UTILIZATION OF NATURAL AND SYNTHETIC CAROTENE AND SYNTHETIC VITAMIN A WITH AND WITHOUT ALPHA-TOCOPHEROL BY CATTLE

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INTRODUCTION

The importance of an exogenous source of vitamin A, or its precursor carotene, in the diet of mammals has long been recognized. As early as 1500 B.C. Egyptian medical men recommended roasted ox liver or the liver of a black cock for the cure of what today would be termed night blindness. Hippocrates, the famous Greek physician, also prescribed liver, suggesting that it be eaten raw after first being dipped in honey. Fresh liver has since been shown to be a rich source of both vitamin A and carotene.

During the past two decades volumes have been written on vitamin A and carotene. Nevertheless, much remains to be learned concerning the conversion of carotene to vitamin A and the utilization of vitamin A by ruminants. Since the recent development of a liver biopsy technique, which makes readily available small cores of liver tissue from the live animal, the assay for vitamin A and carotene content of liver tissues has been considered superior to blood assay. Vitamin A and carotene concentrations in the blood, liver and milk fat are used in studying the utilization of carotene and vitamin A.

In addition to the amount and physical form of the carotene or vitamin A in the diet, a number of dietary factors, such as the amounts of lecithin, fats, bile salts, paraffins, inorganic compounds, and antioxidants have been found to affect the utilization of carotene and vitamin A.

Commercially manufactured products having antioxidant properties are currently being used by the feed industry to stabilize easily oxidizable nutrients. The two most important naturally occurring products having antioxidant properties are the tocopherols and ascorbic acid. The tocopherols are unique among antioxidants in that after absorption through the intestinal wall they are deposited in the fat. Alpha-tocopherol has been shown to be the most effective antioxidant of the various tocopherols.

The objective of these studies was to determine the effect of feeding alpha-tocopherol with natural beta-carotene, synthetic beta-carotene, or synthetic vitamin A to cattle whose basal ration was normal or deficient in carotene.

REVIEW OF LITERATURE

To substantiate earlier observations that carotenes have vitamin A activity, Moore (59, p. 692) fed carotene to rats depleted in liver stores of vitamin A. The presence of stored vitamin A after the rats were fed on purified diets containing carotene indicated that dietary carotene is converted to vitamin A.

Confirmation of the conversion of carotene to vitamin A was soon reported by Wolff et al. (58, p. 27), who observed by intra-liver carotene injections in rats that both carotene and vitamin A concentrations in the liver were increased.

Subsequently, on the belief that an enzyme, "carotenase", was responsible for the conversion of carotene to vitamin A in vivo, the site of action of this enzyme was investigated. It was assumed by Ahmod et al. (2, p. 1195) and DeLuca et al. (21, p. 877) that conversion of carotene to vitamin A took place in the liver. Wiese, Mehl, and Deuel (93, p. 75) later demonstrated that in the rat, ingested carotene is converted to vitamin A in the intestinal wall. Ball and co-workers (3, p. xxiv) in 1947, made the discovery that retinene, or vitamin A aldehyde, is converted to vitamin A in the intestinal wall. This, of course, strengthened the possibility that carotene was

likewise converted to vitamin A in the small intestine. Further evidence that the conversion of carotene to vitamin A occurs in the digestive tract was obtained by Goodwin and Gregory (33, p. 505) when they observed that although the blood plasma of the goat is colorless, when substantial amounts of carotene were given orally, only 7 to 19 per cent of the carotene was excreted in the feces, therefore indirectly indicating that the conversion takes place in the intestinal wall. Research by Glover (32, p. 516), Petal et al. (68, p. 103), and Thompson et al. (87, p. 398) has subsequently shown the intestinal wall to be a site of conversion in the rat, pig, chick and dog.

Popper (72, p. 562) reported that the absorption of vitamin A alcohol in the rat takes place throughout the entire length of the small intestine, whereas Thompson et al. (85, p. 50) state that carotene undergoes a maximum conversion to vitamin A in the middle of the intestine with no conversion occurring anterior to the entrance of the bile duct.

While Bieri et al. (5, p. 32) provided additional information that the small intestine is a site of conversion of carotene, the probability that carotene can be converted to vitamin A in the liver and other tissues should not be overlooked, since many of the experiments discussed above show that body reserves of carotene can

be converted to vitamin A to serve in metabolism.

Reports of utilization of intravenously injected carotene by the chick (10, p. 657), sheep (13, p. 677), calves (43, p. 244), rabbits (10, p. 657), rats (43, p. 244) and goats (53, p. 181) indicate that the conversion of carotene to vitamin A may occur in tissues other than the intestinal wall. Experiments with cattle given carotene solutions intravenously have yielded inconsistent results.

Church et al. (13, p. 677) demonstrated the conversion of carotene to vitamin A in sheep given intravenous injections of carotene solubilized in an aqueous solution of Tween 40 (polyoxyethylenesorbitan monopalmitate). However, they were unable to demonstrate a similar conversion in Hereford, Guernsey, or Holstein calves. Using similar methods, Kon, McGillivray, and Thompson (43, p. 244) were unable to show any appreciable conversion of injected carotene to vitamin A in depleted Ayrshire and Shorthorn calves. Eaton et al. (25, p. 1073) and Warren and Maynard (88, p. 780), however, reported such conversion with aqueous suspensions of carotene injected intravenously in depleted calves. Schuh et al. (78, p. 159) concluded that dairy calves can utilize injected carotene. Their results indicated that intravenously administered carotene is metabolized in a manner similar to that entering the blood

by the intestinal route, which suggested that calves may be able to utilize the carotene present in the blood and other tissues.

Week et al. (90, p. 563) have shown that in the chick carotene and vitamin A, after leaving the small intestine, are transported to the liver mainly through the lymph and to some extent through the portal system. It was suggested that the efficiency of absorption of vitamin A in various species is influenced by the nature and quality of the diluent and by the original state of the vitamin.

Mechanism of Conversion of Carotene to Vitamin A

To explain the inferiority of beta-carotene as compared with vitamin A, one must assume either that half of the carotene molecule is wasted, or that only one out of every two molecules of carotene is converted to vitamin A in vivo. At present the complicated conversion of carotene to vitamin A is not fully understood.

Karr et al. (58, p. 18) were the first to suggest that the addition of two molecules of water at the 15,15' double bond of beta-carotene would yield two molecules of vitamin A ($C_{40}H_{56} + 2H_2O \longrightarrow 2C_{20}H_{29}OH$). This yield is not realized in actuality since on an equal weight basis beta-carotene has a much lower potency than vitamin A. Even under conditions which approach optimum for absorption

and conversion it appears that only about 50 per cent of the carotene ingested by rats is converted to vitamin A. It has been shown by Koehn (42, p. 337) that in the presence of alpha-tocopherol, the conversion of beta-carotene to vitamin A in the rat is almost quantitative. It was suggested that the remainder of the molecule, normally unaccounted for, is oxidized and utilized possibly as short-chain alcohols.

Glover et al. (31, p. xv) postulated that the conversion of carotene may not take place by central fission, but by stepwise degradation from one end of the molecule. To explain the two-fold superiority of beta-carotene over alpha-carotene on this basis, the authors assume that every molecule of the beta-isomer which is degraded gives a single molecule of vitamin A; with alpha-carotene, vitamin A is formed when degradation starts at the end of the molecule remote from the beta-ionone ring, but an active product results when the beta-ionone ring itself is degraded. On the assumption that degradative action can occur equally well at either end of the molecule, it follows that one molecule of vitamin A would be produced from every two molecules of alpha-carotene.

Factors Affecting Utilization

The presence of fat in the diet has been reported to assist absorption of vitamin A and carotene in most animals. Russell and co-workers (77, p. 191) using a normal ration containing 3.83 per cent fat and a low fat experimental ration containing 0.07 per cent fat found by measuring the amount of the vitamin excreted that hens on a normal ration can absorb about 50-60 per cent of the dietary carotene whether fed in the free form or as carotene present in plant tissues. The quantity of carotene absorbed was found to be less on the low fat ration. In the same experiment, the quantity of carotene absorbed increased as increasing amounts of carotene were fed, however, there was a progressive decrease in the percentage absorbed. At the same time, it was observed that when the intake of carotene on the low fat ration was increased 3.5 times the quantity absorbed increased only 1.7 times, whereas under the same conditions on the normal ration, the increase of absorption of carotene was five fold. When vitamin A ester was fed, satisfactory absorption on both the low and normal fat rations occurred. However, dietary fat appeared necessary for the retention of vitamin A in the liver.

Lewis and associates (49, p. 496) compared the

absorption of vitamin A given by stomach tube in an oily suspension and in an aqueous emulsion. They found that young rats given 13,000 I.U. of the vitamin in an oily solution had plasma levels of 900, 1015, and 120 I.U. at 3, 6 and 24 hours later, respectively. Thirty per cent of the vitamin was estimated to be stored in the liver after 24 hours and 23 per cent of the vitamin was lost in the feces; whereas those rats given 13,000 I.U. of the vitamin in an aqueous emulsion had plasma levels of 2,460, 210 and 310 I.U. at 3, 6 and 24 hours later, respectively, with only 5 per cent passing by way of the feces and 58 per cent being stored in the liver at the end of 24 hours.

Kramer and Tarjan (44, p. 295), studying carotene metabolism in rats, reported more efficient utilization of carotene fed in an oil solution than when carrots containing a similar amount of carotene were fed. They reported a recovery of 69 to 90 per cent of the carotene in the feces when carrots were fed as compared with a recovery of 27 to 52 per cent in the feces of rats fed oily solutions of carotene.

Adlersberg and Sobotka (1, p. 255) in studying the effect of emulsification by lecithin on the absorption of vitamin A found that giving 10-25 grams of commercial lecithin to human subjects increased the absorption of both fat and vitamin A. The mean increase in plasma

vitamin A was 31 I.U. per 100 milliliters without lecithin and 153 I.U. with lecithin.

The increased interest in the use of oral dosing with paraffin oil for the prevention of bloat stimulated McDowell and co-workers (54, p. 8) to study the effect of paraffin on carotene absorption. They found that carotene absorption was depressed by the feeding of liquid paraffin. The blood concentrations of vitamin A of cows fed heavy liquid paraffin, at a rate of 150 milliliters daily, reached a maximum depression of 40 per cent after about 16 days of treatment and a 20 per cent reduction in vitamin A ester content of the blood which was parallel with the total blood changes. The carotene level of the butterfat was found to fall by 40-50 per cent while the vitamin A level was slightly lowered and the vitamin A alcohol level of the blood was unaffected. This study was confirmed by McGillvray and associates (56, p. 47) using similar techniques.

The bile salts, sodium salts of glycocholic and taurocholic acids, serve to disperse or emulsify lipids in aqueous solutions, and therefore are presumed to favor enzyme-substrate combinations. Drummond and McWalter (24, p. 236) recognizing the possible importance of bile salts in the absorption of fats, showed that carotene could form a complex with desoxycholic acid. Further evidence was

obtained by Greaver and Schmidt (34, p. 496) who found that when the bile duct was ligated, the rats could utilize oral doses of halibut-liver oil but failed to utilize the carotene. The mechanisms involved are not as yet fully understood. However, it is believed that the bile salts act similarly on the carotenes, as on other lipids, in affecting absorption from the small intestine.

In some cases, emulsifying agents such as the Tweens have been found to increase the speed and efficiency of absorption of preformed vitamin A. Sherman et al. (81, p. 586) compared the utilization by beef cattle of vitamin A from pharamceutical grade halibut liver oil, a corn oil concentrate of synthetic vitamin A palmitate containing 15 per cent Tween 80, and a synthetic pure vitamin A acetate or palmitate dispersed in gelatin beadlets. Those animals receiving the aqueous and gelatin beadlet forms of vitamin A had greater amounts of blood plasma vitamin A at 12 hours after dosing than did those receiving the oil solution of vitamin A. Oil solutions of synthetic vitamin A and natural vitamin A, when administered in equal doses, produced identical blood plasma levels at 12 hours after dosing.

Heaney and Thomas (38, p. 1252) compared the utilization by Hereford steers of carotene and vitamin A from corn oil and vitamin A palmitate in gelatin. When steers

were fed 50,000 I.U. of vitamin A per day, no differences were noted in utilization of the two forms as measured by blood plasma concentrations. However, it was noted that depletion of vitamin A was faster from those animals receiving the corn oil supplementation. It was reported that the addition of yeast induced higher liver stores of vitamin A with a slower depletion time.

Diven and Erwin (22, p. 601) intraruminally injected into sheep aqueous solutions of beta-carotene, vitamin A alcohol and vitamin A acetate dissolved in Tween. Using the liver hepatic stores as criteria for absorption and utilization, they found that vitamin A alcohol and beta-carotene were not equal on an equivalent weight basis as indicated by the Expert Committee on Biological Standardization (29, p. 147-148), but rather that normal sheep injected with vitamin A alcohol stored 3.3 times more vitamin A in the liver than similar animals given beta-carotene. It was also noted that liver stores of vitamin A and beta-carotene were different for normal and vitamin A deficient sheep which may indicate that vitamin A deficiency impairs carotene utilization. It was concluded that vitamin A alcohol was absorbed at a more rapid rate than beta-carotene while the alcohol and acetate forms were absorbed at comparable rates.

Luther et al. (51, p. 362) studying the effect of particle size and type of carrier on the utilization of vitamin A found that the particle size of the vitamin A within the gelatin beadlet affects efficiency of utilization by rats. Particles of vitamin A of two microns or less in diameter dispersed in gelatin resulted in 69 per cent greater liver storage than was obtained with vitamin A in oil. Using 5 micron particles, the superiority was 24 per cent and with 20 micron particles, liver storage was approximately the same as with an oil solution.

The effect of thyroxine on carotene and vitamin A utilization has been given considerable attention over the years. Since thyroxine is noted for its effect in increasing oxygen uptake (O_2 consumption) and the basal metabolic rate of tissues, it is reasonable to assume that this hormone affects vitamin A and carotene utilization by increasing the daily requirements. This anomaly has been aptly demonstrated in rats by Sure and Buchanan (82, p. 521) and Cooper et al. (14, p. 404) in chickens.

Early work by Lemley et al. (48, p. 53) indicated that the efficiency of vitamin A utilization was profoundly influenced by the size of dose. It has since been shown that there is an optimum range of dosage above and below which utilization is less efficient. Vitamin A depleted rats dosed for three days with 63 I.U. per day

of vitamin A stored 11 per cent of the dose in the liver tissues; when raised to 4,000 I.U. per day, efficiency of storage was 38 per cent, but a further increase to 80,000 I.U. per day reduced storage efficiency to 13 per cent.

Davies and Moore (20, p. lxiii) using similar procedures, obtained 0, 13, 15 and 38 per cent recovery of the vitamin in the liver after 48 hours for single doses of 100, 200, 400, and 600 I.U., respectively, from halibut-liver oil.

In another experiment, Moore et al. (60, p. xxxix) gave rats of mixed sexes graded doses of from 10 to 10,240 I.U. of vitamin A acetate per day for five to six weeks. The efficiency of storage, as calculated from the liver and kidneys combined, ranged from less than 2 per cent at the daily dose of 10 I.U. to 80 per cent at doses of 640 I.U. per day.

Week and Sevigne (90, p. 563) found that groups of depleted chicks fed 30,000 I.U. of vitamin A dissolved in corn oil and supplied as the free alcohol, acetate, and undistilled natural esters had mean storage efficiencies of 35.6, 33.6 and 23.8 per cent, respectively. The same authors reported that when rats were dosed with 9,000 I.U. of vitamin A the recovery was 33.2 per cent for the alcohol form, 36.1 per cent for acetate and 29.2 per cent for the natural esters. Frey and Wilgus (29, p. 517) fed laying

pullets, depleted of vitamin A and carotene stores, 2,000 I.U. of vitamin A per pound of ration or beta-carotene in cottonseed oil, carotene in alfalfa, and vitamin A esters from fish liver oil. Analysis of liver samples showed that more vitamin A was stored when fish liver oil was the source of vitamin A than when the source was beta-carotene in cottonseed oil. The least storage of vitamin A was from carotene from alfalfa.

Antioxidants

Quackenbush et al. (74, p. 169) in 1941 found that tocopherol promoted an increased carotene utilization and suggested that the tocopherols functioned in the intestinal tract as antioxidants. Swick and Baumann (83, p. 120) found that high doses of alpha- or gamma-tocopherol and alpha-tocopheryl acetate diminished the storage of vitamin A in the liver and kidneys of rats restricted to a diet deficient in both vitamins A and E. The reserves of vitamin A were used much more rapidly than in control animals given d,l-alpha-tocopherol. The authors indicated that prolonged deficiency of vitamin E led to secondary deficiency of vitamin A as shown by the disappearance of this vitamin from the liver. The deficient rats also developed whitening of the teeth which is indicative of vitamin A deficiency. Sherman (80, p. 134) suggested that tocopherol

was concerned with the utilization of carotene rather than with its protection from oxidation.

Quackenbush et al. (74, p. 169) observed that in rats, alpha-tocopherol was highly effective in preventing autoxidation of carotene in vitro and in promoting a biological response to carotene in ethyl linoleate. They further found the tocopherols responsible for at least a part of the protective action of soybean oil. It was believed that by promoting a biological response to carotene, tocopherol functions as an antioxidant in the gastrointestinal tract rather than as a vitamin. Sherman (80, p. 134) and Patrick and Morgan (64, p. 525) demonstrated the antioxidant role of tocopherol in that carotene was destroyed in the tract in the absence of tocopherol. Phillips and Williams (71, p. 74) found that 0.26 per cent alpha-tocopheryl acetate increased rancidity while higher levels (1.45 per cent) showed antioxidant activity.

Following the demonstration by Mattill (51, p. 141) that auto-oxidation of lard could be prevented by polyphenols, Olcovich (63, p. 105) reported that hydroquinone would stabilize carotene in ethyl oleate or ethyl laurate solutions. Many workers have observed that carotene is less effective as a source of vitamin A when fed in pure solvents than in crude oils.

Kraybill and Shrewsbury (45, p. 103) reported that

carotene was one-fourth as active in butterfat which had been treated with charcoal as in cottonseed oil. The addition of hydroquinone prevented the destruction of carotene during storage, but did not increase the biological potency. They further found that with butterfat treated with Lloyd's reagent (an adsorbing agent), carotene remained stable at 40°C. but it was only one-half as potent biologically as carotene in cottonseed oil. They further suggested that a factor which supplemented the vitamin A activity of the carotene may have been removed by the Lloyd's reagent. Latherbury and Greenwood (46, p. 1665) reported that carotene gave poorer results when administered in coconut oil with or without quinol than in linseed oil.

Dam (18, p. 172) found that muscular degeneration in chicks reared on vitamin E deficient, low fat diets, was completely prevented by the addition of 0.01 per cent alpha-tocopheryl acetate to the diet. Prange (73, p. 330) found an increased deposition of vitamin A similar to that caused by alpha-tocopheryl acetate and alpha-tocopherol in the livers of chicks reared on a vitamin E deficient diet containing 10 per cent cod-liver oil when methylene blue, thiodiphenylamine or Antabuse was added. Contrary results were obtained when the cod-liver oil was replaced by lard. Dam *et al.* (17, p. 299) found that 0.126 per cent

methylene blue, 0.089 per cent thionine, 0.0675 per cent thiodiphenylamine, 0.025 per cent Antabuse, 0.5 per cent nordihydroguretic acid (NDGA) and 0.5 per cent ascorbic acid each afforded partial protection against vitamin E-deficiency symptoms. Dam (16, p. 61) showed that 1.5 per cent inositol, when added to the diet, partially protected chicks from exudative diathesis but did not cause increased tocopherol content of depot fat. Tocopheryl acetate, however, prevented exudative diathesis and increased tocopherol deposition in the body fat. Hydroquinone, ascorbic acid, and diamylhydroquinone failed to increase alpha-tocopherol activity as noted by Swick and Baumann (83, p. 120), but diamylhydroquinone itself slightly depressed storage of vitamin A.

Diphenyl-p-phenylenediamine (DPPD) was shown by Oser and Oser (64, p. 796) to be the most effective of the antioxidants in protecting the tocopherols in poultry rations. However, there was an interference in the hormonal control of parturition in the rat, though there was no histological evidence of injury to the pituitary or direct interference in oxytocin secretion. Draper and Johnson (23, p. 1154) reported complete protection from muscular dystrophy in lambs when DPPD (0.1 per cent of the dry matter intake) was added to the ration. Lower levels gave incomplete protection from the disease. Using biological

tests of vitamin A potency, with chick growth, rat growth and liver storage in rats as criteria, Siedler et al. (79, p. 1023) found 1:2-dihydro-2:2:4-trimethyl-6-ethyoxyquinoline (Santoquin) to be the most effective antioxidant, followed closely by DPPD, butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA with BHT).

Rousseau et al. (76, p. 1671-1682) found that in calves on low carotene intake there was little difference in plasma carotene level regardless of antioxidant used. However, at the higher carotene level, Santoquin was the most effective followed by 2-5 ditertiarybutyl-hydroquinone (DTHH). MN[1]-diphenyl-p-phenylenediamine (DPPD), Santoquin or d-alpha-tocopheryl acetate at 0.01 per cent of the ration, or 25 milligrams 2-methyl-1:4-naphthoquinone (Menadione) fed daily to Holstein and Guernsey cows had no effect on the plasma vitamin A levels or depletion time.

High (37, p. 2) found that when rats were fed 2:6-di-tert-butyl-4-methylphenol and d-alpha-tocopherol at the rate of 10 milligrams per day, there was a significant decrease of approximately 50 to 60 per cent in the vitamin A deposition. On the other hand, the antioxidant had no effect on the utilization of preformed vitamin A. In contrast 10 milligrams per day of DPPD, which is only sparingly fat soluble, had no affect on carotene utilization.

The author concluded that none of the antioxidants interfered with the absorption of carotene.

Chou and Marlatt (12, p. 305) found carotene utilization in the diet of Chinese people was increased by substances such as tocopherols.

Burns and co-workers (9, p. 341) found no difference in plasma carotene and vitamin A levels of heifers when 1.0 mg. of alpha-tocopherol was fed daily with 1.0 mg. of vitamin A and 1.0 mg. of beta-carotene, but did find that 2.0 mg. of tocopherol diminished the efficiency of utilization of beta-carotene.

Gullickson et al. (34, p. 557) and Phillips et al. (70, p. 695) reported no effect on the milk fat production or fat percentage when cows were fed 1 g. of mixed tocopherols daily as a part of the normal ration. Conversely, Harris (36, p. 125) found increases of 27 per cent for milk fat concentration and 21 per cent for total milk production (4% milk) for cows given 1.0 mg. of tocopherol daily supplement to poor quality rations. A similar increase in butterfat production was noted by Marayanan et al. (61, p. 87) both in cows and buffaloes when fed 1 g. tocopheryl acetate per head daily. McGillivray (55, p. 119) in a New Zealand study found a significant increase in the carotene and vitamin A content of milk fat when monozygotic twins were fed 1 g. of

alpha-tocopherol per head daily.

Whiting and Loosli (92, p. 665) studied the effects of tocopherols and cod-liver oil on the fat content of milk and noted that feeding 1 g. of tocopherol per cow per day increased the fat percentage of milk while cod-liver oil at the rate of 5 ounces per cow daily decreased the fat percentage approximately 11 per cent. Feeding tocopherol to cows fed cod-liver oil did not prevent the fall in butterfat percentage. Total milk production was not significantly affected by feeding either tocopherol or cod-liver oil. Feeding tocopherol to cows on pasture slightly increased the fat test of the milk, but had no influence upon total milk production. Ferrando et al. (28, p. 810) also found a significant decrease in the milk fat content of dairy cows when given 50 to 75 g. of cod-liver oil daily. When cod-liver oil was fed for several weeks, a decrease in blood tocopherols to 0.11 to 0.20 milligrams per cent was noted, while 0.25 mg. per cent was considered normal.

The addition of tocopherol (0.5 to 1 g.) to the diet of dairy cows was reported by Latschar et al. (47, p. 503) to increase the concentration of blood serum tocopherol more than 15 per cent. Supplementations of 10 g. daily increased the serum tocopherol concentration four fold. A 30 per cent increase in the tocopherol content of blood

serum and 50 per cent increase in the tocopherol of milk was reported by Parrish (66, p. 251) for dairy cattle supplemented daily with 1 g. per 1,000 pounds of body weight. Dry cows grazing on cereal grass pasture were reported to have serum tocopherol levels approximately 50 per cent higher than a similar group of cows receiving only barn rations. Supplements of 0.5 to 1 g. tocopherols daily during the latter stages of gestation increased the tocopherol content of colostrum about 40 per cent, while 10 g. of tocopherol daily resulted in a four-fold increase. The serum tocopherol level of calves born of tocopherol supplemented dams were reported to be higher at birth than those from cows receiving only barn rations.

Crystalline carotene was found by Parrish et al. (67, p. 55) to be only one-half to two-thirds as potent as vitamin A concentrate in swine rations as determined by blood and liver analyses. Rousseau and co-workers (75, p. 1565-1573) compared carotene from alfalfa fed at a rate of 60 mg/lb. of body weight with vitamin A (alcohol) from a dry carrier and found the value of the carotene was only one-fifth to one-seventh of that of vitamin A (alcohol) as measured by the blood plasma and liver of dairy calves. When carotene was fed at the 180 mg level of intake, it was only one-tenth to one-thirteenth the value of vitamin A acetate. When the level of intake was raised

to 540 mg/lb. of body weight, its value had dropped to one-twentieth to one-twenty-fourth the value of vitamin A acetate.

Baumann, Steenbock, Beeson and Rupel (4, p. 167) reported that 3.3 per cent of the vitamin A ingested on a low carotene ration was secreted into the milk while on a high carotene ration, only 1.3 per cent was secreted. The vitamin A and carotene activity of butter was used as a criterion for measuring the efficiency of conversion of carotene in feeds to milk fat carotene and vitamin A.

Work by Baumann et al. (4, p. 167) showed an inverse relationship between carotene utilization and intake. These workers reported that less than two per cent (1.12%) of the ingested carotene from a winter ration and less than one per cent (0.04%) from a summer ration appeared in the milk fat. Under the conditions of the experiment, the vitamin A secreted did not exceed 1.6 per cent of the ingested carotene from the winter ration, nor 0.7 per cent from the summer ration. In terms of international units, only 8,244 I.U. were secreted for each 240,000 I.U. consumed on the winter ration, and only 14,000 I.U. of the 1,040,000 I.U. ingested on a high carotene summer feeding regime.

Boyer (8, p. 433), in studying the vitamin A and

carotene levels necessary to maintain adequate blood vitamin A, expressed the daily carotene requirements to be 75 μg/Kg. for Holstein yearlings and 125 μg/Kg. body weight for Guernsey yearlings. However, Moore and co-workers (57, p. 533), based on spinal fluid pressure measurements, suggested that Guernsey calves require an intake of 34 μg/lb. (76.1 μg/Kg.), Jersey calves 32 μg/lb. (71.0 μg/Kg.) and Holstein and Ayrshire calves 30 μg. (67.2 μg/Kg.) of carotene per pound of body weight. More recently, Eaton and co-workers (26, p. 462) reported that Holstein calves fed 20, 60, 120 and 140 μg. of carotene/lb. of body weight per day converted carotene to vitamin A 1.4 times more efficiently than Guernsey calves. At the "assay" level, these results indicate that a difference in the ability to utilize carotene does exist among breeds of dairy calves. Hauge et al. (37, p. 63) found that Guernsey cows can utilize carotene in alfalfa as readily as carotene from carrot oil in producing high vitamin A milk fat.

Jones et al. (40, p. 3) found that with normal rations, the blood values for Jerseys ranged from 4.8 to 12.0 μg/ml. of carotene and 0.28 to 0.94 μg/ml. of vitamin A, while those of Holsteins were 6.3 to 14.3 μg. and 0.34 to 1.03 μg/ml. for carotene and vitamin A, respectively. Byers

et al. (10, p. 659) found that blood carotene levels necessary to maintain adequate blood vitamin A were 0.5 to 0.7 μg/ml. for Holsteins and 1.1 to 1.4 μg/ml. for Guernseys.

Jones et al. (40, p. 2) reported that carotene fed to dairy cattle at levels of 130 and 390 μg/Kg. of body weight were sufficient to maintain liver vitamin A at levels of 12.4 and 18.7 μg/g., respectively.

EXPERIMENTAL PROCEDURE

The objectives of this research were: to measure the relative efficiency of utilization of various sources of carotene and vitamin A by dairy cattle; to determine the possible effect of the level of supplementation on *in vivo* utilization; and to study the effect of alpha-tocopherol on *in vivo* vitamin A and carotene utilization.

Some of the animals used in this study were from dams maintained on a suboptimal level of carotene intake and are hereafter referred to as animals having low carotene histories. Before supplementing the rations of experimental animals used in the first four phases of this study, all animals were maintained on a suboptimal level of carotene intake until the blood plasma and liver reserves were depleted.

This study was divided into five phases according to the following treatments: carrot oil (Phase A), alfalfa leaf meal (Phase B), synthetic beta-carotene (Phase C), synthetic vitamin A (Phase D) and synthetic vitamin A and carotene supplemented with d-alpha-tocopherol (Phase E). As indicated in Table 1, the first phase involved eleven Jersey and Holstein cows, all but three having low carotene history, and four sets of identical twin Holstein and Brown Swiss bulls fed controlled amounts of beta-carotene

Table 1
Low carotene animals used in study

Animal Number	Breed	Sex	History	Date of Birth	Carotene or Vit. A Intake	Disposition Phase A	Phase B	Phase C	Phase D	Phase E
G-25	Jersey	F	Low-Carotene	10-23-54	65 γg./kg.	x	x	x	x	x
G-36	Jersey	F	Low-Carotene	11- 8-55	"		x	x	x	
G-27	Holstein	F	Low-Carotene	11-11-54	"	x	x	x	x	
TN-3	Jersey	F	Normal	8- 1-53	"		x	x	x	
G-12	Jersey	F	Low-Carotene	10- 5-52	130 γg./kg.	x	x	x	x	
G-13	Holstein	F	Low-Carotene	10- 6-52	"	x	x	x	x	x
J295	Jersey	F	Normal	2- 6-52	"	x	x	x	x	
J267	Jersey	F	Low-Carotene	5-29-50	"		x	x	x	
H605	Holstein	F	Normal	1-11-53	"	x				
H522	Holstein	F	Normal	9-10-46	"		x	x		
H557	Holstein	F	Normal	5-22-49	390 γg./kg.	x				
G-16	Jersey	F	Low-Carotene	6-22-53	"	x	x	x	x	
G-17	Jersey	F	Low-Carotene	6-27-53	"	x				
G-29	Holstein	F	Low-Carotene	5- 4-55	"	x	x	x	x	x
J266	Jersey	F	Low-Carotene	5-26-55	"	x	x	x	x	x
G-13 B1	Holstein	M	Low-Carotene	11- 8-54	50 γg./kg.	x	x	x	x	
TW-2	Hols.-Guern.	M	Normal	3- 2-55	"		x	x	x	
TI-2	Ayr.-Hols.	M	Normal	8-25-52	"	x				
TR-2	Holstein	M	Normal	4-26-54	"	x				
TJ-2	Jersey	M	Normal	9-18-52	"	x				
TV-2	Br. Swiss	M	Normal	2- 6-55	"	x	x	x	x	
TW-1	Hols.-Guern.	M	Normal	3- 2-55	500 γg./kg.	x	x	x	x	
TI-1	Ayr.-Hols.	M	Normal	8-25-52	"	x				
TJ-1	Jersey	M	Normal	9-18-52	"	x				
TR-1	Holstein	M	Normal	4-26-54	"	x				
TV-1	Br. Swiss	M	Normal	2- 6-55	"		x	x	x	
266B1	Jersey	M	Low-Carotene	11-14-55	"		x	x	x	

Table 1, continued

Animal Number	Breed	Sex	History	Date of Birth	Carotene or Vit. A Intake	Disposition Phase A	Phase B	Phase C	Phase D	Phase E
J224	Jersey	F	Normal	6- 6-46	Normal					x
J222	Jersey	F	Normal	4-16-46	Normal					x
J312	Jersey	F	Normal	4-17-53	Normal					x
J313	Jersey	F	Normal	4-22-53	Normal					x
H547	Holstein	F	Normal	11-10-48	Normal					x
H574	Holstein	F	Normal	10-23-50	Normal					x
TL-1	Jer.-Hols.	F	Normal	5- 5-52	Normal					x
TL-2	Jer.-Hols.	F	Normal	5- 5-52	Normal					x
TS-2	Jer.-Guern.	F	Normal	8- 2-54	Normal					x
TN-2	Jersey	F	Normal	8- 1-53	Normal					x

in carrot oil. Two cows having low carotene histories were supplemented with carotene to 65 μg/kg. of body weight/day; two cows having low carotene histories and two cows having normal histories were supplemented with carotene to 130 μg/kg. of body weight/day; and three cows having low carotene histories and one cow having normal carotene history were supplemented with carotene to 390 μg/kg. body weight/day. Five bulls having normal carotene histories were supplemented with carotene to 50 μg/kg. body weight/day; and their twin mates were supplemented with carotene to 500 μg/kg. body weight/day.

In Phase B of this study, twelve females, all but two having low-carotene histories, and two pairs of identical twin bulls and one pair of other males were maintained on low carotene rations supplemented to various levels of carotene in alfalfa leaf meal. Two cows having low carotene histories and used in Phase A of this study, and two cows having normal carotene histories were supplemented to 65 μg of carotene/kg. body weight/day; four cows having low carotene histories and used in Phase A of this study plus one cow having normal carotene history were supplemented with carotene at the ratio of 130 μg/kg. of body weight/day. Three cows used in Phase A of this study and having low-carotene histories were supplemented to 390 μg. carotene/kg. of body weight/day. Two of the identical

twin bulls used in Phase A plus one other male were supplemented with carotene to 50 μg/kg. of body weight/day while their mates were supplemented to 500 μg/kg. of body weight/day. Phases C and D were the same as Phase B with the exceptions of treatments as shown above and the loss of two animals fed synthetic vitamin A at a level of 130 μg/ kg. of body weight/day.

The beta-carotene used in Phase A was supplied by the Barnett Laboratories Inc. in the form of carrot oil containing, by analysis at the time of feeding, an average of 7,734 micrograms of carotene per ml. of carrot oil. Alpha-tocopherol was added by the manufacturer as an antioxidant. The natural alfalfa leaf meal used in Phase B was ground from pelleted choice dehydrated alfalfa supplied by the Dixon Dryer Co. This leaf meal averaged 217 μg. carotene per g. of meal and had one per cent animal fat stabilized with butylated-hydroxy-anisole (BHA). The synthetic beta-carotene used in Phase C was supplied by Hoffman-LaRoche Company, Inc. as a semi-solid suspension in vegetable oil (Wesson oil) with a potency of 400,000 U.S.P. units of vitamin A per gram and contained BHA or butylated-hydroxy-toluene (BHT) as an antioxidant. Before feeding, this suspension was further diluted with cottonseed oil to contain 3.27 mg. of carotene per ml. The synthetic vitamin A supplied by the Hoffman-LaRoche Co., Inc. used

in Phase D was a dry stabilized vitamin A palmitate (Rovomix A-325) containing 325,000 U.S.P. units per g. of Rovomix and was suspended in a gelatin-sugar-starch base.

The alpha-tocopherol used in Phase E of this study was obtained from Distillation Products Industries as d-alpha-tocopheryl acetate blended in soybean oil meal to contain 44.05 I.U. per g. of supplement (Myvamix Vitamin E Feed Supplement).

During the first feeding period of Phase E, five lactating Jersey and Holstein cows, previously used during Phase D, were fed controlled amounts of vitamin A palmitate. One of the experimental cows completed her lactation ahead of schedule and was removed from the experiment. The four remaining cows were supplemented with one gram of d-alpha-tocopherol per day. Ten normal Holstein and Jersey cows were paired according to breed, age and stage of lactation for periods 2, 3, and 4 of Phase E. Carotene contained in hay and silage, early spring irrigated pasture and fall irrigated pasture was fed and supplemented with one gram of d-alpha-tocopherol/animal/day (22.7 grams Myvamix).

Blood, liver and milk fat samples were taken bi-monthly on the same day for all but Phase E when blood and liver samples were taken at monthly intervals and milk fat samples at ten day intervals. The blood was collected

from the jugular vein and analyzed for carotene and vitamin A by a modification (saponification) of the Kimbles' method (4, p. 1055-1065). The liver samples were taken using the biopsy technique developed by Bone (7, p. 742-752) and were analyzed for carotene and vitamin A using the method developed by Gallup and Hoefer (30, p. 288-290). Milk fat samples were composited by separating milk from three to six milkings. Milk fat was analyzed for carotene and vitamin A via the method approved by the technical committee in charge of the nation-wide butter survey of the United States Department of Agriculture (88, p. 1-10).

In all cases carotene and vitamin A supplements were added to a low carotene ration consisting of ground oats and barley, soybean oil meal, molasses beet pulp and hay containing less than 1.0 to 5.0 p.p.m. carotene (Table 2). Feed analyses for carotene were carried out using the modified A.O.A.C. method (84, p. 219-224) and are reported on an air dry basis. The carotene in the ration of each animal was calculcated at regular intervals (Table 1). One I.U. of vitamin A was considered equivalent to 0.6μg. carotene or 0.344μg. of pure vitamin A acetate. In calculating the vitamin A potency of milk fat, one I.U. of vitamin A was considered equivalent to 0.6μg. carotene or 0.25μg. of vitamin A. Analytical corrections for vitamin

Table 2
Feed analysis - air dry basis

Date	Description	Carotene
		p.p.m.
1955	Ryegrass hay	3.1
1955		2.3
1956	1 year old ryegrass hay	2.0
1956	2 year old ryegrass hay	0.3
1956	Ryegrass hay	3.0
1956	Ryegrass hay	2.3
1956	Ryegrass hay	2.4
4- 4-56	Barley straw	0.6
4- 4-56	Alfalfa leaf meal (2 years old)	18.2
5-21-56	Alfalfa leaf meal	221.0
7-27-56	Alfalfa leaf meal	214.0
7-27-56	Alfalfa leaf meal	217.0
6-26-57	1st cutting alfalfa & grass (weathered)	7.0
7- 9-57	1st cutting alfalfa & grass	5.2
7-13-57	Ryegrass hay	5.0
7-30-57	Ryegrass hay	0.9
3-15-58	Oat & vetch hay	10.3
3-31-58	Alfalfa hay	32.2
4-15-58	Grass silage	41.0*
4-16-58	Oat and vetch hay	14.9

* wet basis

A in milk fat were made on the basis that only 93 per cent of the vitamin was recovered (91, p. 4).

RESULTS AND DISCUSSION

The mean blood plasma, liver and milk fat carotene and vitamin A values for experimental cows fed controlled levels of carotene and/or vitamin A are found in Tables 3 through 8. The values for cows fed carotene are in close agreement with liver carotene and vitamin A values reported by Jones *et al.* (40, p. 2). These workers reported vitamin A values of 12.4 and 18.7 μg/g. of fresh liver for cows fed 130 and 390 μg/kg. of carotene daily, respectively.

The results of this study indicated that blood plasma, liver and milk fat vitamin A and carotene values reflect the ability of the animal to utilize various dietary levels and forms of the vitamin and are, therefore, criteria for measuring the utilization of the vitamin *in vivo*. An analysis of variance indicated (Table 3) that there were statistically significant differences in blood carotene between both the levels fed and carotene sources. The most marked differences in blood plasma carotene were observed when the daily carotene intake varied from the 130 μg. to the 390 μg. level. As shown by the 5% Multiple Range Test in Table 3, blood carotene values from alfalfa leaf meal were significantly higher than those from synthetic vitamin A. Blood plasma carotene values

Table 3
Mean blood carotene from experimental cows

Source	Level fed in μg./Kilogram body weight 65μg.	130μg.	390μg.	Mean
A (Carrot oil)	0.15	0.94	2.82	1.30
B (Alfalfa leaf)	0.63	1.21	2.89	1.58
C (Syn. B carotene)	0.87	0.62	1.83	1.11
D (Syn. Vit. A)	0.16	0.10	0.16	0.14
Mean	0.45	0.72	1.93	

	df	SS	MS	F
Treatment	11	10.8510		
Level	2	4.9288	2.464	19.87**
Source	3	3.5146	1.172	9.45**
Level X Source	6	2.4076	0.401	3.23*
Error	31	3.8440	0.124	

5% Multiple Range Test for Source[1]

	B	A	C
D	1.44 (0.63)	1.16 (0.62)	0.97 (.59)
C	0.47 (0.62)	0.19 (0.59)	
A	0.28 (0.59)		

1 ( ) LSD
** Significant at 1% level
* Significant at 5% level
Blood values in μg./ml.

Table 4
Mean blood vitamin A from experimental cows

Source	Level fed in μg./Kilogram body weight 65μg.	130μg.	390μg.	Mean
A (Carrot oil)	0.10	0.22	0.30	0.21
B (Alfalfa Leaf)	0.19	0.22	0.31	0.24
C (Syn. B carotene)	0.18	0.19	0.28	0.22
D (Syn. Vit. A)	0.22	0.26	0.27	0.25
Mean	0.17	0.22	0.29	

	df	SS	MS	F
Treatment	11	.0392		
Level	2	.0278	.0134	26.80**
Source	3	.0036	.0012	2.40
Level X Source	6	.0078	.0013	2.60*
Error	31	.0152	.0005	

5% Multiple Range Test for Source[1]

	D	B	C
A	.04 (.01)	.03 (.01)	.01 (.01)
C	.03 (.01)	.01 (.01)	
B	.01 (.01)		

1 ( ) LSD
** Significant at 1% level
* Significant at 5% level
Blood values in μg./ml.

Table 5
Mean liver carotene from experimental cows

Source	Level fed in μg./Kilogram body weight 65 μg.	130 μg.	390 μg.	Mean
A (Carrot oil)	0.14	0.89	2.10	0.71
B (Alfalfa leaf)	0.67	1.42	5.86	2.65
C (Syn. B Carotene)	0.68	0.81	2.03	1.17
D (Syn. Vit. A)	0.44	0.44	0.57	0.48
Mean	0.48	0.89	2.64	

	df	SS	MS	F
Treatment	11	29.1029		
Level	2	8.5350	4.2675	8.7520**
Source	3	13.1028	4.3676	8.9573**
Level X Source	6	7.4651	1.2441	2.5514*
Error	29	14.1409	0.4876	

1% Multiple Range Test for Source[1]

	B	C	A
D	2.17 (1.6)	.69 (1.6)	.23 (1.5)
A	1.94 (1.6)	.46 (1.5)	
C	1.48 (1.5)		

1 ( ) LSD
** Significant at 1% level
* Significant at 5% level
Liver values in μg./gram of wet liver

Table 6

Mean liver vitamin A from experimental cows

Source	Level fed in μg./Kilogram body weight 65μg.	130μg.	390μg.	Mean
A (Carrot oil)	0.95	5.72	20.11	8.93
B (Alfalfa leaf)	9.11	15.10	32.00	18.74
C (Syn. B carotene)	6.43	9.86	29.06	15.12
D (Syn. Vit. A)	12.85	37.82	124.37	58.35
Mean	7.33	17.25	51.38	

	df	SS	MS	F
Treatment	11	12149.503		
Level	2	4494.166	2247.0834	862.603**
Source	3	4280.390	1423.4633	546.435**
Level X Source	6	3374.946	562.4910	215.9274**
Error	29	75.557	2.6050	

1% Multiple Range Test for Source[1]

	D	B	C
A	49.2 (1.2)	9.8 (1.2)	6.1 (1.2)
C	43.2 (1.2)	3.6 (1.2)	
B	39.6 (1.2)		

1 ( ) LSD

** Significant at 1% level

* Significant at 5% level

Liver values in μg./gram of wet liver

Table 7

Mean milk fat carotene from experimental cows

Source	Level fed in μg./Kilogram body weight 65μg.	130μg.	390μg.	Mean
A (Carrot oil)	0.43	0.44	1.04	0.62
B (Alfalfa leaf)	0.97	0.42	3.50	1.63
C (Syn. B carotene)	0.33	0.34	0.53	0.40
D (Syn. Vit. A)	0.27	0.19	0.33	0.26
Mean	0.50	0.35	1.35	

	df	SS	MS	F
Treatment	11	9.1705		
Level	2	3.4441	1.7220	25.28**
Source	3	2.3928	0.7926	11.71**
Level X Source	6	3.3336	0.5556	8.15**
Error	17	1.1592	0.0681	

1% Multiple Range Test for Source[1]

	B	A	C
D	1.37 (.66)	.36 (.64)	.14 (.61)
C	1.23 (.64)	.36 (.61)	
A	1.01 (.61)		

[1] ( ) LSD

** Significant at 1% level

Milk fat values in μg./gram of butter fat

Table 8

Mean milk fat vitamin A from experimental cows

Source	Level fed in μg./Kilogram body weight 65μg.	130μg.	390μg.	Mean
A (Carrot oil)	1.63	2.10	3.64	2.46
B (Alfalfa leaf)	3.41	3.18	6.32	4.30
C (Syn. B. carotene)	1.87	1.18	3.16	2.07
D (Syn. Vit. A)	4.33	8.08	8.49	6.97
Mean	2.81	3.63	5.40	

	df	SS	MS	F
Treatment	11	65.8387		
Level	2	44.9687	22.484	21.753**
Source	3	14.034	4.678	4.525*
Level X Source	6	6.836	1.139	1.102
Error	17	1.0336	0.060	

1% Multiple Range Test for Source[1]

	D	B	A
C	4.9 (.61)	4.5 (.60)	.39 (.57)
A	4.5 (.60)	1.8 (.57)	
B	2.6 (.57)		

1 ( ) LSD

** Significant at 1% level

* Significant at 5% level

Milk fat values in μg./gram of butter fat

obtained from cows fed carrot oil were significantly higher than those values obtained from cows fed synthetic vitamin A. Blood plasma carotene values from cows fed alfalfa leaf meal and synthetic beta-carotene were not significantly different while those values from cows fed the synthetic vitamin were significantly lower than the values obtained from cows fed beta-carotene in alfalfa leaf meal, synthetic beta-carotene, or carrot oil. This may be expected since it was reported by Jones et al. (40, p. 1) that blood plasma carotene values reflect the dietary carotene intake. In addition, there is no evidence to indicate that vitamin A will undergo reversal to carotene. The low blood plasma carotene values for all three levels of synthetic vitamin A fed with a low carotene hay (1 p.p.m.) resulted in blood plasma carotene values of 0.10 to 0.16 μg/ml. Assuming that the efficiency of utilization of the vitamin is indirectly proportional with the dietary intake, these values may nave been lower had synthetic vitamin A been restricted from the diet.

Blood plasma vitamin A values (Table 4) obtained for cows fed 65 μg. of carrot oil per Kg. of body weight per day were significantly lower than all other values. No other significant differences in blood vitamin A values were observed for carrot oil between source or within

source comparisons at the 65 and 130 μg. levels of carotene. However, the blood vitamin A values obtained when cows were fed beta-carotene from carrot oil, alfalfa leaf meal, and synthetic beta-carotene at the 390 μg. level, were significantly higher ($P > 0.01$) than those obtained when synthetic vitamin A was fed. There was no significant difference in blood vitamin A potency regardless of source. The blood vitamin A values of cows fed synthetic vitamin A did not change significantly as the dietary intake of the vitamin increased. Assuming that the blood plasma vitamin A potency has a physiological function, it appears that the dairy cow would utilize vitamin A and carotene at diminishing rates as the level of intake is increased.

As indicated by the values in Table 5 and 6, liver vitamin A and carotene increased with the level fed and varied significantly with the source of the vitamin. Liver carotene values from cows fed alfalfa leaf meal were higher than those from the other three sources. Liver carotene values from cows fed synthetic beta-carotene were not significantly higher than those values obtained when carrot oil or synthetic vitamin A was fed. There was no significant difference between the liver carotene values when cows were fed carrot oil and synthetic vitamin A.

As shown in Table 6 significantly higher liver vitamin A values were obtained at the 390 μg. level of

carotene intake compared to the 65μg. level. Liver vitamin A values varied significantly ($P > 0.01$) depending on the source of the vitamin. The significantly higher liver vitamin A values obtained from cows fed alfalfa leaf meal as compared to those values obtained when fed synthetic beta-carotene, and the higher vitamin A values obtained by feeding synthetic beta-carotene as compared with those values for carrot oil was apparently associated with the ability of the animal to convert carotene to vitamin A. The high liver vitamin A values obtained by feeding synthetic vitamin A demonstrated the ability of the dairy cow to store larger amounts of the vitamin when fed in this form as compared to the three forms of beta-carotene.

To discuss the superiority of one carrier of beta-carotene with another, one must assume that the antioxidants added as stabilizers have equal stabilizing abilities. Then too, one must assume that the increased storage of vitamin A is due to increased efficiency in utilization. The antioxidants added to those supplements (page 28) have been shown to have varied effects on carotene and vitamin A utilization (12, p. 305; 79, p. 1023). The increased liver storage of vitamin A indicated, under the conditions of this experiment, that synthetic vitamin A palmitate, in a gelatin beadlet form, had a higher biological value than

that reported for vitamin A alcohol by the Expert Committee on Biological Standardization (27, p. 147-148), who reported beta-carotene to be equivalent to half that of vitamin A. The results in Table 4 show that dairy cows given vitamin A palmitate stored four times more liver vitamin A on an equal weight basis than the same cows fed beta-carotene. The vitamin A values of the milk fat, on the other hand, were in good agreement with the 2:1 ratio.

Analyses of milk fat (Tables 7 and 8) indicated that both carotene and vitamin A increased significantly with the dietary levels and source of the vitamin. Synthetic vitamin A produced butterfat significantly higher in vitamin A than any of the other three sources of the vitamin. Alfalfa leaf meal produced milk fat of higher carotene and vitamin A value than beta-carotene in carrot oil or synthetic beta-carotene. The low biological value for carrot oil and synthetic beta-carotene as measured by the blood plasma, liver and milk fat indicated the oily carrier to lower rather than increase the rate of absorption and utilization of beta-carotene in dairy cattle. This is contrary to work reported by Russell et al. (77, p. 211) using chickens, Lewis and associates (49, p. 486) and Kramer et al. (44, p. 295) using rats, all of whom reported increased absorption and utilization with injected carotene in oily solutions.

Correlation coefficients among means (Table 9) indicated a positive correlation between the amount of the vitamin fed, blood plasma, carotene and plasma vitamin A, liver carotene and liver vitamin A. There was a positive correlation between liver vitamin A and the amount of the vitamin fed. However, there was no correlation between liver vitamin A and blood plasma vitamin A levels. There were positive correlations between milk fat carotene, blood carotene and liver carotene, and between milk fat vitamin A, blood vitamin A, and liver vitamin A. There was no correlation between blood carotene and liver vitamin A and milk vitamin A, which further substantiates the belief that vitamin A does not undergo the reversal to carotene in vivo.

Carotene and vitamin A values from experimental bulls (Tables 10 and 11) indicated a significant increase in carotene and vitamin A potency of blood plasma when the level fed was increased from 50 to 500 μg/kg. body weight/day. This increase was, however, not proportional to the dietary intake of the vitamin. While the level of intake was increased by ten-fold, the mean plasma carotene and vitamin A increased by only 4.7 and 1.9-fold, respectively. There was no significant difference in blood plasma carotene ($P < 0.05$) between sources of the vitamin. However, there was a significant difference with source in vitamin

Table 9
Correlation coefficient of means of Data, Tables 3 through 8

	Liver Carotene	Liver Vit. A	Blood Plasma Carotene	Blood Plasma Vit. A.	Milk Carotene	Milk Vit.A	Vit. A Source
Liver Carotene	---	0.04	0.84**	0.63*	0.94**	0.22	0.63*
Liver Vit. A	0.04	---	-0.12	0.48	0.00	0.79**	0.59*
Blood Plasma Carotene	0.84**	-0.12	---	0.63*	0.72*	-0.04	0.67*
Blood Plasma Vit. A	0.63*	0.48	0.63*	---	0.46	0.62*	0.82**
Milk Carotene	0.94**	0.00	0.72*	0.46	---	0.23	0.48
Milk Vit. A	0.22	0.79**	-0.04	0.62*	0.23	---	0.46
Vitamin A Source	0.63*	0.59*	0.67*	0.82**	0.48	0.46	---

** Significant at 1% level
* Significant at 5% level

Table 10
Mean blood plasma carotene from experimental bulls

Source	Level fed in μg./Kilogram body weight 50 μg.	500 μg.	Mean
A (Carrot oil)	0.23	2.02	1.12
B (Alfalfa leaf)	0.29	1.69	0.99
C (Syn. B carotene)	0.20	0.61	0.40
D (Syn. Vitamin A)	0.22	0.12	0.17
mean	0.23	1.11	

	df	SS	MS	F
Treatment	7	4.7845		
Level	1	2.1134	2.1134	11.442**
Source	3	2.3854	0.7951	4.3048*
Level X Source	3	0.2857	0.0952	0.5154
Error	20	3.6954	0.1847	

5% Multiple Range Test for Source[1]

	A	B	C
D	.95 (.97)	.82 (.95)	.23 (.90)
C	.72 (.95)	.59 (.90)	
A	.13 (.90)		

1 ( ) LSD
** Significant at 1% level
* Signifi cant at 5% level

Table 11
Mean blood plasma vitamin A from experimental bulls

Source	Level fed in μg./Kilogram body weight 50 μg.	500 μg.	Mean
A (Carrot oil)	0.15	0.29	0.22
B (Alfalfa leaf)	0.10	0.34	0.27
C (Syn. B carotene)	0.15	0.32	0.23
D (Syn. Vitamin A)	0.28	0.34	0.31
mean	0.19	0.32	

	df	SS	MS	F
Treatment	7	.0455		
Level	1	.0046	.0046	46.00**
Source	3	.0326	.0108	108.00**
Level X Source	3	.0084	.0028	28.00**
Error	20	.0026	.0001	

1% Multiple Range Test for Source[1]

	D	B	C
A	.09 (.01)	.05 (.01)	.01 (.01)
C	.08 (.01)	.04 (.01)	
B	.04 (.01)		

1 ( ) LSD
** Significant at 1% level

A potency of the blood ($P > 0.01$). Synthetic vitamin A produced vitamin A values significantly higher than the three sources of beta-carotene. Alfalfa leaf meal produced plasma vitamin A values significantly higher than the other two sources of beta-carotene.

As shown in Tables 12 and 13, bulls receiving 500 µg. of carotene/kg. body weight per day had liver carotene values significantly higher than those receiving the 50 µg. level. Beta-carotene in carrot oil produced the highest liver carotene values ($P > 0.01$) followed closely by beta-carotene in alfalfa leaf meal. Synthetic vitamin A produced the highest liver vitamin A values ($P > 0.01$). Alfalfa leaf meal resulted in liver carotene values significantly higher than carrot oil while carrot oil gave liver carotene values significantly higher than synthetic beta-carotene. All liver vitamin A values were significantly higher at the 500 microgram level of intake. Liver carotene increased three-fold and liver vitamin A eight-fold with a ten-fold increase in level fed. At the 50 µg. level of intake, synthetic vitamin A increased liver vitamin A values six-fold. When the level fed was increased by ten-fold, liver stores were increased fifteen-fold and produced values eleven times greater than that produced by beta-carotene.

Table 12
Mean liver carotene of experimental bulls

Source	Level fed in μg./Kilogram body weight 50μg.	500μg.	Mean
A (Carrot oil)	0.32	1.62	0.97
B (Alfalfa leaf)	0.48	1.40	0.94
C (Syn. B Carotene)	0.35	0.76	0.55
D (Syn. Vitamin A)	0.30	0.31	0.30
mean	0.36	1.02	

	df	SS	MS	F
Treatment	7	1.9670		
Level	1	.6147	.2049	12.052**
Source	3	.8712	.2904	17.082**
Source X Level	3	.4811	.1604	9.435**
Error	17	.2890	.0170	

1% Multiple Range Test for Source[1]

	A	B	C
D	.67 (.39)	.64 (.38)	.26 (.36)
C	.41 (.38)	.41 (.36)	
B	.03 (.36)		

1 ( ) LSD
** Significant at 1% level
Liver values in μg./gram of wet liver

Table 13
Mean liver vitamin A from experimental bulls

Source	Level fed in μg./Kilogram body weight 50 μg.	500 μg.	Mean
A (Carrot oil)	2.24	16.44	9.34
B (Alfalfa leaf)	2.18	23.98	13.08
C (Syn. B carotene)	1.82	11.82	6.82
D (Syn. Vitamin A)	12.35	189.04	100.60
mean	4.65	60.32	

	df	SS	MS	F
Treatment	7	26383.6759		
Level	1	4137.3296	4137.3296	357.498**
Source	3	10385.5578	346.1852	299.131**
Source X Level	3	11860.7885	3953.5961	341.620**
Error	17	196.7423	11.5730	

1% Multiple Range Test for Source[1]

	D	B	A
C	93.87 (10.58)	6.26 (10.32)	2.52 (9.84)
A	91.35 (10.32)	3.74 (9.84)	
B	87.61 (9.84)		

[1] ( ) LSD
** Significant at 1% level
Liver values in μg./gram of wet liver

The carotene and vitamin A potency of the blood plasma, liver and milk fat of experimental cows fed vitamin A palmitate supplemented with one gram of d-alpha-tocopherol per day are found in Tables 14 through 17.

Feeding one gram of d-alpha-tocopherol per day resulted in a 65, 55, and 50 per cent increase in vitamin A potency of the blood plasma, milk fat, and liver vitamin A activity, respectively, and an average increase in vitamin A potency of the blood plasma, liver and milk fat of 57 per cent. Due to the small number of animals used in this experiment and the few observations obtained, the only values indicating a statistically significant difference were those for liver vitamin A potency. Based on the findings of the Expert Committee on Biological Standardization (27, p. 147-148) that 0.3μg. of vitamin A equal 1 I.U. of vitamin A activity, a 57 per cent increase in biological potency of the vitamin would mean that 1 I.U. of vitamin A activity is equal to almost 0.2 (0.19)μg. of vitamin A or a 36 per cent increase in the efficiency of utilization when supplemented with one gram of d-alpha-tocopherol. Blood plasma carotene values increased 90 per cent while the liver and milk fat carotene potency decreased by 69 and 20 per cent, respectively. Inasmuch as the general trend was toward an increase in vitamin A potency and decrease in carotene potency of the liver and milk

Table 14

Mean carotene and vitamin A values of blood plasma and liver from experimental and control cows

Animal Number	Vit. E fed/day	Carotene and Vitamin A intake/kg./b.w.	Blood Plasma				Liver			
			Carotene		Vitamin A		Carotene		Vitamin A	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
	I.U.		μg./ml.		μg./ml.		μg./g.		μg./g.	
February 1958 - Low Carotene Hay Ration										
J266	991	296 I.U. - A**	0.42	1.10	0.26	0.44	0.74	0.01	107.5	187.0
G-29	991	296 " "	0.15	0.00*	0.23	0.14	0.33	0.18	147.0	180.0
G-13	991	296 " "	0.08	0.27	0.22	0.45	0.74	0.41	64.2	29.4
G-25	991	49 " "	0.09	0.07	0.21	0.48	0.61	0.15	8.2	5.9
Mean	991	185 " "	0.19	0.36	0.23	0.38	0.61	0.19	67.3	100.6
March 1958 - Normal Hay and Silage Ration										
J224	991	2.25 Mg. - C***	3.60	4.90	0.49	0.43	5.48	----	72.9	----
J222	None	2.05 " "	7.42	8.16	0.33	0.81	11.03	11.40	117.0	103.0
J312	991	2.30 " "	9.73	10.84	0.57	0.97	10.60	11.80	127.0	109.0
J313	None	2.56 " "	7.70	8.64	0.40	0.69	7.32	12.00	115.0	130.0
H547	991	2.10 " "	3.38	4.90	0.20	0.32	5.85	7.40	143.0	155.0
H574	None	2.15 " "	5.32	7.15	0.28	0.58	1.49	7.97	45.1	64.8
TL-1	991	2.30 " "	10.30	9.60	0.74	0.88	12.45	15.40	116.0	118.0
TL-2	None	2.07 " "	7.20	8.80	0.68	0.89	10.40	13.25	105.0	137.0
TS-2	991	2.14 " "	3.17	5.33	0.33	0.62	6.24	6.44	54.8	61.6
TN-2	None	1.84 " "	4.75	12.50	0.40	1.11	11.57	14.10	69.3	78.2
Mean	Treated	2.22 " "	6.18	7.07	0.47	0.58	8.12	10.26	102.74	110.9
	Control	2.13 " "	6.48	9.05	0.42	0.82	8.36	11.74	90.28	102.6

* Trace (not readable)

** International Units Vitamin A Palmitate

*** Milligrams Carotene

Table 15

Mean carotene and vitamin A values of blood plasma and liver from experimental and control cows (continued)

Animal Number	Vit. E fed/day	Carotene and Vitamin A intake/kg./b.w.	Blood Plasma				Liver			
			Carotene		Vitamin A		Carotene		Vitamin A	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final
	I.U.		µg./ml.		µg./ml.		µg./g.		µg./g.	
May 1958 - Spring Pasture (early growth)										
J299	991		12.80	11.80	1.15	0.94	18.90	18.90	207.0	219.0
	None		10.90	10.10	0.85	0.66	18.00	16.40	134.0	125.0
J312	991		15.20	14.20	1.65	0.86	16.30	17.60	184.0	167.0
J320	None		11.30	11.60	0.80	0.66	13.20	14.30	164.0	179.0
H547	991		8.40	7.10	0.58	0.60	11.00	10.00	170.0	171.0
H574	None		10.20	7.70	0.68	0.45	11.40	9.60	87.0	84.0
TL-1	991		14.71	14.00	1.43	1.02	26.20	23.00	158.0	174.0
TL-2	None		12.80	10.60	1.91	0.62	20.40	17.40	139.0	173.0
TX-1	991		12.00	13.50	1.74	0.94	15.70	15.40	110.0	125.0
TN-2	None		18.00	15.60	2.11	1.47	18.40	17.70	112.0	94.0
Mean	Treated		12.62	12.12	1.31	0.87	17.62	16.98	165.8	171.2
	Control		12.64	11.12	1.27	0.77	16.28	15.08	127.2	131.0
September 1958 - Fall Pasture (irrigated pasture)										
J299	991		8.65	13.70	0.62	0.93	9.60	13.05	127.0	129.0
J222	None		6.05	9.65	0.42	1.05	9.57	4.88	74.5	64.0
J313	991		8.42	9.85	0.54	0.93	9.00	9.88	95.2	98.2
J320	None		7.92	10.58	0.56	1.09	7.65	10.38	126.0	110.0
H547	991		4.53	5.46	0.40	0.31	3.47	6.38	112.0	118.0
H574	None		4.68	6.97	0.47	0.47	4.96	5.73	54.0	61.8
TL-1	991		11.10	14.40	0.80	1.30	9.88	15.60	134.0	129.0
TL-2	None		9.00	11.95	0.68	1.41	5.93	11.80	110.0	106.0
TU-2	991		8.65	12.20	0.79	1.11	7.43	8.23	114.0	93.0
TN-2	None		12.60	16.75	1.11	2.05	10.85	13.50	89.0	70.5
Mean	Treated		8.27	11.12	0.63	0.92	7.88	10.63	164.0	113.44
	Control		8.05	11.18	0.65	1.21	7.79	9.26	90.7	82.46

Table 16

Mean carotene and vitamin A values of milk fat from experimental and control cows

Animal Number	Vit. E fed/day	Vitamin A intake/kg./b.w.	Milk Fat					
			Carotene		Vitamin A		I.U. Vitamin A	
			Initial	Final	Initial	Final	Initial	Final
	I.U.		μg./g.		μg./g.		μg./g.	
February 1958 - Low Carotene Hay Ration								
J266	991	296 I.U. - A*	0.40	0.26	4.18	12.15	17.39	49.03
G-29	991	296 " "	0.35	0.29	6.37	16.79	26.07	67.64
G-13	991	99 " "	0.15	0.21	11.12	6.79	44.75	27.51
G-25	991	49 " "	0.50	0.37	3.81	3.75	16.07	15.62
Mean		185 " "	0.35	0.28	6.37	9.87	23.57	39.95
March 1958 - Normal Hay and Silage Ration								
J224	991	2.25 mg. - C**	4.60	5.30	6.00	5.90	31.67	32.43
J222	None	2.05 " "	4.00	4.93	6.68	5.98	33.39	32.14
J312	991	2.30 " "	2.40	4.73	7.32	6.79	33.28	35.04
J313	None	2.56 " "	4.00	8.00	5.94	7.50	30.43	43.33
H547	991	2.10 " "	1.60	2.03	8.08	8.41	34.99	37.02
H574	None	2.15 " "	2.10	3.43	6.00	7.66	27.50	36.36
TL-1	991	2.30 " "	6.00	5.67	6.84	6.75	37.36	36.45
TL-2	None	2.07 " "	4.80	----	6.78	----	35.12	----
TS-2	991	2.14 " "	1.50	4.27	5.44	5.39	24.26	28.68
TN-2	None	1.84 " "	3.40	10.10	6.46	5.49	31.51	38.79
Mean	Treated	2.22 " "	3.22	4.40	6.74	6.48	32.31	33.92
	Control	2.13 " "	3.66	6.62	6.37	6.66	31.59	30.12

* Trace (not readable)

** International Units Vitamin A Palmitate

Table 17

Mean carotene and vitamin A values of milk fat from experimental and control cows

Animal Number	Vit. E fed/day	Vitamin A Intake/kg./b.w.	Milk Fat					
			Carotene		Vitamin A		I.U. Vitamin A	
			Initial	Final	Initial	Final	Initial	Final
	I.U.		μg./g.		μg./g.		μg./g.	
May 1958 - Spring Pasture (early growth)								
J299	991		9.00	8.50	6.00	6.54	39.00	40.33
J222	None		12.80	7.00	9.12	7.78	57.81	42.79
J312	991		6.60	6.10	9.84	9.24	50.36	47.13
J320	None		9.20	6.10	8.00	8.31	47.33	43.41
H547	991		3.00	1.40	8.30	8.78	38.20	37.45
H574	None		5.60	3.10	9.60	9.83	47.73	44.49
TL-1	991		11.00	9.10	9.84	9.60	57.69	53.57
TL-2	None		8.20	7.70	10.08	8.65	53.99	47.43
TX-1	991		13.10	9.30	6.15	7.27	46.43	44.58
TN-2	None		5.80	6.10	7.54	7.80	39.83	41.37
Mean	Treated		8.54	6.88	8.03	8.29	46.34	44.62
	Control		8.32	6.00	8.87	8.47	49.34	43.90
September 1958 - Fall Pasture (irrigated pasture)								
J299	991		7.00	8.30	7.02	8.31	39.75	47.07
J222	None		4.10	6.80	8.66	7.30	41.47	40.47
J313	991		6.80	7.93	9.72	10.08	50.21	53.54
J320	None		5.00	5.77	7.06	7.96	36.57	41.46
H547	991		1.40	1.73	9.36	9.41	39.77	40.52
H574	None		2.60	3.73	10.04	10.31	44.49	47.46
TL-1	991		9.40	9.73	10.22	10.73	56.55	59.14
TL-2	None		7.40	8.90	9.14	10.35	48.89	56.23
TU-2	991		3.80	6.13	7.08	8.19	34.65	42.98
TN-2	None		13.40	14.90	6.62	8.93	48.81	60.55
Mean	Treated		5.68	6.76	8.68	9.34	44.19	48.65
	Control		6.50	8.02	8.30	8.97	44.06	49.23

fat, it would appear that there was a biologically significant increase in the utilization of vitamin A whereas carotene utilization was suppressed. It is, therefore, suggested from these limited data that while the main role of alpha-tocopherol in the utilization of the vitamin may be as an antioxidant, it may act secondarily as a co-factor for the enzyme system "carotenase" responsible for the conversion of carotene to vitamin A. This would not only account for the lowered carotene value noted in these tables but helps to explain the high biological value which appears to prevail for the vitamin A palmitate.

The results of supplementing one gram of d-alpha-tocopherol daily to lactating Jersey and Holstein cows receiving carotene from hay and silage, early spring pasture, or fall pasture are found in Tables 14 through 17. An analysis of covariance showed no significant differences in the carotene and vitamin A potency of milk fat between the treated and control cows as measured by the values obtained for each treatment.

The results herein are, therefore, contrary to the findings of McGillivray (55, p. 119) who found one gram of alpha tocopherol fed per day to cows on pasture to increase the vitamin A activity of New Zealand butter.

The slight variation between vitamin A activity of butter from control and treated cows illustrated in

Figure 1 represents the normal, expected variation among samplings and among cattle and is not, therefore, indicative of the experimental treatments. With the exception of the early fall values, the seasonal fluctuations in the vitamin A activity of butter shown by both the treated and control groups receiving carotene in hay, silage or pasture correspond very closely with those seasonal variations reported by Weswig and co-workers (92, p. 425) for Oregon-produced butter. The high vitamin A and carotene values obtained during September and October for both the supplemented and unsupplemented groups is probably due to the lush irrigated pastures that were saved exclusively for the experimental animals and no doubt contained considerably more carotene than most pastures for this time of year.

The blood and liver carotene and vitamin A values of cows fed one gram of d-alpha-tocopherol daily as part of the normal ration had no effect on the carotene and vitamin A potency of blood plasma and liver tissue. These results agree with the work reported by Gullickson et al. (34, p. 557) and Phillips et al. (70, p. 695) but disagree with the findings of Harris (36, p. 125) and Maroyanan et al. (61, p. 87).

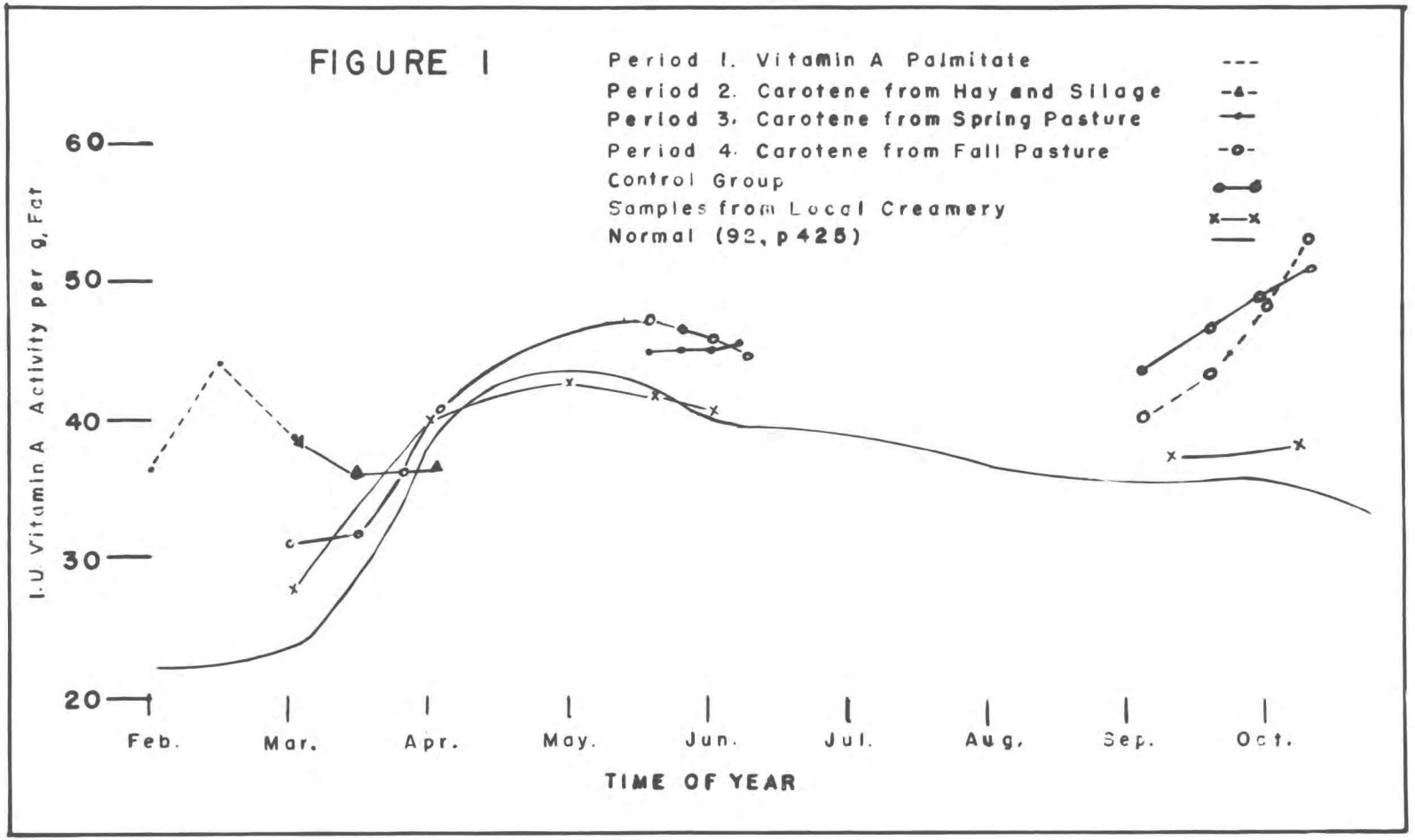
FIGURE I
Period 1. Vitamin A Palmitate
Period 2. Carotene from Hay and Silage
Period 3. Carotene from Spring Pasture
Period 4. Carotene from Fall Pasture
Control Group
Samples from Local Creamery
Normal (92, p 425)
I.U Vitamin A Activity per g. Fat
60
50
40
30
20
Feb.
Mar.
Apr.
May.
Jun.
Jul.
Aug.
Sep.
Oct.
TIME OF YEAR

SUMMARY

This report presents the results of feeding controlled levels of beta-carotene in carrot oil, alfalfa leaf meal, synthetic beta-carotene or vitamin A palmitate to lactating Holstein and Jersey cows, and to Holstein and Brown Swiss bulls. The levels of supplementation for cows were 65, 130, and 390 μg/kg. body weight per day; and 50 and 500 μg/kg. body weight for bulls.

The carotene and vitamin A contents of blood plasma were found to reflect the level of supplementation regardless of source with vitamin A palmitate producing the highest plasma vitamin A values, averaging 0.25 μg/ml. from cows and 0.31 μg/ml. from bulls. The highest blood plasma carotene values from cows (1.58 μg/ml.) was from feeding alfalfa leaf meal. The highest mean blood plasma carotene value from bulls was obtained when the highest level of carrot oil was fed (2.02 μg/ml.).

Liver vitamin A and carotene of cows and bulls increased significantly ($P > 0.01$) with the level fed regardless of the source of the vitamin. Alfalfa leaf meal produced the highest liver carotene values (2.65 μg/g.) for cows. Vitamin A palmitate produced liver vitamin A values for both cows (58.35 μg/g.) and bulls (100.60 μg/g.) significantly higher than the other sources

of supplementation.

Alfalfa leaf meal gave the highest mean liver vitamin A values of the three sources of supplementary carotene fed to both cows and bulls.

Milk fat carotene and vitamin A increased significantly with the dietary levels and sources of the vitamin. Vitamin A palmitate produced milk fat vitamin A of the highest values, while alfalfa leaf meal produced milk fat of higher carotene and vitamin A values than carrot oil or synthetic beta-carotene. The low biological value for beta-carotene in carrot oil and synthetic beta-carotene as measured by the three criteria used in this study indicates that the oily carrier may have decreased the rate of absorption or utilization of the vitamin with dairy cattle.

Positive correlations were found among the amounts of the vitamin fed, blood plasma carotene, blood plasma vitamin A, liver carotene and liver vitamin A. There were positive correlations between milk fat carotene, milk fat vitamin A and liver vitamin A and the amounts of the vitamin fed. There was no correlation between blood carotene and either liver vitamin A or milk vitamin A.

The supplementation of one gram of d-alpha-tocopherol per day to lactating Jersey and Holstein cows fed low carotene rations supplemented with three levels of vitamin A palmitate increased the vitamin A potency of the blood

plasma, liver and milk fat but lowered the carotene potency of the liver and milk fat. A 90 per cent increase in the carotene potency of the blood plasma of cows coupled with the decreased liver and milk fat carotene after receiving alpha-tocopherol for approximately one month indicated that alpha-tocopherol interfered with the utilization of carotene in dairy cattle.

Feeding one gram of alpha-tocopherol to lactating cows receiving their carotene intakes from hay, silage, or spring and fall pastures had no effect on the carotene and vitamin A potency of milk fat. There was no significant difference noted between the blood plasma and liver carotene and vitamin A values of the supplemented and control cows.

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