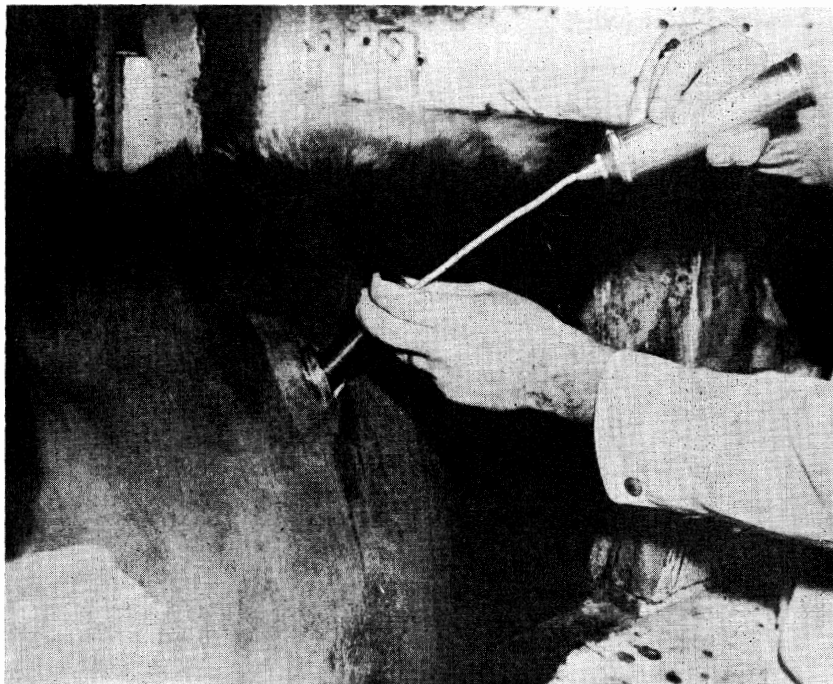
Figure 3.—Equipment was designed to obtain a small sample of liver tissue. After application of a local anesthetic, the trocar could be inserted through the right side and the liver surface located by means of a special flashlight.

This technique was used successfully with little injury or death loss in the collection of more than 800 samples from cattle at this station. Other biopsy techniques have been reported by Garner, Loosmore and Allcroft, and Dyer *et al.* (26). The advantage of the technique described herein would be in the relatively large size of the sample obtained, and the homogeneity of the sample with surrounding liver tissue.

Few adverse post-operative effects were noted. Infection in the area of the operation was usually prevented by applying antibiotic before the incision was sutured. Considerable hemorrhage was found to develop upon the removal of the liver sample in a few instances, and in several cases death resulted. This was caused by sampling in proximity to a major artery. Occasional swelling and infection were noted, but in general, the effects of the operation were minor.

Liver Stores of Vitamin A in Cattle

Successful development of the liver biopsy technique made it possible to estimate liver reserves of vitamin A in the living animal and correlate these with blood data. Repeated samples could be taken from the same animal to follow the depletion or repletion of liver stores. Several problems inherent to the biopsy technique warranted further study, such as: (a) effect of repeated sampling on liver tissue near the biopsy site and on the animal, (b) validity of this technique in estimating liver vitamin A concentration, and (c) variations in vitamin A reserves according to location in the liver.

Repeated biopsies were made on individual cows at monthly intervals for 12 months in certain studies. Females were biopsied shortly before and after calving, with little apparent affect. Steers in metabolism trials were successfully biopsied shortly before being placed in stalls. Young calves appeared easiest to sample by using the laparotomy technique. So great is the recuperative ability of liver tissue that repeated sampling had little effect on the lobe of the liver from which the sample was removed, as evidenced by postmortem examination of livers from cattle which had undergone repeated biopsies.

Liver samples were taken at monthly intervals for a 12-month period from two cows and two steers (27). The animals were then slaughtered and their livers examined for tissue damage. A very limited amount of scar tissue was observed, with only a few adhesions. This was not believed to be sufficient to affect the integrity or function of liver tissue in the region where the samples were taken.

In another study, liver samples were taken periodically (total of 17 samples) from Hereford females over a 43-month period during a depletion study. No ill effects were noted other than rather severe adhesions of the liver and peritoneum, and marked development of superficial scar tissue near the point of incision, making subsequent biopsies more difficult. Each cow completed two gestation-lactation periods and conceived 3 times during the study. Some local infection was noted in isolated cases, but this could be reduced by performing the operation under aseptic conditions.

Since a biopsy sample is from only a small area of one lobe of the liver, it seemed necessary to determine if this truly reflected vitamin A concentration in the entire liver. Only a few studies have been made on the distribution of vitamin A in bovine liver. To gain information on the homogeneity of liver stores, four livers from freshly-slaughtered cattle were obtained (27). Samples (one gram in size) were taken in

duplicate from 6 locations in various parts of the liver and representing each lobe. The mean vitamin A values and standard error of the 12 samples from each liver were as follows:

Liver Sample	Vitamin A	Standard Error
	(mcg. per gm., wet basis)	
A	14.3	.72
B	8.2	.53
C	66.3	2.53
D	12.2	1.04

Using Bartlett's test for homogeneity of variances from which the standard errors were calculated, it appeared that there was little chance that the variances were from the same population. The standard error appeared to increase with the vitamin A concentration of the liver. Although there was not enough data to show which location in the liver was most reliable in measuring vitamin A stores, it appeared that the location from which the biopsy samples were taken (dorsal lobe) was as representative as any other site. The average deviation from the average analysis of vitamin A content was 15 percent. Hence, a sample taken from the liver via the biopsy technique, should not vary more than 15 percent from the average vitamin A content per gram of the entire liver. However, on the average, a maximum error of 30 percent could occur.

A further study was undertaken to determine the accuracy and precision with which the biopsy sample estimates the vitamin A content of the entire liver (28). In two experiments, a total of 18 cow livers were obtained from a packing plant at time of slaughter and frozen until analyses could be made. After thawing, two samples were taken with the biopsy instrument from the area of the dorsal lobe which approximated the actual field of sampling in the intact animal. The remainder of the liver was then finely ground and mixed, and duplicate samples were taken for analyses. Vitamin A was then determined in duplicate on each sample, making a total of 8 determinations on each liver.

There was considerable variation in the vitamin A content between duplicate samples. The livers proved to be unusually rich in vitamin A, in contrast to that observed in most experimental cattle. Large errors in sampling and analysis appear to have occurred. The biopsy samples and the random samples of ground liver had mean vitamin A contents of 572 and 461 mcg. per gram, dry matter, respectively. The biopsy samples, therefore, contained 111 mcg. more vitamin A than the random samples,

or a difference of 24 percent. Statistical analysis revealed that for each method, the majority of the variance observed between analyses was accounted for by differences in vitamin A content between livers— which would be anticipated. The error mean square for all liver data was used to set confidence limits on means of the 18 livers. From this, a standard error of the difference ($t_0 5$) was found to be 111 ± 26 mcg. of vitamin A. Extrapolating from this data, a mean square for interaction was used as the more appropriate term, giving a standard error of the difference of 111 ± 57 mcg.

It is possible that as the vitamin A concentration of the liver increases, differential storage may occur in certain locations. Also, errors in sampling and chemical analysis are undoubtedly magnified at higher levels of vitamin A concentration. Ground and mixed samples appeared a somewhat more precise estimate of liver vitamin A as indicated by a lower error mean square. It is possible that some stratification may occur from dorsal to ventral surface of the liver. This component analysis would indicate that the duplicate biopsy samples taken from the dorsal lobe were as precise a measure of the vitamin A concentration of that area as the ground samples were of the entire liver. When working with lower concentrations of vitamin A, less variation would be expected.

In further studies, a regression of liver weight on body weight of steers was determined (27). Such a relationship would make it possible to estimate liver weight from live animal weight. Hence, the total vitamin A liver reserve might be estimated from a single biopsy sample. Data for this study was obtained at a packing plant where steers from feedlot experiments were slaughtered. Data were obtained on the livers from 88 steers. Regression coefficients for the two years were nearly the same. In each of the two trials in which data were collected, for each 100 pounds change in body weight of the steers, the liver changed by 1.01 and 0.891 pounds, respectively. Standard deviations from regression were nearly identical for the two years. The average liver weight was found to be 1.067 percent of body weight, with a range of ± 0.004 percent. It is recognized that such an estimate may not be accurate with other classes of cattle, *i.e.*, thin cows or suckling calves.

Relationship of Plasma Vitamin A and Carotene to Liver Vitamin A

Plasma levels of carotene and vitamin A are relatively easy to determine and are widely used to estimate the vitamin A status of the animal. Data obtained at this station and elsewhere suggest that a wide range in plasma carotene may occur due to fluctuations in intake of

the animal. Plasma and liver carotene or vitamin A levels may show less variation. The amount of vitamin A stored in the liver is an important factor in assessing the nutritional status of the animal and its dietary needs. Since the liver biopsy technique described is somewhat difficult and time-consuming, and therefore not suitable for use in the field or with large numbers, the possibility of relationship between plasma and liver vitamin A levels was investigated.

Data on the relationship of plasma vitamin A and carotene with liver vitamin A was studied using blood or liver samples obtained from 56 pregnant cows placed on rations devoid of carotene about six months prepartum (29). Also included were blood and liver values from these cows and their calves at parturition and at the end of three months' lactation. Data were obtained from 176 steers fattened in drylot on different carotene intakes. Vitamin A values from blood samples taken at the termination of the experiment were compared with liver samples taken at slaughter. Individual variation among cows and steers during one experiment was studied by ranking the animals, within lots, at each bleeding, according to plasma vitamin A values. Repeatability estimates were conducted on these data.

Data presented in Table 7 show a highly significant correlation ($P<.01$) between plasma carotene and liver vitamin A in samples from

Table 7.—Relationships of Plasma Vitamin A and Carotene to Liver Vitamin A in Different Age Groups of Beef Cattle.

	Number of Cattle	Mean Values for—			Correlation of Liver Vitamin A with—	
		Plasma Carotene (mcg./ 100 ml.)	Plasma Vitamin A (mcg./ 100 ml.)	Liver Vitamin A (mcg./gm. D.M.)	Plasma Vitamin A	Plasma Carotene
COWS						
Fall after summer grass	56	121.5	33.6	290.8	.038	.609**
After 6 mo. on depletion rations	55	24.4	12.7	56.3	.512**	−.219
SUCKLING CALVES						
Nursing dams in drylot	48	20.7	8.1	4.9	.340*	−.018
FATTENING STEERS ON DIFFERENT INTAKES OF CAROTENE						
Low carotene basal	30	43.4	15.7	2.3	.163	.312
+ 1.2 lbs. alfalfa hay	29	70.0	23.9	4.3	.146	.219
+ .8 lbs. dehyd. alf. meal	29	70.2	21.9	4.9	.464*	.157
+ 2.4 lbs. alf. hay	30	78.1	26.4	6.1	−.018	.064
+ 1.6 lbs. dehyd. alf. meal	30	87.7	25.1	9.2	.322	.339
+ 3.2 lbs. dehyd. alf. meal	28	124.5	28.2	10.6	.393*	.379*

*Significant at the 5 percent level of probability.
**Significant at the 1 percent level of probability.

beef cows at the end of the summer grazing season—a long period of high carotene intake. In contrast, low negative correlations existed after a depletion period in drylot, or with young suckling calves nursing dams subsisting on relatively low carotene intakes. Positive correlations were observed with fattening steers, but these assumed significance only when steers were receiving relatively high carotene intakes, *i.e.,* more than 3 pounds of dehydrated alfalfa meal per head daily for 165 days.

Plasma and liver vitamin A were positively correlated to a significant extent (P<.05) only after beef cows had been depleted for a relatively long period, or with young calves nursing dams in drylot. With fattening steers, except for one lot, a significant positive correlation (P<.05) was observed only at the highest level of carotene intake.

It appears that blood carotene may be significantly associated with liver vitamin A after a long period of relatively high carotene intake. Vitamin A in the liver and plasma are associated to a high degree only after a depletion period, or with long-fed steers on high carotene intakes. Thus the past history of the animal must be known if plasma levels of carotene or vitamin A are used to predict liver stores. At best, the correlations show a rather wide degree of error from influences other than those accounted for by the association of the two factors.

Rousseau *et al.* (30) studied the relationship of plasma and liver vitamin A in 97 dairy calves fed depletion diets vs. minimum levels of carotene. When plasma vitamin A and liver vitamin A were expressed as logarithms, a positive linear relationship between the two variables was found, with applicable limits for prediction of approximately 3.6 and 25.8 mg. per 100 ml. of plasma. Thomas and Moore (31) found that the plasma vitamin A of dairy calves reflected carotene intake up to a level of about four times the minimum requirements. In rachitic calves and calves fed synthetic diets, plasma vitamin A gave no reliable estimate of carotene intake or liver stores. Frey *et al.* (32) , working with Hereford steers, found no simple relationship between serum levels and hepatic stores of vitamin A, and suggested that body mechanisms controlling serum levels may be different from those controlling hepatic stores.

There was no apparent trend for individual cattle to maintain high or low plasma vitamin A levels. Repeatability estimates, within lots for each class were —0.25 for cows and 0.06 for steers (29) . Thus allotting experimental cattle according to initial levels of plasma vitamin A would be of little or no value in removing variation at subsequent bleedings.

Utilization of Intravenously-Administered Carotene by Calves and Lambs

Cattle normally circulate large amounts of b-carotene in the blood and much is deposited in the liver, kidney, and fatty tissues. Sheep, on the other hand, are known to convert most of the carotene ingested to vitamin A; practically none is found in the blood stream.

It is commonly believed that most of the carotene in the diet is converted into vitamin A at the intestinal wall (33). This is borne out by studies in which sections of intestinal wall tissue have been shown to convert b-carotene into vitamin A *in vitro*. The possibility of carotene conversion at other sites in the body has not been adequately demonstrated.

Using a prepartion of carotene dispersed in water with "Tween 40",* an attempt was made to show differences between cattle and sheep in ability to convert carotene to vitamin A within the body (34). Single doses were given intravenously to 8 yearling wethers which had been on a low-carotene depletion diet for 8 weeks, and similarly to 7 four-month-old Hereford calves suckling dams which had been on low-carotene rations. The calves were at a critical point in vitamin A nutrition as evidenced by plasma levels and deficiency symptoms.

Blood samples collected prior to carotene administration and at frequent intervals for 10 days thereafter, were analyzed for carotene and vitamin A. The dramatic difference in response between species when carotene was administered at approximately 7 times the minimum daily requirement is shown in Figure 4.

In wethers, the increase in plasma vitamin A after carotene injection was highly significant; the mean values at 3, 6, 9, and 12 hours after injection being approximately twice that of the initial value. Blood carotene, after a sharp initial rise, fell rapidly during the first 6 hours after injection, then declined slowly for the remaining 10 days to a level approaching zero.

In calves, a different picture appeared. Carotene injection caused no significant increase in plasma vitamin A, while plasma carotene rose sharply and then declined to a level near 85 mcg. per 100 ml. This was maintained for 2 to 24 hours following injection, and then declined slowly. No liver storage of vitamin A in calves could be determined when liver samples taken one month before treatment and three days after

---

*Crystalline carotene, supplied by Barnett Laboratories, Long Beach, Calif., was dispersed with polyexthylene 20 sorbitan monopalmitate (Tween 40).
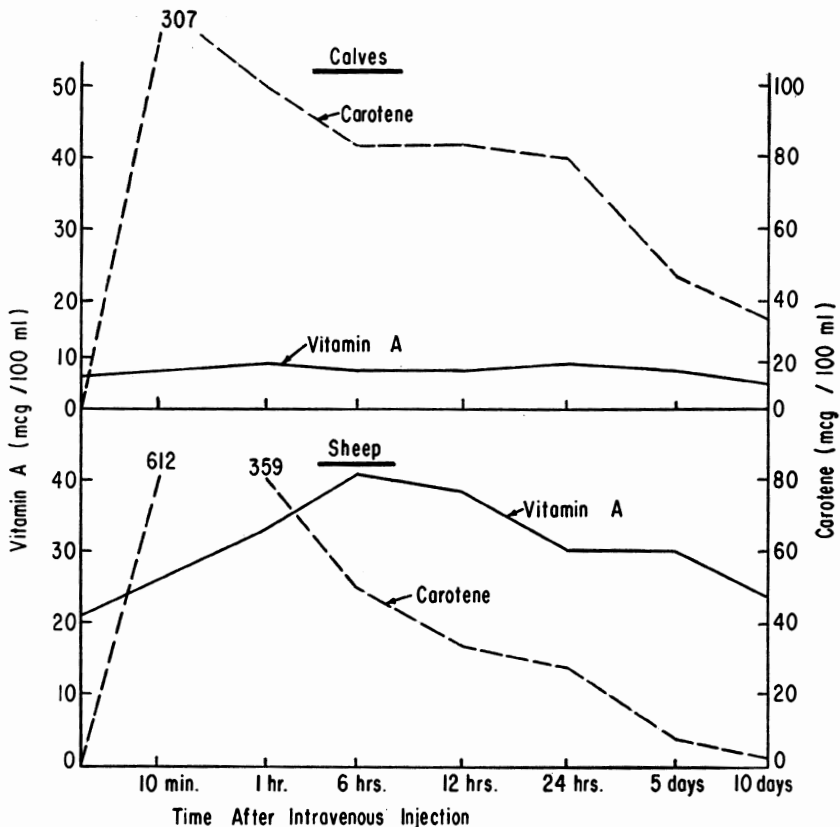
Figure 4.—Plasma carotene and vitamin A of calves and sheep after intravenous injection of carotene.

injection were compared. Advanced vitamin A deficiency symptoms (diarrhea, muscular incoordination, and partial blindness) in certain of the calves were not relieved; in fact, they appeared to intensify during the period following injection. However, in other studies with dairy calves at this station, vitamin A deficiency symptoms could be reversed to some extent by carotene injections (35). Other workers have observed no apparent conversion of carotene given calves and swine subcutaneously or intravenously (36). The form in which carotene is administered may be a possible explanation of the difference in results obtained.

These data indicate a wide difference between cattle and sheep in ability to convert carotene into vitamin A. It has been suggested that

animals which have carotene normally circulating in the blood are less efficient converters (at the intestinal wall site) than are animals without circulating carotene. While this may be true, data from this study suggest that in certain species carotene may be converted into vitamin A at sites other than the intestinal wall; thus the blood remains relatively free of carotene. Further work is in progress at this station to elucidate the site (s) of such conversion. This study raises considerable question as to the nutritional significance to cattle of the carotene deposited in large amounts in adipose tissue during the lush pasture season.

Effect of Dietary Phosphorus on Carotene Utilization

An examination of several years' data with range beef cattle revealed a tendency for phosphorus intake and plasma carotene to be inversely related, *i.e.,* where phosphorus intakes were suboptimal, plasma carotene levels tended to rise. Since phosphorus has many functions in the metabolism of nutrients, it was postulated that a deficiency of phosphorus might depress carotene conversion, thereby resulting in increased levels of plasma carotene.

An analysis of blood data from range beef cows at the Wilburton Experiment Station (southeastern Oklahoma), where phosphorus is known to be deficient in the native grass forage, showed that increasing levels of dietary phosphorus (as dicalcium phosphate) resulted in lower plasma carotene levels (46). Unfortunately, no plasma vitamin A values were obtained.

More detailed experiments under controlled conditions were undertaken at the Stillwater station (47). Lambs were depleted of phosphorus as indicated by plasma levels, and were then supplemented with varying amounts of phosphorus and carotene. Similar studies were undertaken with beef steers. While not significant, a trend for an inverse relationship between plasma carotene and dietary phosphorus was noted.

Although more research is needed, such a relationship would emphasize the importance of adequate carotene for cattle on phosphorus-deficient range. Conversely, adequate phosphorus for cattle on low carotene rations is also indicated. Phosphorus deficient areas are widely prevalent in the Southwest. The phosphorus problem is often more acute during drought, and this might conceivably increase the already existing danger of vitamin A deficiency.

Effect of Ration on Carotene and Vitamin A Levels in Blood and Milk of Hereford Cows and the Blood of Their Calves

Extensive studies have shown that under range conditions, low levels of plasma carotene and vitamin A may occur among individuals with no gross or outward symptoms of a deficiency. Average plasma levels of carotene as low as 57 mcg. per 100 ml., and vitamin A as low as 10.9 ml., have been observed. The lowest levels under range conditions at this station have occurred during March when dietary intake was negligible and the cows had recently calved and were nursing calves. Payne and Kingman (37) reported that beef heifers with less than 97 mcg. carotene per 100 ml. showed symptoms of deficiency. Davis and Madsen (38), working with Shorthorn heifers, postulated the critical level was 25 mcg. per 100 ml. for plasma carotene and 16 mcg. for vitamin A.

To establish a pattern for plasma vitamin A and carotene at parturition and for one month thereafter, an experiment was undertaken with nine mature Hereford cows fed under carefully controlled conditions and sampled frequently before and after calving (39). The cows were confined to stalls and were fed 3 pounds of cottonseed cake per head daily plus mature, weathered, range grass hay (cut in December and containing less than 5 mcg. b-carotene per gram). Blood samples were taken from the cows at weekly intervals until parturition, daily thereafter for 10 days, and on the 20th and 30th day post-partum. The cows were on the low carotene rations for 75 to 100 days prior to calving. Blood samples were taken from the calves at birth before they had opportunity to nurse, every 4 hours for 24 hours, daily for 9 days, and at 20 and 30 days of age. Samples of colostrum were taken at parturition and milk samples were taken daily for 4 days, and at 10, 20, and 30 days post-partum. All cows received the low carotene basal plus 5.0 pounds alfalfa hay per head daily for Lot 1 as a source of carotene, 2.5 pounds alfalfa hay for Lot 2, and none for Lot 3. The carotene intakes were calculated to be 106, 75 and 38 mg. per head daily during the 30-day lactation period.

The results of this experiment are shown in Table 8. Daily blood determinations indicated a drop in carotene after parturition, reaching a low point on the 3rd to 5th days. Other workers have noted a decrease in plasma carotene and vitamin A during the last few weeks of pregnancy and early lactation in dairy cows (14). The reason for this phenomenon is not known.

Plasma vitamin A, on the other hand, failed to show as marked a decrease as carotene during the first week after calving. The lowest

Table 8.—Plasma Carotene and Vitamin A of Cows and Calves on Three Carotene Intakes for 30 Days Postpartum.
(mcg. per 100 ml.)

	Parturition	Number of Days Postpartal			
		1	10	20	30
COWS					
Plasma carotene					
Lot 1	96	83	68	81	90
Lot 2	59	53	61	60	65
Lot 3	42	39	36	47	44
Plasma vitamin A					
Lot 1	18.3	15.7	14.7	13.4	16.0
Lot 2	11.6	16.1	16.2	12.9	17.2
Lot 3	12.2	12.6	7.8	13.3	13.3
CALVES					
Plasma carotene					
Lot 1	3.4	8.7	4.7	15.9	21.0
Lot 2	0.0	4.0	4.0	8.2	7.5
Lot 3	0.0	0.2	4.0	5.0	4.0
Plasma vitamin A					
Lot 1	4.2	11.5	10.0	10.4	10.1
Lot 2	4.3	9.2	14.0	12.7	8.2
Lot 3	2.3	9.4	12.6	6.0	7.5
Colostrum and milk vitamin A					
Lot 1	114.2	63.5	10.7	7.0	4.4
Lot 2	37.3	35.4	9.4	14.6	13.3
Lot 3	96.8	54.2	9.8	10.0	8.4

*Lot 1 received 107 mg.; Lot 2, 75 mg.; and Lot 3, 37 mg./head daily of carotene from ration.

values for plasma vitamin A were found within the first 20 days following parturition. All cows showed increased levels by the 30th day. Nearly all calves were born with little or no plasma carotene, but showed increases after the first day. Plasma carotene levels were somewhat higher for calves from supplemented dams of Lots 1 and 2, at 20 days of age.

Vitamin A in the plasma of cows of the two supplemented groups (Lots 1 and 2) failed to show a consistent advantage over the basal group (Lot 3). Carotene intakes varying from 37 to 106 mg. per head daily for 30 days after calving had little effect on plasma vitamin A levels at the termination of the test.

With calves, all vitamin A values were low at birth, but improved rapidly within 24 hours to levels of about 10 mcg. per 100 ml. These did not vary thereafter for Lot 1 calves, but tended to rise and then de-

cline for calves in Lots 2 and 3. At 30 days of age, both plasma carotene and vitamin A of the calves reflected the carotene intake of their dams. None of the calves, however, showed symptoms of vitamin A deficiency and the cows appeared normal throughout the trial. Colostrum vitamin A levels dropped rapidly by the end of the first day, and by the 10th day, the vitamin A potency of milk from cows of all lots was essentially the same. No correlation could be found between milk samples taken at 10, 20, and 30 days post-partum and blood levels of vitamin A of the calves. Errors in completely milking the quarter and in sampling may have confounded the results.

The results show no ill effects on cows or calves, even though blood carotene and vitamin A were in a range considered sub-optimal. Plasma carotene levels of the cows and their calves were positively correlated with carotene intake. Both plasma carotene and vitamin A declined shortly after parturition, and then rose during the subsequent 30-day lactation period. The plasma carotene and vitamin A level of the calves increased rapidly during the first few days and then decreased to a level which was generally maintained throughout the first month of life.

Despite the relatively low blood levels of carotene and vitamin A observed in this study, all cows produced normal calves and raised them successfully. Hence, seasonal declines in plasma vitamin A and carotene may occur without the appearance of clinical symptoms of a deficiency in cows or their calves.

Long-Time Depletion Studies with Beef Cows

Few long-time studies have been conducted with beef cattle to show the rate of depletion of liver stores. Guilbert and Hart reported that nine to twenty months were required to deplete 12 beef steers that had been given ample opportunity to store vitamin A (1). Jones and associates at the Texas station have reported extensive data with calves and year-lings (1). Using night blindness as the criterion, time of depletion varied from 178 days on the average with yearlings, to 83 to 90 days with calves. Little research has been done with mature beef cows.

In November, 1950, an experiment was initiated with 4 weaner heifer calves which had nursed their dams on native grass the previous summer (40). The objective was to study the rate of depletion of liver vitamin A and carotene. The calves were approximately 8 months of age when weaned and were maintained in drylot for 41 months, at which time gross symptoms of vitamin A deficiency were observed. The rations fed contained varying combinations of cottonseed meal, milo,

oats, dried beet pulp, cottonseed hulls, wheat straw, and weathered range grass hay harvested in the late fall. All feeds were either devoid of or extremely low in carotene content upon chemical analysis. Salt and a mineral mix of bone meal, limestone, and salt were made available during the test. The rations were calculated to meet N.R.C. allowances for all known requirements during growth, gestation, and lactation, with the exception of carotene.

Blood and liver samples were taken at monthly intervals for 8 months, followed by samples taken at approximately 6-month intervals for the remainder of the experiment. The heifers were bred in the summer of 1951, and re-bred in 1952 and 1953. They conceived readily when placed with the bull. The average blood and liver vitamin A values obtained in the succession of samples are shown graphically in Figure 5.

Figure 5.—Changes in plasma vitamin A and carotene and liver vitamin A of beef cows maintained 41 months on low carotene intakes.
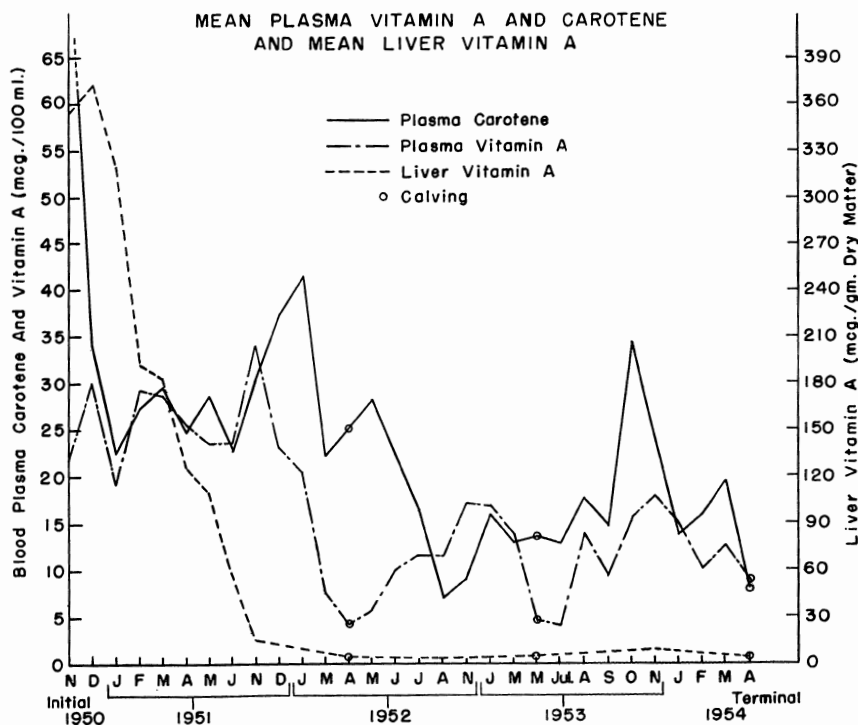
The females exhibited remarkable ability to grow normally and carry on reproductive functions, even when severely depleted of liver vitamin A. The vitamin A requirements of beef cows for maintenance and reproduction appeared to be quite low. This is evident from the extremely low carotene contents of the feed used and the consistently low vitamin A nutrition of the female after initial depletion of liver stores (which occurred at the end of 12 months in drylot).

The cows conceived successfully three times. Two live calves were born to each cow during first and second pregnancies, with little difficulty at calving and only one case of a retained placenta. At approximately 6 to 7 months of the third pregnancy, all 4 cows aborted. The aborted fetuses showed pathological changes characteristic of those reported by other researchers for vitamin A deficiency (1). There was extensive edema and disintegration of the fetus, and placental membranes showed marked necrotic changes (See Figure 6).

Some muscular incoordination, weakness, lacrimation, and diarrhea were observed in the cows shortly after abortion. Massive intravenous and oral doses of vitamin A were given the cows after abortion and recovery to apparently normal condition followed rapidly. Thereafter, the cows were placed on lush, native pasture and exposed to a bull. All conceived readily and calved the following spring. However, one calf produced from these matings was blind from birth and autopsy revealed characteristic degeneration of the optic nerve, as often seen in advanced stages of vitamin A deficiency. Calves from other cows were normal, and 2 of the cows were used for several years in other range experiments.

Although all calves produced by the cows during the first and second pregnancies on low carotene rations were normal at birth, they developed varying degrees of incoordination, weakness, diarrhea, lacrimation, bulging eyes, nyctalopia, and respiratory infections, within six to eight weeks of age. Prompt supplementation of the calves with high potency fish liver oil caused rapid improvement, with the exception of several incidents of complete blindness which had progressed too far before treatment was initiated. Since later milk was almost devoid of vitamin A potency, rapid onset of symptoms of a deficiency occurred in calves within three or four weeks of age.

The results of these and foregoing studies demonstrate that beef females may store up enough vitamin A reserve in the liver to meet their requirements for maintenance for long periods. Beef cows in a relatively poor state of vitamin A nutrition conceived without difficulty and

Figure 6.—Abortion at about 3 months prepartum was observed in beef cows in low vitamin A nutrition, but only after a long period (over 40 months) on low carotene rations. Note the edematous and decomposed fetus.

maintained pregnancy up to the time vitamin A reserves were almost completely exhausted. Calves from cows deficient in vitamin A, however, suffered from a deficiency at an early age unless supplemented. It appears that long periods of low vitamin A nutrition, even to the point of severe deficiency symptoms, may not be permanently detrimental to the beef female.

Effect of Liver Reserves of Vitamin A on Carotene Requirements During Lactation

Vitamin A stores in the liver of beef cattle act as a reserve against a deficiency in the ration. The extent to which the lactating beef cow may use such liver stores is not known. Eaton *et al.* (41) have shown that the vitamin A content of the liver of pigs and lambs was significantly higher at birth and at 30 days of age when the dams received supplementary vitamin A during late gestation. Other work with rats (42) in-

dicates that the vitamin A content of the milk of the mother, and liver stores of the nursing offspring, are more influenced by amount of vitamin A in the mother's diet during lactation than by her liver reserves. With beef cows which normally store large reserves of vitamin A on lush, summer pasture, the question of its availability or transfer to the suckling calf becomes important.

Two tests were conducted to study the effect of different levels of carotene intake on liver stores of cows at parturition, and the effect of maternal liver stores on vitamin A content of the milk, blood, and liver of their calves at three months of age (43). In the first experiment, 10 two-year-old heifers in the second and third months of pregnancy, were selected. In the second experiment, 20 two-year-old heifers were used.

During gestation and lactation, these heifers received a low-carotene ration calculated to meet N.R.C. requirements except for carotene. Heifers of the basal lot received no supplemental carotene throughout the experiment, whereas those in Lot 4 received carotene via capsule during the entire experiment at levels of 60 mcg. carotene daily per pound of body weight during gestation, and 330 mcg. during lactation. Lot 2 heifers received carotene at the above level only during the lactation period, and Lot 3 heifers only during gestation. Blood, liver, and milk samples were obtained at intervals during the test, and growth data and health of cows and calves were observed closely. Only the results from the second experiment are shown here since more numbers were involved and the data is typical of results obtained in both trials (Table 9).

In each trial, initial liver stores of the heifers were high, reflecting a liberal carotene intake during the previous summer. Wide variations in liver stores were observed among individual heifers at the start of the experiment, with a seven-fold variation in initial stores in the second trial, even though all cows were of comparable age and treatment prior to the test. This tremendous variation in liver stores of range cows in the fall has been consistently observed at this station. This poses an economic problem of supplementing the entire herd in order to protect a few individuals that may have poor ability to accumulate large liver stores.

During gestation, liver stores of all heifers declined, and supplementation with carotene at levels of 60 mcg. per pound per head daily did not affect either liver stores of heifers at parturition, or of their calves at birth or 3 months. Supplementing heifers of Lots 2 and 4 with

Table 9.—Average Liver, Plasma, and Milk Vitamin A of Beef Cows on Various Carotene Treatments, and Liver and Plasma Vitamin A of Their Calves.

	Lot 1 No Carotene	Lot 2 Carotene During Lactation only*	Lot 3 Carotene During Gestation only*	Lot 4 Carotene Throughout Experiment*
COWS				
Liver vitamin A (mcg./gm. D.M.)				
5 months before calving	303	308	151	252
At parturition	141	138	56	112
3 months after calving	31	111	10	84
Plasma vitamin A (mcg./100 ml.)				
5 months prepartum	32.5	30.0	27.4	34.0
At parturition	17.3	12.2	13.4	16.8
3 months postpartum	15.5	26.5	16.9	30.4
Colostrum and milk vitamin A (mcg./100 ml.)				
At parturition	144	225	128	133
3 months postpartum	3.8	8.6	3.3	7.2
CALVES				
Liver vitamin A (mcg./gm. D.M.)				
At birth	4.0	5.8	2.7	2.9
At 3 months	3.3	15.9	1.7	13.3
Plasma vitamin A (mcg./100 ml.)				
At birth	4.9	3.8	6.2	4.9
At 3 months	4.5	12.6	5.6	11.5

*60 mcg./lb during gestation and 300 mcg. during lactation.

300 mcg. per pound of carotene daily for 3 months during lactation appeared to spare liver stores of vitamin A, although further declines in liver levels during lactation showed an accelerated rate of depletion, with no apparent benefit from carotene supplied prior to calving.

Liver stores of vitamin A in the newborn calves were all exceptionally low and showed no benefit from carotene supplementation of the dams prior to calving. This work supports the view that extremely high intakes of carotene are necessary to obtain a substantial increase in placental transfer of vitamin A (44). In contrast, there was an apparent benefit to calves in Lots 2 and 4, to 3 months of age, from high levels of carotene supplied their dams. Plasma vitamin A levels of the heifers showed no effect of carotene supplementation prior to calving (Lot 3), but did reflect the supplementation with carotene after calving (Lots 2 and 4). Vitamin A in the colostrum was variable, but there was some tendency for later milk to show an increase in potency from

carotene supplementation (Lots 2 and 4) parallel to that observed in the blood. Calf plasma vitamin A levels were all low at birth, but showed a marked increase in Lots 2 and 4 at 3 months of age.

Typical vitamin A deficiency symptoms were observed in three calves in Lot 1, and three calves in Lot 3. None of the cows showed symptoms of a deficiency in either experiment. A carotene allowance of 60 mg. per day during gestation was ineffective in helping heifers in Lots 3 and 4 maintain liver stores or plasma vitamin A levels during 5 to 6 months of gestation. When the carotene allowance was increased to 300 mg. during lactation, improved plasma vitamin A values of cows and their calves resulted, with a decrease in rate of liver depletion. Mobilization of liver vitamin A by lactating beef cows on a low carotene intake (Lots 1 and 3) was inadequate to provide sufficient vitamin A for their calves, as evidenced by plasma and liver levels of vitamin A and deficiency symptoms in the calves. Carotene was not fed at high enough levels to provide for sufficient placental transfer to protect the newborn calf. High intakes of carotene during lactation appear necessary to provide adequate vitamin A for the suckling calf and to prevent liver depletion in the dam.

Effect of Plane of Nutrition on Carotene Requirements of Beef Cows

Calf losses are frequently attributed to vitamin A deficiency when cattle are wintered on low-quality roughages or during a drouth. Under such conditions, vitamin A deficiency may be complicated by lack of other nutrients, particularly energy, protein and minerals. Little is known concerning the effect of other deficiencies on vitamin A. Accordingly, two experiments were conducted to study the effect of low levels of nutrient intake on the depletion of liver vitamin A stores in beef cows during gestation and their carotene requirements during early lactation (45).

From the experimental herd, two-year-old heifers (approximately 750 pounds in weight), which had grazed native grass the previous summer, were selected for the test. They were assigned to one of two levels of nutrient intake, designated as "low" or "adequate" according to N.R.C. standards for protein and energy. The rations fed (per head daily) during gestation were: "low level", 1.0 pound cottonseed meal plus weathered grass hay; and "adequate level", 2.5 pounds cottonseed meal plus 4.0 pounds ground milo and weathered hay. After parturition, one-half of the cows on each ration received either 30 mg. of carotene per head daily, or 150 mg. (about one-third, and one and two-thirds, of

the N.R.C. recommended allowance for beef cows nursing calves). Blood and liver samples were taken periodically and the cows were continued on test until the calves were three months of age (See Figure 7). A summary of part of the data obtained is given in Table 10.

The low plane of nutrition caused much greater loss of body weight up to parturition, with less gain during lactation than the adequate feed level. This loss resulted in lighter birth weights of calves from the low level heifers and less gain of calves to three months of age. Blood levels of carotene and vitamin A in the cows and their calves were not affected by low feed levels. Cows fed at the high level had somewhat lower liver reserves of vitamin A at parturition, perhaps a reflection of their greater vitamin A needs due to heavier body weight. Death losses occurred in calves from cows on each treatment, except where adequate feed and high carotene supplementation was practiced.

Within the levels used in this experiment, supplemental carotene failed to maintain liver stores of vitamin A in the cows, irrespective of plane of nutrition. The high level of carotene decreased the rate of depletion of liver stores and increased plasma vitamin A. There was some occurrence of diarrhea among calves in all lots.

Table 10.—Effect of Plane of Nutrition and Level of Carotene Intake During Lactation on Performance of Beef Cows and Their Calves.
First Experiment

	Low Level		Adequate Level	
	4.5 mg*	22.0 mg*	3.9 mg*	19.0 mg*
Number of Cows Compared	12	12	12	12
Cow and Calf Weights (lb.)				
Cow wt. loss to parturition	−116	−129	−63	−44
Wt. gain during lactation	+31	+32	+78	+58
Calf birth wt.	58	56	65	64
Gain to 12 weeks of age	69	67	88	95
Calf death loss	2	2	2	0
Plasma Vitamin A (mcg./100 ml.)				
Cows—Initial (6 mo. pre-partum)	34	34	33	36
Parturition	16	15	15	15
Terminal (3 mo. postpartum)	18	24	18	22
Calves—At Birth	8.0	8.0	7.2	7.2
Terminal	5.8	5.1	5.5	5.8
Liver Vitamin A (mcg./gm. D.M.)				
Cows—Parturition	154	115	115	88
Terminal	85	85	44	51

*Carotene per 100 lbs. body weight.

Figure 7.—The effect of plane of nutrition on requirements for carotene by beef cows during gestation and lactation was studied on two trials. Results indicate no increased carotene requirement due to a "low" plane (above) vs. an "adequate" feed level (below).

It would appear from these data that for production of healthy calves, range beef cows may need a daily intake of 20 mg. or more carotene per cwt. during the first 3 months of lactation, regardless of plane of nutrition. The tests indicate that the carotene requirement of the beef cow does not increase when rations low in protein and energy are fed.

Carotene Requirements of Beef Cows During Lactation

Research at this station has shown the inadequacy of supplementing beef cows during pregnancy with enough carotene to influence the vitamin A nutrition of the calf at birth or during early lactation. Also, 300 mg. of carotene per head daily during lactation, while not sufficient to prevent some loss of liver vitamin A in the cow, may be more than necessary in order to maintain the beef cow and protect her suckling calf. Thus it was necessary to consider the carotene requirement of the dam during early lactation.

Accordingly, two experiments were undertaken in an attempt to establish the dietary carotene requirements of lactating beef cows (48). In each test, mature Hereford cows of similar age and past history were selected from the experimental herd in the fall, after grazing native grass pasture the previous summer. They were confined to small, dirt pens and were fed milo, cottonseed meal, and weathered range grass hay (devoid of carotene), with minerals *ad lib.* After parturition, the concentrate allowance was increased to meet their requirements for protein, energy, and minerals. Blood samples were taken at monthly intervals and liver samples were taken 6 months prepartum (first experiment only), at parturition, and at 3 months post-partum. The experiment was terminated when the calves reached 3 months of age.

In the first trial, the cows received three levels of carotene during the 3 months of lactation from a carrot oil concentrate* fed individually every third day in amounts necessary to supply 10, 20, and 30 mg. per cwt. per day (approximately 100, 200, and 300 percent of current N.R.C. recommended allowances). In the second trial, the cows were divided into 3 lots and received 0, 5, and 10 mg. carotene per cwt. daily during the first three months of lactation.

The average results of certain data obtained are shown in Table 11. Liver stores of vitamin A were high when the cows started the first experiment at the end of the summer grazing season. A rapid drop in liver stores during gestation is apparent from the data shown. A similar decline is evident in plasma vitamin A. Thus the cows were in a par-

Table 11.—Effect of Different Levels of Carotene Intake on Performance of Beef Cows During Early Lactation.

	1st Experiment†			2nd Experiment†		
	10	20	30	0	5	10
Number of cows per treatment	7	7	7	5	5	6
COWS						
Plasma vitamin A (mcg./100 ml.)						
5-6 mo. before calving	35.8	34.4	34.9	5.0††	19.0	22.5
Parturition	10.3	9.2	7.1	15.4	12.1	18.5
3rd mo. lactation	20.8	23.2	26.8	10.0	14.3	16.2
Plasma carotene (mcg./100 ml.)						
Parturition	27.1	29.2	30.6	37.9	46.7	55.1
3rd mo. lactation	193.9	267.2	292.1	23.9	114.0	275.4
Liver vitamin A (mcg./gm., D.M.)						
5-6 mo. before calving	312.0	293.0	226.0			
At parturition	27.1	26.2	21.7	64.0	80.7	78.6
3rd mo. lactation	13.7	11.6	16.0	23.8	11.4	46.5
CALVES						
Plasma vitamin A (mcg./100 ml.)						
At birth	4.5	4.4	4.5	17.4	16.4	14.5
3 mo. of age	5.5	9.0	8.3	4.6	4.8	6.3
Plasma carotene (mcg./100 ml.)						
3 mo. of age	33.5	34.8	33.1	12.1	29.1	41.3
Liver vitamin A (mcg./gm. D.M.)						
3 mo. of age	2.6	3.7	5.2	4.5	5.4	8.2
Death loss to 3 months	2	1	0	2	0	0

†N mber of mg. carotene daily per 100 pounds body weight.
††Samples believed to be spuriously low in vitamin A for unknown reasons.

tially depleted state at parturition, although not as deficient as other experimental cows which were maintained for much longer periods on low-carotene rations. Nevertheless, they appeared to have lost over 90 percent of their liver stores (per gram of liver tissue) by calving time. At parturition, cows in the second trial had higher liver vitamin A levels than those in the first trial, apparently due to a more favorable summer grazing season prior to the test.

In the first trial, supplementing cows with 10, 20, and 30 mg. carotene per cwt. per day during lactation caused plasma carotene to increase rapidly, while vitamin A responded more slowly. By the end of the third month of lactation, vitamin A values were all within a range considered normal, with both carotene and vitamin A reflecting the increasing levels of carotene intake. Liver vitamin A of the cows continued to decline even at the highest level of carotene administered. Only

slight variations occurred in liver vitamin A stores of the cows at the end of the experiment. Calf plasma vitamin A levels were dangerously low at birth, and rose only slightly during the three-month experimental period. Calves from cows supplemented with 20 and 30 mg. per cwt. showed highest plasma levels at three months. Plasma carotene varied only slightly, while liver levels increased in proportion to carotene given the dams, although all levels would be considered low.

A similar pattern was observed in the second trial. Cows in this experiment started lactation with greater liver stores and higher plasma carotene and vitamin A levels than cows in the previous trial. Cows fed no supplemental carotene during lactation declined in plasma vitamin A and carotene to dangerously low levels; cows fed 5 to 10 mg. carotene per cwt. had increased in plasma carotene, but remained low in vitamin A. Liver vitamin A declined on all treatments. Plasma vitamin A levels of the calves were somewhat higher at birth than during the first experiment, but declined to lower levels at 3 months of age. Plasma carotene in the calf directly reflected the level of carotene intake of the dam.

Rather severe cases of diarrhea occurred in first trial in calves from cows on the 10 and 20 mg. levels. In the second experiment it occurred among calves in all lots. Of considerable significance was the death loss encountered, which was heaviest in the first experiment in the 10 mg. lot and in the second test in the non-supplemented lot.

While the results of these studies are not entirely consistent, some recommendations as to carotene requirements of beef cows nursing calves might be drawn. None of the levels fed during lactation prevented a further decline in liver vitamin A stores of cows, although this may be of little consequence, depending on the length of time the cow must nurse her calf on a low-carotene diet. For cows entering lactation with low liver reserves, a level of 30 mg. per cwt. daily early lactation appears necessary to maintain adequate blood levels in cows and prevent death losses among the calves. With cows entering lactation with greater vitamin A reserves (as in the second test), 10 mg. of carotene per cwt. daily appeared adequate. Generally, the above levels were necessary to maintain plasma levels above 5 mcg. per 100 ml. in the calf and prevent death loss. None of the levels of carotene given the dams appeared to affect appreciably the liver levels of the suckling calf.

In neither trial was there evidence of vitamin A deficiency among the cows. All conceived readily when exposed to bulls under pasture conditions following the experiment. Thus the lowest levels used

here appeared adequate to meet the maintenance needs of the cow. Hence, it may be more efficient and economical to supplement the calf directly than to attempt to feed the cow the necessary carotene to nourish the calf, at much higher cost.

Wheeler and associates in Oregon (49), in a 3-year study, showed that mature beef cows receiving 1.5 mg. of carotene per 100 pounds per day during the winter performed satisfactorily, although plasma carotene and vitamin A levels were higher in cows supplemented with 5, 15, or 25 mg. carotene. In an 8-year study at New Mexico, Watkins and Knox (50) could show no advantage from feeding range cows a supplement containing 23 percent dehydrated alfalfa meal. In their studies, range forage appeared to be adequate in carotene for calving and early lactation. In a 3-year Colorado study, range supplements containing additional vitamin A or alfalfa did not improve conception rates, although weaning weights were slightly higher than for the control lots (51).

Carotene Requirements of Fattening Steers

Most rations for fattening cattle in the Southwest are based on milo, cottonseed meal, and roughage low in carotene. To supply carotene and other factors, alfalfa hay and dehydrated alfalfa meal are commonly added to the ration. Dry, stabilized vitamin A is also available at relatively low cost.

Extensive tests at the Texas station by Jones *et al.* (1) have shown the length of time necessary to deplete steer calves of vitamin A. Obviously, the carotene needs of fattening cattle depend on amount of liver storage, length of fattening period, age, and possibly other factors.

Five winter experiments were undertaken with steer calves fattened in drylot for 160 to 170 days to determine: (a) effect of adding a crude carotene concentrate to rations containing yellow corn, cottonseed meal, sorghum silage, and a limited amount of alfalfa hay, and (b) the relative value of alfalfa hay and dehydrated alfalfa meal when substituted for cottonseed meal in rations based on milo and limited amounts of sorghum silage. An estimate of the carotene requirements of fattening calves was possible from these studies.

A total of 190 weaner steer calves were used. The calves were obtained at weaning from large commercial herds or from the Experiment Station herd. All calves were full-fed either yellow corn or sorghum grain, with 1.5 pounds of cottonseed meal, and limited amounts of sor-

ghum silage. Varying amounts of either alfalfa hay or dehydrated alfalfa meal pellets were fed to provide different carotene intakes.

The possible benefits from adding a crude carotene concentrate to a fattening ration containing yellow corn, cottonseed meal, sorghum silage, and 1.0 pound of alfalfa hay per head daily was investigated (52). Additional carotene was added to a basal ration containing approximately 30 mg. to provide an intake of about 47 mg. per calf per day. It was estimated that the carotene in the basal ration was adequate according to N.R.C. allowances. Adding carotene to the basal ration did not increase feedlot performance of the steer calves. Thus, the amount of carotene supplied by the basal ration was apparently adequate for the 165-day feeding period.

In additional studies, sorghum grain replaced corn in rations containing cottonseed meal, sorghum silage, and varying amounts of alfalfa hay or dehydrated alfalfa meal pellets (53). The two alfalfa products were compared on an equal-protein basis when each replaced one-fourth or one-half of the cottonseed meal. In addition, dehydrated alfalfa meal pellets replaced all cottonseed meal. In most trials, blood samples were taken at the beginning, mid-way, and at completion of the study. Liver stores of vitamin A were obtained from samples taken at slaughter. Adequacy of the ration in terms of vitamin A was established by blood levels, liver stores at the completion of the trial, appearance of vitamin A deficiencies, and feedlot performance.

In the first trial, one lot of steers received ground yellow corn, while two lots received ground milo with protein and alfalfa hay supplements in addition to sorghum silage, as follows:

Lot 1—Ground milo plus 1.85 pounds cottonseed meal.

Lot 2—Ground milo plus 1.5 pounds cottonseed meal plus 1.0 pound alfalfa hay.

Lot 3—Yellow corn plus 1.5 pounds cottonseed meal plus 1.0 pound alfalfa hay.

Average daily gains, and blood and liver data are shown in Table 12. The results indicate that rations based on milo, sorghum silage, and cottonseed meal (as fed Lot 1) were not adequate in carotene for satisfactory performance. The addition of 1.0 pound of alfalfa hay per steer daily (Lot 2) resulted in increased gains and blood carotene and vitamin A levels. A comparison of the value of milo vs. yellow corn (Lot 2 vs. Lot 3) showed that the rather high intake of yellow corn (11 pounds per head daily) improved the blood and liver levels of carotene and vitamin A. Liver levels were low on all treatments, but tended to reflect the differences in carotene intake from the ration.

Table 12.—Comparison of Yellow Corn and Milo, With and Without Alfalfa Hay, for Fattening Steer Calves (165 Day Trial, 10 Calves per Lot.)

	Lot 1	Lot 2	Lot 3
Average daily gain (lb).	2.03	2.26	2.24
Estimated daily carotene intake, (mg./head)*	3.78	20.2	32.2
Plasma carotene at end of trial (mcg./100 ml.)	10.0	40.0	81.0
Plasma vitamin A at end of trial (mcg./100 ml.)	7.7	15.1	20.5
Liver vitamin A at end of trial (mcg./gm., D.M. basis)	0.9	2.2	3.3

*Estimated from carotene content of feeds and average amounts consumed during trial.

At the completion of the trial, several steers in Lot 1 showed definite symptoms of a vitamin A deficiency. An opaque condition of the cornea, followed by complete blindness, was observed in one steer (See Figure 8). Excessive lacrimation, anasarca, and convulsions when excited, were evident in several steers. Feed consumption and rate of gain of Lot 1 calves declined toward the end of the trial. None of the steers in Lots 2 and 3 showed any outward signs of a deficiency. In this trial, the daily carotene intake of Lot 2 steers (about 20 mg. per head) was apparently adequate.

Three additional trials were conducted in which alfalfa hay replaced one-fourth and one-half of the cottonseed meal on an equal-protein basis, and dehydrated alfalfa meal replaced one-fourth, one-half, and all of the protein supplement. Milo, sorghum silage, and varying amounts of cottonseed meal were also fed.

The results are presented in Table 13 according to trials, since the actual amounts of alfalfa hay and dehydrated alfalfa meal pellets fed varied from year to year. Also, the results appeared to differ in relation to the carotene content of the basal ration. Certain trends may be noted in all trials, however.

In trial 3, average daily gains varied somewhat, but increased with increasing levels of carotene intake from alfalfa hay or dehydrated alfalfa meal. The difference in gain between calves of Lot 1 and others receiving alfalfa was statistically significant $(P<.05)$.

There was no consistent pattern for daily gains in the following two trials. This is perhaps explainable in the basis of the carotene in the silage fed, and the initial blood values of the steer calves. Symptoms of vitamin A deficiency were noted in steers in the basal lot in trial 3 only (thin and watery diarrhea, night blindness, edematous swelling of

Figure 8.—Ocular changes were noted early in vitamin A deficient calves born to beef cows carried on low carotene rations for long periods. Such changes caused by constriction of the optic nerve were irreversible.

hocks and forelegs, and convulsions toward the termination of the experiment). The only signs of a deficiency in the basal group in trials 4 and 5 were a mild edema of the foreleg and brisket noted in calves at time of slaughter (See Figure 9). Carcass grades were not affected by treatment, although a few carcasses in the basal lot had to be trimmed before passing government inspection.

Plasma carotene levels reflected the increase in intake of carotene from alfalfa hay or dehydrated alfalfa meal. Plasma vitamin A also varied directly with carotene intake, although considerable variation is apparent in the data. Liver stores of vitamin A at the end of the feeding period were low for all treatments, but were improved somewhat by the higher carotene intakes.

Table 13.—Effect of Varying Amounts of Carotene from Alfalfa Hay and Dehydrated Alfalfa Meal in Steer Fattening Rations.

	When Substitution Level of Alfalfa for C. S. Meal was—					
	None	¼ A'f. Hay	¼ Dehyd. Alf. Meal	½ A'f. Hay	½ Dehyd. Alf. Meal	All Dehyd. Alf. Meal
TRIAL 3 (166 days)						
Avg. daily gain (lb.)	1.88	1.95	2.05	2.10	2.13	2.12
Est. daily carotene intake* (mg.)	12.1	28.6	34.8	42.9	57.8	95.6
Plasma carotene (mcg./100 ml.)						
Initial	103	98	90	101	124	98
Mid-point of trial	33	55	67	65	79	128
Final	22	51	49	77	94	145
Plasma vitamin A (mcg./100 ml.)						
Mid-point	15.4	20.1	19.8	21.9	21.4	20.9
Final	13.5	23.2	23.3	27.4	29.4	33.1
Liver vitamin A (mcg./gm., D.M.)	1.7	3.3	3.8	8.2	6.0	16.4
TRIAL 4 (163 days)						
Avg. daily gain (lb.)	2.29	2.35	2.34	2.25	2.26	2.25
Est. daily carotene intake*(mg.)	29.1	40.8	40.9	54.2	46.2	68.0
Plasma carotene (mcg./100 ml.)						
Initial	132	120	122	105	104	141
Mid-point	48	72	64	63	72	125
Final	60	90	76	80	86	124
Plasma vitamin A (mcg./100 ml.)						
Initial	31.2	30.9	29.6	28.7	28.7	34.1
Mid-point	19.7	28.4	25.6	20.4	17.0	30.2
Final	18.5	25.9	22.1	26.9	21.2	25.6
Liver vitamin A (mcg./gm. D.M.)	2.3	3.6	3.9	3.9	7.3	4.1
TRIAL 5 (166 days)						
Avg. daily gain (lb.)	2.10	2.05	2.18	2.16	2.18	2.05
Est. daily carotene intake* (mg.)	20.2	42.0	49.6	62.3	79.4	124.4
Plasma carotene (mcg./100 ml.)						
Initial	110.7	186.9	169.1	152.9	196.8	123.9
Mid-point	80.8	88.7	102.3	103.7	98.6	116.0
Final	47.6	71.0	82.8	77.3	82.5	95.0
Plasma vitamin A (mcg./100 ml.)						
Initial	28.7	35.1	32.7	33.6	35.2	30.0
Mid-point	29.8	29.6	34.4	34.9	33.0	31.6
Final	15.0	22.8	24.2	24.8	24.6	26.7
Liver vitamin A (mcg./gm. D.M.)	2.8	5.8	7.1	6.2	14.2	11.2

*Calculated from feed consumed and carotene content of feeds analyzed three times during trial.

Figure 9.—Fattening steers and beef cows long depleted of vitamin A showed edema of the foreleg and brisket (anasarca). Several steers in experimental lots without additional carotene were partially condemned upon slaughter.

Data from these experiments suggest that plasma carotene levels below 40 mcg., and vitamin A values less than 15 mcg. per 100 ml., respectively, are indicative of a vitamin A deficiency in feedlot cattle. Such blood levels are likely to be accompanied by signs of a deficiency and reduced rate of gain. Carotene intakes of 2.4 to 3.5 mg. per cwt. appeared necessary to support satisfactory gains and feedlot performance for steer calves fed approximately 165 days in drylot. This level is lower than that reported by Jones *et al.* from extensive Texas experiments but higher than that reported by Guilbert and Hart for maintenance (1, 2). Differences in liver vitamin A stores at the start of the feeding period undoubtedly influence dietary requirements.

Liver storage was improved only at the highest levels of carotene intake in trials 3 and 5; while in trial 4, there was little difference in liver stores due to carotene intake. Approximately 1.0 pound of alfalfa hay or dehydrated alfalfa meal pellets appear sufficient in a milo, cottonseed meal, sorghum silage ration to maintain steer calves in an adequate state of vitamin A nutrition.

Recently, an increasing number of vitamin A deficiencies have been observed among long-fed steers in midwestern feedlots, despite what appears to be adequate carotene in the rations (54). In a Purdue test, supplementing steer fattening rations composed of ground ear corn, soybean meal and minerals with 2,000 to 32,000 International Units of

vitamin A per head daily generally improved feed intake, daily gains, and plasma vitamin A levels (55).

The fattening tests reported in this bulletin were conducted during the winter, whereas the Purdue tests were during the hot summer months. During the summer, the added stress of hot weather might increase the vitamin A needs of fattening steers. It has also been suggested that certain factors in the ration might interfere with the conversion of carotene to vitamin A in the animal body. There is some evidence that large amounts of nitrate, as might occur in the corn plant due to heavy nitrogen fertilization, may be converted to nitrate in the paunch and interfere with conversion of carotene to vitamin A (56).

SUMMARY

Extensive experiments at the Oklahoma Agricultural Experiment Station over a 10-year period have shown that:

- Carotene in native grass is most abundant from May to July and declines rapidly by October and November. Essentially no carotene remains during the winter months.

- The plasma carotene level in beef cows rises sharply as green grass appears in the spring, followed by a slower upturn in vitamin A. Plasma carotene falls off after October; whereas vitamin A is maintained at levels of 25 to 30 mg. per 100 ml. by virtue of liver stores. Plasma carotene and vitamin A levels in the nursing calf reflect the changes observed in the maternal blood, but at a slower rate.

- No significant difference was observed in plasma carotene and vitamin A among cattle of the Hereford, Angus, and Shorthorn breeds, when handled under similar conditions.

- Steers fattened on pasture build up high plasma levels of carotene (5 times higher than in drylot) and slightly higher plasma vitamin A than those fattened in drylot. The effect of this greater storage of carotene and vitamin A on lush pasture was still apparent after 63 days on drylot rations.

- Supplementing weathered range grass with carotene-rich feeds for growing beef calves and yearlings has given no consistent boost in gains, although the vitamin A blood picture has been improved.

- Unlike many other nutrients, the amount of carotene in the ration does not seem to affect its availability to cattle. Varying

dietary levels of carotene are digested to about the same degree (40 percent apparent digestibility).

● A liver biopsy technique has been developed by which 1.5 to 2 grams of liver tissue can be removed from the live animal. Repeated sampling has had little effect on the animal and this technique can be used to follow changes in liver reserves of vitamin A.

● Liver biopsy samples, while varying somewhat from the entire liver in vitamin A and carotene content, estimate these stores with sufficient precision to be useful in vitamin A studies (15 percent variation from average content per gram as compared to the entire liver). With steers, it was determined that liver weights were 0.891 to 1.01 percent of live body weight, and thus some estimate can be made of total liver stores.

● Correlations show that plasma carotene and liver vitamin A are significantly associated only after a long period of high carotene intake. In contrast, plasma and liver vitamin A appear to be significantly correlated only after a long depletion period. or with long-fed steers on high carotene intakes. Hence, plasma carotene and vitamin A are valuable as indicators of liver vitamin A only if the previous treatment of the animal is known. There was no apparent tendency for individuals to maintain high or low vitamin A blood levels.

● In contrast to lambs, vitamin A depleted calves made little use of intravenously-administered carotene. This suggests a fundamental difference between species in the sites of carotene conversion, and raises questions as to the nutritional value of the carotene deposited in the fatty tissues of cattle during a lush grazing season.

● The characteristic drop in plasma carotene and vitamin A at parturition and shortly after, as noted by other workers with dairy cows, was observed with beef cows. Plasma carotene and vitamin A levels of the suckling calf to 30 days of age reflect the dietary carotene intake of the dam.

● Beef females may subsist for long periods (up to 43 months) on low-carotene rations before showing signs of severe deficiency. This long period of carotene deprivation did not affect conception, and abortions did not occur until the sixth to seventh month of the third pregnancy. Live calves born to such cows exhibited the classical symptoms of vitamin A deficiency

within 6 to 8 weeks of age. Cows severely depleted of vitamin A showed marked improvement when treated and placed on good pasture.

● Liver reserves of vitamin A in the mature beef cow are poorly mobilized to protect the young, suckling calf. The carotene intake of the dam during lactation is much more important than her liver reserves before, or after, parturition.

● Low planes of nutrition (protein and energy) do not appear to increase the vitamin A requirements of the beef female during the last 6 months of pregnancy and the first 3 months of lactation.

● Experimental evidence suggests that a lack of phosphorus may adversely affect the conversion of carotene to vitamin A in cattle.

● The carotene requirement of beef cows during the first 3 months of lactation depends on liver reserves built up during the previous grazing season. Cows with low liver reserves at the start of the wintering period may require 10 mg. or more of carotene per 100 pounds of body weight, whereas cows having ample liver stores as they approach calving may get by with 5 mg. or less.

● Weaner steer calves fattened for 165 days on a ration of yellow corn, cottonseed meal, sorghum silage, and only a small quantity of alfalfa hay, did not respond to additional carotene in the ration. When milo replaced corn, so that the daily carotene intake was less than 20 mg. per head, additional carotene was beneficial. The carotene requirement of fattening steer calves, averaging about 650 pounds during the winter feeding period, appears to be about 2.5 to 3.5 mg. per 100 pounds of body weight per day for a 165-day drylot period.

LITERATURE CITED

1. Jones *et al.*, Texas Agr. Exp. Sta. Bul. 630; Schmidt, H., Am. J. Vet. Res. 2:373:1941; Moore, L. A., J. Dairy Sci. 22:803:1939; Hart, G. H. and H. R. Guilbert, Calif. Agr. Exp. Sta. Bul. 560; Payne, Merle G. and H. E. Kingman, J. Animal Sci. 6:50:1947; and Guilbert, H. R. and G. H. Hart, J. Nutr. 8:25:1934.

2. Guilbert, H. R. and G. H. Hart, J. Nutr. 10:409:1935.

3. Page, H. M., E. S. Erwin, T. R. Varnell and C. B. Roubicek, Am. J. Phys. 194:313:1958; Thomas, J. W. and L. A. Moore, J. Dairy Sci. 35:687:1952; Rousseau *et al.*, J. Dairy Sci. 37:1376:1954; Frey, Paul R., Rue Jensen and W. E. Connell, J. Nutr. 34:421:1947.

4. Frey, Paul R. and Rue Jensen, J. Nutr. 32:133:1946.

5. Braun, W. and B. N. Carle, J. Nutr. 26:549:1943; Spielman *et al.*, J. Dairy Sci. 29:707:1946 and 29:520:1946; Wise, G. H., M. J. Caldwell and J. S. Hughes, Science 103:616:1946.

6. Moore, L. A., J. Dairy Sci. 22:803:1939; Moore, L. A., J. F. Sykes, W. C. Jacobson and H. C. Wiseman, J. Dairy Sci. 31:533:1948; Boyer *et al.*, J. Dairy Sci. 25:433:1942.

7. Boyer, P. D., P. H. Phillips, N. S. Lundquist, C. W. Jensen, and I. W. Rupel, J. Dairy Sci. 25:433:1942.

8. Davis, Russell E. and L. L. Madsen, J. Nutr. 21:135:1941; Repp, W. W. and W. E. Watkins, New Mex. Bul. 420; Madsen, L. L. and R. E. Davis, J. Animal Sci. 8:625:1949; Payne, Merle G. and H. E. Kingman, J. Animal Sci. 6:50:1947.

9. Kuhlman, A. H. and W. D. Gallup, J. Dairy Sci. 25:688:1942; Ronning *et al.*, J. Dairy Sci. 36:52:1953; Ronning, Magnar, E. R. Berousek, Jack L. Griffith and Willis D. Gallup, Okla. Agr. Exp. Sta. Tech. Bul. T-76.

10. National Research Council, Recommended Nutrient Allowances for Beef Cattle. Washington, D. C., 1959.

11. Morrison, F. B., *Feeds and Feeding,* 22nd Ed., Morrison Publ. Co., Ithaca, N.Y.

12. Davis, Russell E. and L. L. Madsen, J. Nutr. 21:135:1941; Madsen, L. L. and R. E. Davis, J. Animal Sci. 8:625:1949.

13. Madsen, L. L. and R. E. Davis, J. Animal Sci. 8:625:1949 (Abs); Mohler, J. R., Report of the Chief of the Bureau of Animal Industry, 1939, p. 18.

14. Sutton, T. S., H. E. Kaeser and P. A. Soldner, J. Dairy Sci. 28:933:1945; Kuhlman, A. H. and W. D. Gallup, J. Dairy Sci. 27:633:1944.

15. Guilbert, H. R. and G. H. Hart, J. Nutr. 10:409:1935.

16. Nelson, A. B., O. B. Ross, A. E. Darlow, W. D. Campbell and R. W. MacVicar, Okla. Agr. Exp. Sta. Bul. B-418.

17. Savage, D. A. and V. G. Heller, U.S.D.A. Tech. Bul. 943; See also: Repp, Ward W. and W. E. Watkins, J. Animal Sci. 17:398:1958.

18. Nelson *et al.*, Okla. Agr. Exp. Sta. Bul. B-418.

19. Long, Robert A., W. A. Van Arsdell, R. W. MacVicar and O. B. Ross, Okla. Agr. Exp. Sta. Tech. Bul. T-43.

20. Hoeffer, J. A., W. D. Gallup and J. C. Hillier, Okla. Agr. Exp. Sta. Feeder's Day Rpt. 1946.

21. Ross, O. B., A. B. Watts and A. E. Darlow, Okla. Agr. Exp. Sta. Feeder's Day Rpt. 1949.

22. Stephens, D. F., O. B. Ross, R. W. MacVicar, W. D. Campbell, and A. E. Darlow, Okla. Agr. Exp. Sta. Feeder's Day Rpt. 1948.

23. Baker, Frank H., 1953, Ph.D. Thesis, Oklahoma State University; Church, David C., 1956, Ph.D. Thesis, Oklahoma State University.

24. Guilbert, H. R. and G. H. Hart, J. Nutr. 10:409:1935.

25. Whitehair, C. K., D. A. Peterson, W. J. Van Arsdell and O. O. Thomas, J. Am. Vet. Med. Assn. 121:285:1952.

26. Garner, R. J., Vet. Record 62:729:1950; Loosmore, R. M. and Allcroft, R., Vet. Res. 63:414:1951; Erwin, E. S., I. A. Dyer, T. O. Meyer and K. W. Scott, J. Animal Sci. 15:428:1956.

27. Van Arsdell, 1952, Ph.D. Thesis, Oklahoma State University.

28. Church, D. C., 1956, Ph.D. Thesis, Oklahoma State University.

29. Daniel, Odell G., 1957, Ph.D. Thesis, Oklahoma State University.

30. Rousseau *et al.*, J. Dairy Sci. 37:1376:1954.

31. Thomas, J. W. and L. A. Moore, J. Dairy Sci. 35:687:1952.

32. Frey, Paul R., Rue Jensen and W. E. Connell, J. Nutr. 34:421:1947.

33. Kon, S. K. and S. Y. Thompson, Brit. J. Nutr. 5:114:1951.

34. Church, D. C., R. W. MacVicar, J. G. Bieri, F. H. Baker and L. S. Pope, J. Animal Sci. 13:678:1954.

35. Schuh, J. D., M. Ronning and W. D. Gallup, J. Dairy Sci. 42:159:1959.

36. Klosterman, Earle W., D. W. Bolin and Merle R. Light, J. Animal Sci. 8:624:1949 (Abs.); Warner, R. G. and L. A. Maynard, J. Animal Sci. 11:780:1952 (Abs.); Eaton *et al.*, J. Dairy Sci. 34:1073:1951; Hentges, J. F., Jr., R. H. Grummer and D. K. Sorenson, J. Animal Sci. 11:794:1952 (Abs.).

37. Payne, Merle G. and H. E. Kingman, J. Animal Sci. 6:50:1947.

38. Davis, Russell E. and L. L. Madsen, J. Nutr. 21:135:1941.

39. Van Arsdell, W. J., O. B. Ross and R. W. MacVicar, J. Animal Sci. 9:546:1950.

40. Baker, Frank H., R. W. MacVicar, L. S. Pope, C. K. Whitehair and W. D. Campbell, Okla. Agr. Exp. Sta. Misc. Pub. MP-31, p. 59; Church, D. C., L. S. Pope, R. W. MacVicar, C. K. Whitehair and W. D. Campbell, Okla. Agr. Exp. Sta. Misc. Pub. MP-43, p. 19.

41. Eaton, H. D., J. A. Christian, F. C. Daugherty, A. A. Spielman and L. D. Matterson, J. Animal Sci. 8:224:1949.

42. Henry, K. M., S. K. Kon, E. H. Mawson, J. E. Stainer and S. Y. Thompson, Brit. J. Nutr. 3:301:1949.

43. Baker, F. H., R. W. MacVicar and L. S. Pope, Proc. Soc. Exp. Biol. and Med. 83:571:1953; Baker, F. H., L. S. Pope and R. W. MacVicar, J. Animal Sci. 13:803:1954.

44. Braun, W. and B. N. Carle, J. Nutr. 26:549:1943; Spielman *et al.,* J. Dairy Sci. 29:707:1946; Wise, G. H., M. J. Caldwell and J. S. Hughes, Science 103:616:1946.

45. Church, D. C., L. S. Pope and R. W. MacVicar, J. Animal Sci. 15:1078:1956.

46. Ross, O. B. and W. D. Gallup, J. Animal Sci. 8:628:1949 (Abs.).

47. Thomas, O. O., 1952, Ph.D. Thesis, Oklahoma State University.

48. Daniel, O. G., 1957, Ph.D. Thesis, Oklahoma State University; Pitts, J. L., 1958, M. S. Thesis, Oklahoma State University; Pope, L. S., J. L. Pitts, and George Waller, Okla. Agr. Exp. Sta. Misc. Pub. MP-51; Daniel, O. G., L. S. Pope, R. W. MacVicar and W. D. Campbell, Okla. Agr. Exp. Sta. Misc. Pub. MP-48.

49. Wheeler, R. R., P. H. Weswig, W. F. Brannon, F. E. Hubert and W. A. Sawyer, J. Animal Sci. 16:525:1957.

50. Watkins, W. E. and J. H. Knox, J. Animal Sci. 13:491:1954; Repp, W. W. and W. E. Watkins, J. Animal Sci. 17:398:1958.

51. Daugherty *et al.,* Colo. Gen. Series Paper 670.

52. Pope, L. S., R. D. Humphrey, A. E. Darlow, V. G. Heller, O. B. Ross and R. A. Long, Okla. Agr. Exp. Sta. Bul. B-428.

53. Church, D. C., 1956, Ph.D. Thesis, Oklahoma State University; Pope, L. S., R. W. MacVicar, Lowell Walters and V. G. Heller, Okla. Agr. Exp. Sta. Bul. B-522.

54. Crane, F. M., Proc. Semi-Annual AFMA Nutr. Council, Nov., 1960.

55. Perry, T. W., W. M. Beeson and R. C. Holz, J. Animal Sci. 19:1283:1960 (Abs.); See however: Kolari *et al.,* Minn. Mimeo. Rpt., 1960.

56. Perez, C. B. and C. D. Story, J. Animal Sci. 19:1311:1960 (Abs.); O'dell *et al.,* J. Animal Sci. 19:1280:1960 (Abs.).