THE RELATIVE BIOPOTENCY OF FERMENTATION BETA-CAROTENE, CRYSTALLINE BETA-CAROTENE AND VITAMIN A FOR POULTRY

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By

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ABSTRACT

THE RELATIVE BIOPOTENCY OF FERMENTATION BETA-CAROTENE, CRYSTALLINE BETA-CAROTENE AND VITAMIN A FOR POULTRY

By Cal J. Flegal

Experiments were designed to determine the utilization of a newly-developed beta-carotene product produced by a fermentation process involving mating of opposite types of the heterothallic mold, Blakeslea trispora. Comparison was made with the ANRC Vitamin A Reference Standard, vitamin A palmitate and commercially available crystalline and synthetic beta-carotene.

Day-old commercial White Rock cockerels were depleted of their vitamin A reserves and then placed on the test diets. Observations were made on growth, feed efficiency, vitamin A plasma concentration, vitamin A liver concentration, and survival on a vitamin A deficient ration after having been on the test diets. Experiments were also conducted to determine the vitamin A reserves of turkeys by placing day-old poults on a vitamin A deficient ration and observing vitamin A plasma and liver concentrations at various intervals.

In the first experiment, the levels of the fermentation beta-carotene ranged from 514 to 1,433 IU, crystalline beta-carotene from 548 to 1, 453 IU and the ANRC Vitamin A Reference Standard from 539 IU to 1,298 IU per pound of diet. In all diets, as the vitamin A activity rose, mean body weights became larger, feed efficiency improved, plasma vitamin A concentration increased and liver vitamin A storage and

survival time increased. At any given dietary level, the crystalline beta-carotene and the fermentation beta-carotene produced similar results for all criteria observed. Neither beta-carotene product, however, performed as well as the ANRC Vitamin A Reference Standard.

In the second experiment, the levels of the fermentation beta-carotene ranged from 765 IU to 2,070 IU, crystalline beta-carotene from 810 IU to 2,050 IU, and the ANRC Vitamin A Reference Standard from 1,320 IU to 2,090 IU per pound of diet. The lowest level of the fermentation beta-carotene and the lowest three levels of the crystalline beta-carotene did not support the rate of growth obtained with the other diets. There was little difference in the feed efficiency between diets regardless of product or level. With each product, as the vitamin A activity increased in the diet, plasma and liver vitamin A concentrations increased. There were few significant differences in survival time. At any given dietary level of vitamin A activity, the crystalline beta-carotene and the fermentation beta-carotene produced similar results with respect to the criteria used. However, at any given comparable dietary level of vitamin A activity, plasma and liver concentrations of vitamin A were higher in those chicks receiving the ANRC Vitamin A Reference Standard than either beta-carotene product.

In Experiment III, the levels of fermentation beta-carotene were 8,950 IU and 21,350 IU per pound of diet and the levels of the vitamin A palmitate were 10,400 IU and 21,700 IU per pound of diet. The liver vitamin A concentration was significantly higher from the vitamin A palmitate than from each comparative level of the fermentation beta-carotene.

In these experiments, the beta-carotene products produced similar results for all the criteria used in evaluation, but the ANRC Vitamin A Reference Standard and vitamin A palmitate were more effective than were any of the beta-carotene products in promoting blood plasma and/or liver concentration of vitamin A.

Turkey poults were found to have large body reserves of vitamin A. The plasma vitamin A concentration dropped from an initial value of about 140 micrograms per 100 ml of plasma to about 12 micrograms per 100 ml of plasma; liver vitamin A concentration dropped from an initial value of about 40 micrograms per gram to less than one microgram per gram in 40 days on vitamin A deficient rations.

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INTRODUCTION

Vitamin A is one of the oldest known vitamins. However, until as recently as 15 years ago, fish liver oils were the only major source of vitamin A. These products differed widely in their potency, stability and purity as well as their supply and price. Therefore, significant dependence was placed on plant carotenes to supply a major portion of poultry and livestock vitamin A requirements.

As a result of extensive scientific investigations, today both synthetic vitamin A and synthetic beta-carotene are being commercially produced for feed supplementation. Related carotenoids also are now being used to impart a desirable yellow-orange color to such consumable products as eggs, poultry meat, and dairy products.

In 1957, research workers at the United States Department of Agriculture Northern Utilization Research and Development Division developed a new method for producing beta-carotene. This new beta-carotene product is produced by a fermentation process involving mating of opposite types of the heterothallic mold, Blakeslea trispora.

This beta-carotene product may have an economic advantage over synthetically produced sources of vitamin A. Therefore, if this new product could be utilized successfully by poultry and other livestock, the farmer-producer should realize a lower cost of production.

The purposes of the present experiments were: (a) to determine the efficiency of utilization of this newly-developed, beta-carotene product by growing chicks, (b) to investigate some of the relationships of

vitamin A and beta-carotene in growing chicks, and (c) to investigate body reserves of vitamin A in the turkey poult.

REVIEW OF LITERATURE

Vitamin A was first postulated by McCollum and Davis in 1915. Later, Steenbock and Boutwell (1920) showed that naturally-occurring carotenoids had vitamin A activity. However, it was not until 1931 that Karrer, Morph and Schopp, and Karrer and Morph determined the structure of these two products. In 1937, Kuhn and Morris produced vitamin A synthetically. Finally, in 1950, Karrer and Eugster synthesized beta-carotene.

Probably no other nutrient has been so extensively studied as a source of vitamin A for poultry and other domestic animals as has carotene. These studies have produced widely varying results.

The relative performance of beta-carotene in species other than poultry has also been considered. Guilbert et al. (1940) reported a carotene to vitamin A ratio, based on weight, of 6:1 at levels of intake to provide for freedom from night blindness but little or no storage, and a ratio of 10:1 to provide significant liver storage for horses, cattle, sheep and swine. Similar results were obtained by Braude et al. (1941) for fattening pigs where 300 IU/lb of feed from beta-carotene provided for less liver storage than 100 IU/lb of feed as preformed vitamin A.

Gray et al. (1940) compared the U.S.P. reference oil, vitamin A alcohol, the separated esters, and beta-carotene using as a basis of comparison liver storage of vitamin A in rats after feeding a known amount of vitamin A. Greatest recovery was from the U.S.P. reference oil, then separated esters, then alcohol; beta-carotene ran a poor last.

Much of the beta-carotene was plainly visible in the feces. Small amounts of beta-carotene were absorbed efficiently but larger amounts were absorbed less efficiently. With fattening lambs, Hoefer and Gallup (1947) reported similar results based on blood levels and liver storage of vitamin A after feeding carotene concentrates, alfalfa meal, or fish liver oil.

Based on the classic four-week rat curative growth assay, Marusich et al. (1961) found that pure beta-carotene in the form of stabilized beadlets was fully active and that 0.6 mcg. of all-trans beta-carotene equals one IU of vitamin A. However, when carotene levels higher than those necessary for growth were fed to rats for seven weeks in order to induce liver and kidney storage of vitamin A, only one-third to one-fourth as much of the vitamin A from the beta-carotene was stored as when the equivalent levels of vitamin A were fed. In further studies, Marusich and Bauernfeind (1963) found that rats fed 4,540 IU beta-carotene per pound of feed had about one-third as much liver storage of vitamin A as did rats fed dry vitamin A at the same level. This indicated an effective carotene to vitamin A ratio of 6:1 on a weight basis. At 9,080 IU per pound of feed, liver vitamin A storage from carotene was about one-fifth as high as with the same level of vitamin A. This represents a carotene to vitamin A ratio of 10:1 based on weight.

Gurcay et al. (1950) compared crystalline carotene and crystalline vitamin A acetate, using both growth rate and liver storage of vitamin A. Turkey poults were placed on a vitamin A deficient diet for two weeks and then on the test diets for six weeks. Based on

International Units of vitamin A, crystalline vitamin A acetate was about four times as efficient as corresponding levels of crystalline carotene in supporting growth. At intakes higher than those needed for normal growth, approximately 4,000 IU per pound from crystalline vitamin A acetate were equivalent to feeding 30,000 IU/lb from carotene based on liver storage of vitamin A. Poults which received crystalline vitamin A acetate had about twice as much vitamin A in the blood plasma as those which received corresponding levels of crystalline carotene.

Chavey et al. (1964) fed turkey poults diets containing stabilized vitamin A and beta-carotene based on vitamin A equivalents. The beta-carotene found in dehydrated alfalfa leaf meal or in a dehydrated flowering aquatic meal blend was not converted to vitamin A to any appreciable extent at either four or eight weeks of age based on liver storage of vitamin A. Levels of vitamin A found in the liver at eight weeks of age were 364 IU for the poults fed 2,000 IU per pound of stabilized vitamin A. At an equivalent level of beta-carotene from either alfalfa leaf meal or the aquatic meal blend, only traces of liver vitamin A were found.

Nestler et al. (1948) compared crystalline carotene, vitamin A alcohol, vitamin A ester and vitamin A acetate using liver stores of vitamin A as criteria. Vitamin A products were fed from 0 to 10 weeks of age to quail. The crystalline carotene was utilized only

one-third to one-seventh as efficiently as vitamin A alcohol, one-half to one-tenth as efficiently as the vitamin A ester and one-fourth to one-seventeenth as efficiently as the vitamin A acetate. Considerable individual variation in vitamin A liver stores were noted in quail on the same diet and with the same parental background.

Castano et al. (1951) compared crystalline carotene and vitamin A acetate and found that crystalline carotene was about one-third to one-fifth as effective as vitamin A acetate, based on vitamin A liver storage in chicks.

Harvey et al. (1955) found that preformed vitamin A was always more effective than carotene in any given similar preparation used orally, as measured by liver stores and survival time in chicks.

Gledhill and Smith (1955) found substantially higher liver stores of vitamin A (about twice as much), significantly greater gains, better feed efficiency and lower mortality in chicks fed dry vitamin A from one day to ten weeks of age, as compared to chicks fed carotene from alfalfa at levels of 1,000 IU per pound of feed.

Laughland and Phillips (1955) studied the utilization of vitamin A and carotene by normal and deutectomized chicks. They found that liver stores after 8, 15, 22, and 29 days were 5 to 10 times higher when vitamin A acetate was fed at 10,000 IU per pound of feed than when 5.6 mg. beta-carotene (9,933 units) was fed.

Ely (1959) reported that dry vitamin A was about 2.6 times as effective in promoting chick liver storage as was the provitamin. Carotenes from alfalfa leaf meal and/or corn meal or from purified beta-carotene concentrate were poorly utilized as a source of vitamin A up to 31 days by chicks. Olson et al. (1959) compared several dry vitamin A sources and vitamin A feeding oil with carotene from cereal grass and obtained similar results. At levels of 100 to 1,200 IU per pound of feed no differences were obtained in growth between products. However, based on liver stores of vitamin A and survival time, the cereal grass was greatly inferior. These authors concluded that in short-term experiments, growth may not be a reliable criterion for evaluation of vitamin A availability. Judging by liver storage of vitamin A, feeding oils and dehydrated cereal grass were the poorest sources of vitamin A. Gelatin-coated dry vitamin A preparations were superior to preparations in which the vitamin was coated with wax or fat, or absorbed on vegetable protein.

Erasmus et al. (1959) reported that at corresponding levels, stabilized vitamin A produced vitamin A liver storage about ten times as high as that produced by beta-carotene in chicks which had been artificially infected with coccidiosis. Erasmus et al. (1960) found that a level of 800 units of stabilized vitamin A per pound of feed was as effective in maintaining feed consumption and growth rate in chicks artificially infected with coccidiosis, as was a level of 2,400 units from dry beta-carotene beadlets.

Halloran (1960) reported that 2,000 IU of vitamin A per pound of diet from alfalfa leaf meal was insufficient to maintain vitamin A liver levels in 9-month-old laying pullets or 19-month-old laying hens.

However, Zimmerman et al. (1961) found that carotene from alfalfa was used satisfactorily as a sole source of vitamin A in laying hens.

In broiler-type chicks fed diets containing 2,500 and 5,000 IU of vitamin A per pound of diet, Marusich et al. (1961) found that vitamin A liver storage was only one-third to one-fourth as large from the feeding of beta-carotene as from the feeding of equivalent levels of dry vitamin A. Similar results were also reported by Marusich et al. (1963) in comparing dry beta-carotene beadlets and dry vitamin A beadlets. At levels of 2,500 and 5,000 IU per pound of feed, based on vitamin A liver storage, beta-carotene affords about one-third to one-fourth the storage as true vitamin A at both levels of feeding, revealing an effective beta-carotene to vitamin A ratio of 6 to 8:1, calculated on a weight basis. In this same experiment, when 2,500 and 5,000 IU per pound of feed were fed as dehydrated alfalfa meal or dry vitamin A, vitamin A provided liver stores two to four times higher than beta-carotene based on percent deposition of vitamin A in the liver. This yields an effective beta-carotene to vitamin A ratio of 4 to 8:1 calculated on a weight basis.

Utilizing chicks, Olsen et al. (1964) found that, with growth, livability, and liver storage as criteria, the minimum requirement is at least 600 IU of vitamin A per pound of diet. However, data from chicks fed crystalline beta-carotene indicated that the minimum requirement was in excess of 350 micrograms or 600 IU per pound; that is, that carotene is a less efficient source of vitamin A activity.

As far as growth rate in the normal chick and in the rat is concerned, most investigators have reported that carotene is as efficient as vitamin A (Wilson et al., 1936; Record et al., 1937; Reynolds et al., 1947). It is, therefore, at higher levels of feeding, where liver storage of vitamin A is measurable, that carotene appears to perform less effectively than vitamin A.

The relative performance of beta-carotene and vitamin A with respect to blood levels of vitamin A has also been reported.

Gurcay et al. (1950) reported that turkey poults which were fed crystalline vitamin A acetate had about twice as much vitamin A in the blood plasma as those which received corresponding levels of crystalline carotene.

In comparing crystalline vitamin A acetate and crystalline carotene using chicks, at comparative levels, vitamin A acetate was more effective in causing higher concentrations of the vitamin in the blood plasma (Castano et al., 1951).

A comparison of various criteria for measuring the biological activity of different sources of vitamin A activity is also of interest. McCoord et al. (1934) observed that the concentration of vitamin A in the blood of rats was no indication of the amount that may be stored in the liver. Krause (1949) found that with rats it was difficult to assess the body's need or its reserve of vitamin A on the basis of blood level per se. Lewis et al. (1942) observed that growth of rats

increased until it reached a maximum when the diet level of vitamin A was increased from 0 to 25 IU daily; it remained here at a maximum up to 1,000 IU daily. The average plasma vitamin A concentration also increased with increasing levels of vitamin A intake, reaching an optimum at 50 IU per day and remained at this level with intake up to 1,000 IU per day. There was no liver storage at intakes of 10 IU or less per day, slight storage at 25 IU per day and increasingly larger storages at higher intakes.

In turkeys, Guilbert et al. (1934) found a direct correlation between liver storage, level of vitamin A in the diet, growth, mortality, and the survival of pen-mates when birds were later placed on the vitamin A deficient ration. Also, large variations in liver storage were found among individuals on the same feed. Record et al. (1937), in observations of growing chicks, found that 50 to 100 micrograms of carotene or 80 to 160 IU of vitamin A from cod liver oil per 100 grams of diet were required for normal growth. However, several times these "minimum" amounts were needed to obtain liver storage.

In observations of growing chicks, Johnson et al. (1948) found that the critical storage level of vitamin A in the livers of chicks studied appeared to be two or three IU per gram and a high storage of vitamin A in the liver was not required for satisfactory growth. Johnson et al. (1947) had previously determined that growing chicks grew well when their livers contained from 3 to 10 IU of vitamin A per gram.

Castano et al. (1951), in experiments with chicks, observed that the vitamin A in the blood was related to vitamin A intake and that some minimum level of vitamin A must be maintained in the blood before measurable liver storage occurred. Further, a high storage of vitamin A in the liver was not essential for satisfactory growth provided sufficient vitamin A was in the daily diet. Therefore, at low intake most of the vitamin A was conceivably employed in biological functions and at higher levels liver storage took place. In this same trial, between 500 and 1,000 IU per pound of diet allowed maximum growth while raising the vitamin A in the diet further increased the vitamin A liver storage. In contrast, Harms et al. (1955) observed no difference in average weights of chicks fed dietary levels of vitamin A ranging from 500 to 4,000 IU per pound of feed but liver storage was increased as the dietary level of vitamin A increased, however, a depletion period was not utilized prior to the use of the test diets by Harms et al. (1955) while Castano et al. (1951) placed the chicks on vitamin A depletion diet for 14 days prior to the employment of the test diets.

In recent years, vitamin A deficiency has been implicated in several disease conditions in poultry, in addition to its long-standing connection with growth, general well-being, and integrity of the epithelial tissue. This has stimulated considerable research in the area of disease and vitamin A.

Heilborn et al. (1944) has expressed the opinion that vitamin A deficiencies render the altered epithelium more vulnerable to both

bacterial and parasitic attack. Pande and Krishnamurtz (1959) found that insufficient vitamin A favored infestation of chickens with Ascaridia galli. The latter damages the intestinal mucosa and causes a state of conditioned vitamin A deficiency.

Two reports (Bergdoll, 1964; Patterson and McInnis, 1964) have shown that high levels of vitamin A have been beneficial in treating laying hens showing infestation with Capillaria columbae.

Erasmus et al. (1959) and Erasmus and Scott (1960) reported that chicks infected with coccidiosis required more vitamin A and had lowered liver levels of vitamin A. Chicks that recovered from coccidiosis regained their appetite and grew faster if their diet contained 8,000 IU of vitamin A per pound compared to 800 IU. Scott et al. (1961) also reported that coccidiosis in chicks reduced liver levels of vitamin A and the results strongly indicated that adequate vitamin A nutrition above the minimum levels needed for growth was of primary importance in prevention of the severe lesions and losses from CRD as well as coccidiosis. Panda et al. (1964) reported that the feeding of three to seven times the NRC requirements of vitamin A during the acute phase to chicks infected with coccidiosis resulted in significantly larger body weight gains than did the feeding of vitamin A at the NRC recommended level.

As measured by growth of chicks, Panda et al. (1962) reported that in preliminary studies with infectious bronchitis, high levels of vitamin A were beneficial. Squibb and Veros (1961) showed that

vitamin A therapy of chicks with adequate reserves of vitamin A did not improve weight gain or lessen mortality during the period of Newcastle disease involvement.

Abbott et al. (1960), in an experiment with turkeys, found an increased incidence of mycoplasma lesions in embryos from eggs from vitamin A deficient turkeys over those from turkeys fed adequate levels of vitamin A. In chicks, Boyd and Edwards (1962) observed that vitamin A had no influence on the course of mycoplasma or E. coli infections and mycoplasma and E. coli infections do not have any direct influence on vitamin A absorption or metabolism in the chick. However, Boyd and Edwards (1962) also reported that mortality was greater in mycoplasma infected vitamin A-deficient chicks than in mycoplasma infected chicks receiving adequate vitamin A.

MATERIALS AND METHODS

Experiment I: Comparison of three dietary levels of fermentation beta-carotene, crystalline beta-carotene, synthetic beta-carotene and the ANRC Vitamin A Reference Standard.

This experiment was designed to determine a suitable depletion period for chicks and to establish practical levels of vitamin A.

The sources of vitamin A activity in this experiment were:

Fermentation beta-carotene -- This product was produced and supplied by the United States Department of Agriculture Northern Regional Laboratory, Peoria, Illinois, as dry fermentation solids. Analysis of the fermentation product at the U. S. Department of Agriculture Northern Regional Research Laboratory indicated that it contained 85 percent all-trans beta-carotene, six percent neo-beta-carotene B and the remainder consisted of unidentifiable material.

Synthetic beta-carotene -- This product was supplied by the Hoffman-LaRoche, Incorporation, Nutley, New Jersey as dry beta-carotene beadlets, type 2.4-S. It was a dispersion of beta-carotene and vegetable oil in a matrix of gelatin and carbohydrate that contained 48 percent all-trans beta-carotene, 21 percent neo-beta-carotene B, 20 percent neo-u-carotene and the remainder consisted of unidentifiable material.

Crystalline beta-carotene -- This product was supplied by General Biochemicals, Chagrin Falls, Ohio as 100 percent all-trans beta-carotene.

Vitamin A -- This product was the Animal Nutrition Research Council Reference Standard (Ames, 1965).

All products were prepared for the experiment by diluting the appropriate premix concentration with soybean oil meal containing two per cent of a 2:3 mixture of BHA and BHT. These premixes were chemically analyzed, packaged under nitrogen in sealed glass containers and stored under refrigeration (-10° F) in the dark until they were used.

Commercially obtained one-day-old Cobb's Strain White Rock cockerels were placed into heated, raised-wire chick batteries and placed on the basal depletion diet (Table 1). The brooding temperature was initially 90 to 95° F and was decreased 5 degrees F each week until room temperature (about 70° F) was reached. At regular intervals, a few chicks were removed and blood samples taken to determine plasma vitamin A concentration. After 17 days (Figure I), the vitamin A plasma level was very low. The birds were then placed in equalized weight groups, wing-banded, and three replications of ten birds each were randomly distributed in chick batteries. The chicks were placed on the test diets for four weeks. The intended and assayed vitamin A activity[1] per pound of the test diets were as follows:

1/ Vitamin A activity for the beta-carotene products is expressed as IU per pound and was based on the conversion of 0.6 microgram of beta-carotene to one IU of vitamin A activity.

Table 1. Composition of basal depletion diet used in Experiment I

Ingredient	Percent
Ground white milo	60.0
Soybean oil meal, 44% protein	34.0
Steamed bone meal	2.0
Limestone, ground	1.5
Salt, iodized	.5
Vitamin premix	2.0†

† The premix contained the following per pound of ration:

200 mg riboflavin
500 mg dl-calcium pantothenate
1,250 mg niacin
2,000 mg choline chloride
0.5 mg vitamin B_{12}
4,000 I.C.U. vitamin D_3
200 mg procaine penicillin
1 gm $MnSO_4$

Sufficient soybean oil to make up the two percent

Calculated analysis:

	Unit	
Protein	%	20.6
Fat	%	1.98
Fiber	%	3.79
Calcium	%	1.2
Phosphorus	%	.66
Methionine	%	.31
Cystine	%	.31
Lysine	%	1.20
Prod. energy	Cal/lb	922

Treatment of ration	I.U. Vitamin A Activity/lb. Intended	Assayed†
Basal	0	0
Basal + fermentation beta-carotene	400	514
" + " " "	700	836
" + " " "	1,225	1,433
Basal + crystalline beta-carotene	400	548
" + " " "	700	876
" + " " "	1,225	1,453
Basal + ANRC Vitamin A Reference Standard	400	539
" + " " " " "	700	819
" + " " " " "	1,225	1,298

	First week††		Last 3 weeks††
Basal + synthetic beta-carotene	400	3,655	1,342
" + " " "	700	7,055	1,916
" + " " "	1,225	11,500	2,320

†Average of analytical data obtained from feed samples analyzed at the U.S. Department of Agriculture Northern Utilization Research and Development Division.

††This premix was inadvertently mixed with approximately ten times the requested amount of vitamin A activity. The change between the first week and the last three weeks was made in an attempt to correct the amount of vitamin A activity in the finished feed.

The diets were mixed once weekly. They were prepared by adding appropriate amounts of the beta-carotene or vitamin A premixes and the soybean oil meal-antioxidant diluent to the basal depletion diet. Approximately one-half of the weekly needs was fed and the remainder was put in covered metal containers, placed in the dark and refrigerated (-10° F). After one-half week, the feed not consumed was removed and replaced by the portion which had been kept refrigerated. Each diet

was sampled at the time of mixing, at mid-week, and at the end of each week for determination of vitamin A activity. The samples were packaged under nitrogen in sealed containers and kept refrigerated (-10° F) until analyzed.

The chicks were individually weighed at weekly intervals and feed consumption per lot was recorded. Blood samples obtained by heart puncture were taken from each chick[2] at the end of the second and fourth weeks on the experimental diets for plasma vitamin A determinations.

After having been on the test diets four weeks, one-half of the birds on each diet were randomly selected, sacrificed, livers removed, wrapped in Saran Wrap and aluminum foil and refrigerated at about -10° F until assayed for vitamin A. The remaining birds were placed on the basal depletion ration and survival in days was recorded.

The plasma vitamin A determinations were made using antimony trichloride according to the method outlined by Yudkin (1941). All lobes of each liver were sectioned approximately at the midline and a portion from this area was removed for analysis. Individual liver vitamin A concentrations were determined according to the method described by Gallup and Hoefer (1946). A Bausch and Lomb Spectronic-20 spectrophotometer was used.

Individual body weights, vitamin A blood plasma concentrations, vitamin A liver concentrations and survival were subjected to analysis of variance (Snedecor, 1956); then Duncan's multiple range test (1955)

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2. At the end of two weeks, individual blood samples were taken from only one replicate. Pooled samples were obtained from the other replicates.

was used to determine which means were significantly different at the .01 and .05 levels of probability.

Experiment II: Comparison of four dietary levels of fermentation beta-carotene, crystalline beta-carotene and the ANRC Vitamin A Reference Standard.

Based on an analysis of the results obtained in Experiment I, Experiment II was conducted similar to Experiment I with the following exceptions.

At one day of age, the chicks were placed on the basal depletion diet for 15 days. Also, 0.24 mg/lb vitamin K, 20 mg/lb zinc and 1½ per cent cottonseed oil, at the expense of milo, were added to the basal depletion diet shown in Table 1. Blood samples for vitamin A determinations were taken only after the birds had been on the test diets four weeks. The intended and assayed vitamin A concentrations of the test diets were as follows:

	I.U. Vitamin A Activity/lb.	
	Intended	Assayed†
Basal	0	0
Basal + fermentation beta-carotene	800	765
" + " " "	1,120	1,035
" + " " "	1,568	1,565
" + " " "	2,195	2,070
Basal + crystalline beta-carotene	800	810
" + " " "	1,120	1,090
" + " " "	1,568	1,510
" + " " "	2,195	2,050
Basal + ANRC Vitamin A Reference Standard	800	1,030
" + " " " " "	1,120	1,320
" + " " " " "	1,568	1,590
" + " " " " "	2,195	2,090

† Average of analytical data from feed samples analyzed at the U. S. Department of Agriculture Northern Utilization Research and Development Division.

Experiment III: Comparison of relatively high dietary levels of fermentation beta-carotene and vitamin A palmitate.

The purpose of this experiment was to determine the relative ability of the fermentation beta-carotene and a vitamin A product to influence vitamin A liver concentration at relatively high feed levels fed for a one-week period.

The sources of vitamin A activity used in this trial were:

Fermentation beta-carotene - previously described.

Vitamin A palmitate - This material was purchased from Distillation Products Industries, Rochester, New York in the form of PGB-250 Dry Vitamin A Feed Supplement.

Commercially obtained day-old Cobb's strain White Rock cockerels were placed into heated, raised-wire chick batteries and placed on the depletion diet as used in Experiment II. After seven days, the birds were placed in equalized weight groups, wing-banded, and four replications of 10 birds each were randomly distributed in chick batteries. The chicks were placed on the test diets for one week. The intended and assayed vitamin A activity per pound of the test diets were as follows:

	Vitamin A activity/lb. of diet	
	Intended	Assayed†
Basal	0	0
" + fermentation beta-carotene	10,000	8,950
" + " " "	25,000	21,350
" + PGB-250 dry vitamin A	10,000	10,400
" + " " " " "	25,000	21,700

†Average of analytical data from feed samples analyzed at the U. S. Department of Agriculture Northern Utilization Research and Developmental Division.

After one week on the test diets, all birds were sacrificed, livers removed, wrapped in Saran Wrap and aluminum foil and refrigerated at about -10° F until assayed for vitamin A. Assay procedures and analytical interpretations were identical to those used in Experiments I and II.

Experiment IV: The effects of a vitamin A deficient diet on turkey poults' plasma and liver vitamin A concentration.

The purpose of this experiment was to examine the effects of a vitamin A deficient diet on the plasma and liver vitamin A concentration of turkey poults.

The turkey poults used in this experiment were obtained from eggs that were hatched from the turkey flock maintained at the Michigan State University Poultry Research Farm and consisted of both Beltsville Small White and Broad Breasted Bronze varieties. The breeding flock had been fed the regular turkey breeder diet used at the farm which contained the following:

Ingredients	Percent of ration
Ground yellow corn	50.0
Ground heavy oats	10.0
Soybean oil meal, 44% protein	10.0
Wheat std. middlings	5.0
Wheat bran	5.0
Alfalfa meal, 17% protein	5.0
Fish meal	4.0
Meat and bone scraps, 50%	2.0
Dried yeast	2.0
Dried whey	1.5
Ground limestone	3.0
Dicalcium phosphate	1.5
Salt, iodized	.5
Manganese sulfate, 70%	.025
Dry vitamin A, 5,000 IU/gm	.15
Dry vitamin D_3, 1,500 ICU/gm	.15
Choline chloride, 25%	.10
Vitamin B_{12}, 6 mg/lb	.06
Vitamin E, 20,000 IU/lb	.04
BHT	.0125
Niacin	10 grams
Riboflavin	1.0 gram

Calculated analysis:

Protein	%	16.6	Phosphorus	%	.7
Fat	%	3.1	Vitamin A	IU/lb	6,900
Fiber	%	5.1	Vitamin D	ICU/lb	1,020
Calcium	%	2.2			

This experiment consisted of two trials.

Trial A -- A blood sample, collected by heart puncture, was obtained from several of the one-day-old turkey poults, before they had consumed any feed, to determine vitamin A plasma concentration. The remainder of the poults were randomly distributed into heated, raised-wire chick batteries and placed on the basal depletion diet shown in Table 2. At regular intervals, several (five or more) poults were removed from the pens and blood samples obtained to determine plasma vitamin A concentration of the pooled samples.

Trial B -- This trial was conducted similar to Trial A. However, at certain intervals, the same birds from which blood samples had been taken were sacrificed, livers removed, wrapped in Saran Wrap and aluminum foil and refrigerated at about -10° F until assayed for vitamin A concentration. A pooled sample of the liver samples was analyzed as previously described.

Table 2. Composition of basal depletion diet used in Experiment IV

Ingredient	Percent of ration
Ground white milo	38.8
Soybean oil meal, 44% protein	55.2
Steamed bone meal	2.0
Limestone, ground	1.5
Salt, iodized	.5
Vitamin premix	2.0†

† The premix contained the following per pound of ration:

200 mg riboflavin
500 mg dl-calcium pantothenate
1,250 mg niacin
2,000 mg choline chloride
0.5 mg vitamin B_{12}
4,000 I.C.U. vitamin D_3
200 mg procaine penicillin
1 gm $MnSO_4$

Sufficient soybean oil meal to make up the two percent.

Calculated analysis:

	Unit	
Protein	%	28.02
Fat	%	1.55
Fiber	%	4.78
Calcium	%	1.25
Phosphorus	%	.73
Methionine	%	.41
Cystine	%	.428
Prod. energy	Cal/lb	850.3

RESULTS

Experiment I

The effect on plasma vitamin A concentration when the day-old chicks were placed on the basal depletion diet can be seen in Figure I. The concentration dropped very rapidly and almost in a straight line during the first seven days from an initial value of 194 micrograms per 100 ml to about 43 micrograms per 100 ml of plasma. The decline in vitamin A concentration was less rapid from 7 to 14 days when the plasma vitamin A concentration dropped from about 43 micrograms per 100 ml to about five micrograms per 100 ml.

Observations were also made on the effects of the basal depletion ration on the appearance of the chicks prior to placement on the test diets. No visible effects were observed during the first four to six days. At about seven days, the chicks appeared to be extremely nervous. At about the same time, mortality at a rate greater than "normal" appeared and continued at an excessive rate until the birds were placed on the test diets. At about 12 to 14 days, a portion of the chicks exhibited a general weakness, staggering gait, and ruffled plumage. These conditions became more general the longer the birds were kept on the basal ration culminating in death of those chicks not placed on the test diets.

The effect of the test diets on weight gain and feed efficiency are shown in Tables 3-1, 3-2, 3-3 and 3-4. Significant differences (Tables 3-1 and 3-2) occurred in mean body weights both after two and four weeks on the test diets.

Figure 1. Plasma vitamin A concentration of chicks.

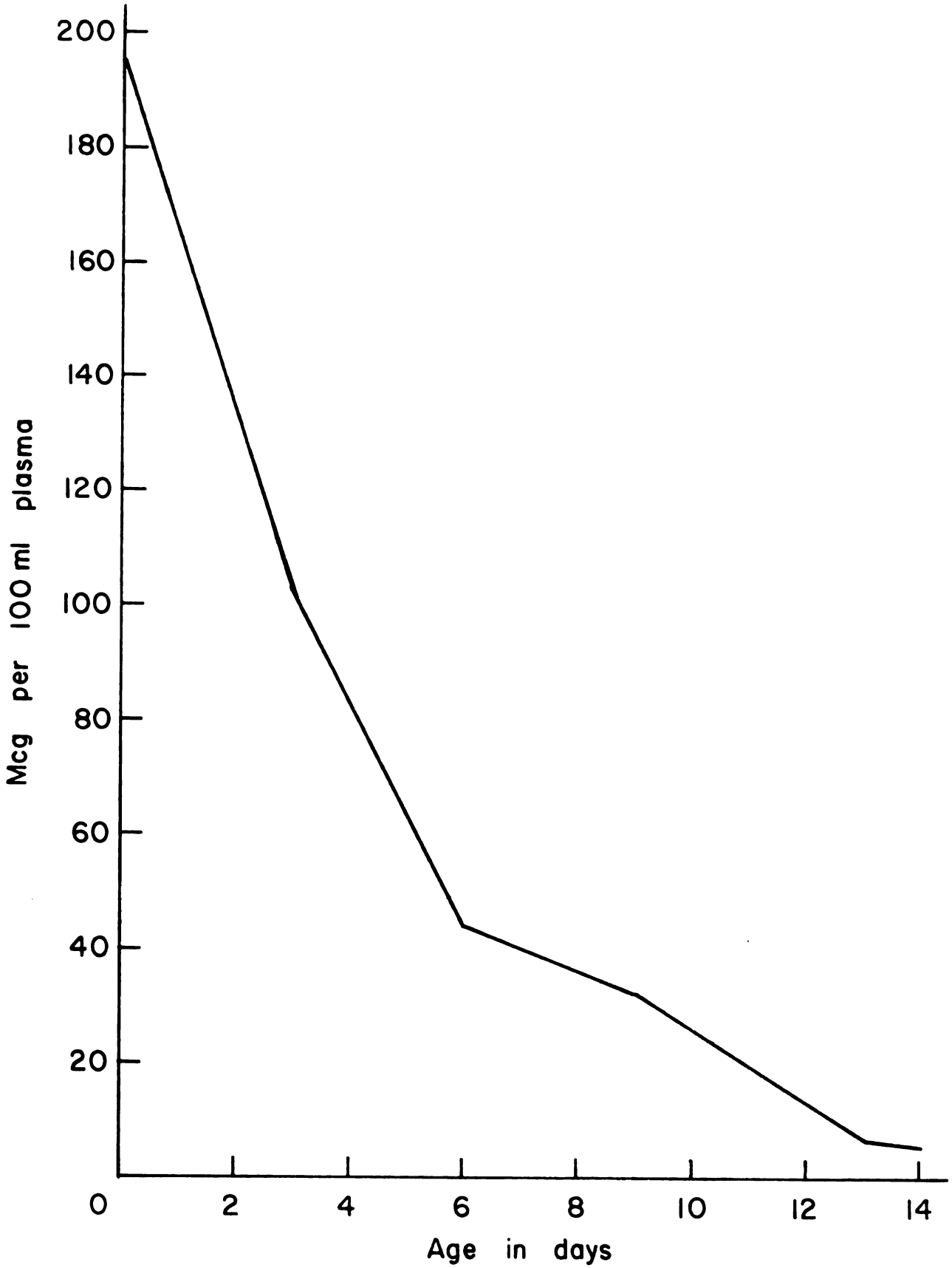


Table 3-1. Two-week chick weights

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† body weights (gms.)
Fermentation beta-carotene	514	358.7 DFe
" " "	836	376.5 Dde
" " "	1,433	434.2 ABCbc
Crystalline beta-carotene	548	315.9 Ef
" " "	876	377.8 CDde
" " "	1,453	384.0 CDde
Synthetic beta-carotene	--††	456.9 ABabc
" " "	--	464.2 ABabc
" " "	--	484.1 Aa
ANRC Vitamin A Reference Standard	539	416.3 BCDcd
" " " " "	819	453.1 ABabc
" " " " "	1,298	461.8 ABabc

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

†† Omitted due to incorrect value assigned to premix. See text for complete explanation.

Table 3-2. Four-week chick weights and feed efficiency

Treatment of basal ration	I.U. Vit. A/lb Assayed	Mean† (body weights (gms)	Feed effic. (Lbs. feed/ lb. gain in body wt.)
Fermentation beta-carotene	514	618.2 DEd	3.41
" " "	836	666.9 CDd	3.10
" " "	1,433	771.7 ABab	2.70
Crystalline beta-carotene	548	517.3 Fe	3.77
" " "	876	608.7 DEd	3.12
" " "	1,453	682.8 BCDcd	2.80
Synthetic beta-carotene	--††	662.8 CDd	2.93
" " "	--	776.8 ABab	2.57
" " "	--	822.2 Aa	2.49
ANRC Vitamin A Reference Standard	539	742.7 ABCbc	2.71
" " " " "	819	770.8 ABab	2.67
" " " " "	1,298	801.0 Aab	2.55

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

†† Omitted due to incorrect value assigned to premix. See text for complete explanation.

Table 3-3. Analysis of variance of two-week chick weights

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	333	2,686,206		
Subclass	35	939,077	26,381	4.50**
Treatment	11	798,344	72,577	12.38**
Replication	2	28,217	14,108	2.41
Total int.	22	112,516	5,114	.87
Error	298	1,747,130	5,863	

** Significant at the .01 level of probability.

Table 3-4. Analysis of variance of four-week chick weights

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	292	6,198,466		
Subclass	35	2,394,540	68,415	4.62**
Treatment	11	2,034,914	184,992	12.50**
Replication	2	36,064	18,032	1.22
Total int.	22	323,563	14,707	.99
Error	257	3,803,926	14,801	

** Significant at the .01 level of probability.

It appeared that both the product and the level of vitamin A activity in the diet had an influence on mean body weight. Mean body weights after both two and four weeks on the test diets were larger as the vitamin A activity of the diet was increased for all the products tested. The crystalline beta-carotene fed at 548 IU per pound of ration resulted in mean body weights that were significantly smaller than those resulting from all other diets. The synthetic beta-carotene (which was mixed at levels much higher than the other diets) resulted in the largest mean body weights although these were not significantly larger than most of the other mean body weights.

The fermentation beta-carotene and the crystalline beta-carotene resulted in mean body weights that were not significantly different at each corresponding level of dietary vitamin A activity with the exception that has been noted above. However, there was a trend toward larger mean body weights at each of the corresponding dietary levels of vitamin A when comparing the fermentation beta-carotene to the crystalline beta-carotene.

At corresponding dietary levels of vitamin A activity, the ANRC Vitamin A Reference Standard resulted in significantly larger mean body weights (after both two and four weeks on the test diets) than the crystalline beta-carotene. This was also true when comparing the fermentation beta-carotene to the ANRC Vitamin A at the two lowest dietary levels.

Feed efficiency was closely associated with rate of gain; the faster rate of gain producing the best feed efficiency. The ANRC Vitamin A Reference Standard resulted in a considerably better feed conversion at the two lowest levels than did either the fermentation beta-carotene or the crystalline beta-carotene.

The effects of the test diets on vitamin A plasma concentrations are shown in Tables 3-5, 3-6 and 3-7. The product and the level of vitamin A activity had an influence upon mean plasma vitamin A concentration. In general, as the feed level of vitamin A activity was raised, the mean plasma vitamin A concentration increased. In most instances the plasma vitamin A concentration was higher after four weeks on the test than after two weeks for all products at each level tested.

After having been on the test diets two weeks, only the lowest levels of the fermentation beta-carotene and the crystalline beta-carotene failed to raise the plasma vitamin A concentration above the depleted value. However, after four weeks on the test diets, all diets had increased the vitamin A plasma concentrations to a level above the depleted value. Few significant differences in plasma vitamin A concentrations were observed at two weeks. The ANRC Vitamin A Reference Standard increased the plasma vitamin A concentration more, although not always significantly, at each comparable level than did either the fermentation beta-carotene or the crystalline beta-carotene but not more than did the synthetic beta-carotene.

After four weeks on the test diets, at each comparable level of vitamin A activity in the feed, all of the beta-carotene products resulted in similar plasma vitamin A concentrations, except that the highest level of crystalline beta-carotene resulted in significantly smaller (P 0.01) plasma vitamin A concentration than the other two beta-carotene products at the highest feed level of vitamin A activity.

Table 3-5. Chick plasma vitamin A concentrations

Treatment of basal ration	I.U. Vit. A/lb.	Means† Mcg/100 ml blood plasma 2 wks.††	4 wks.
Fermentation beta-carotene	514	6.7 De	16.0 Efg
" " "	836	13.5 DEde	18.9 DEefg
" " "	1,433	21.0 BCDbcd	30.5 Bb
Crystalline beta-carotene	548	7.9 De	15.4 Eg
" " "	876	15.6 CDcd	18.2 DEefg
" " "	1,453	14.2 CDde	24.2 CDcd
Synthetic beta-carotene	--†††	13.7 CDde	16.4 Eefg
" " "	--	25.8 BCbc	20.5 CDEdef
" " "	--	31.5 ABab	25.8 BCbc
ANRC Vitamin A Reference Standard	539	21.9 BCDbcd	21.1 CDEde
" " " "	819	28.1 ABCb	30.3 Bb
" " " "	1,298	41.1 Aa	50.7 Aa

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

†† From one replication only.

††† Omitted due to incorrect value assigned to premix. See text for complete explanation.

Table 3-6. Analysis of variance of two-week chick plasma vitamin A concentrations

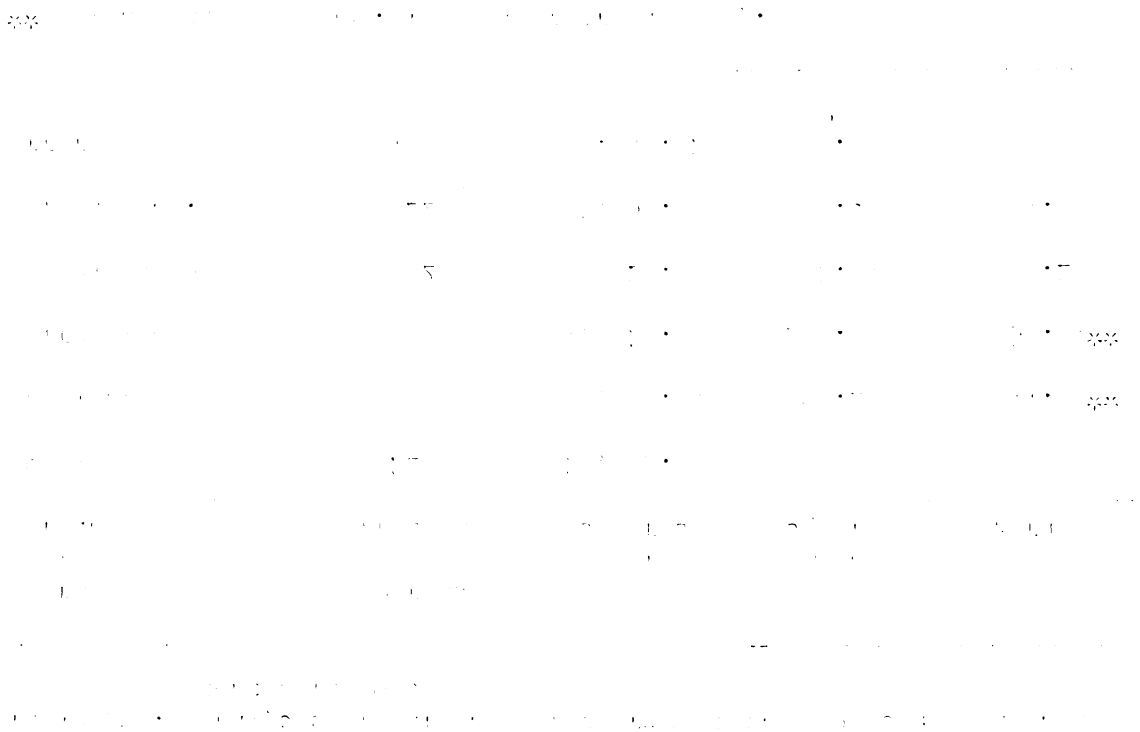
Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	60	8,698	145	
Treatment	11	5,562	506	9.37**
Error	49	3,136	64	

** Significant at the .01 level of probability.

Table 3-7. Analysis of variance of four-week chick plasma vitamin A concentrations

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	223	28,375.36		
Subclass	35	19,434.34	555.27	11.68**
Treatment	11	17,323.35	1,574.85	33.11**
Replication	2	25.50	12.75	.27
Total int.	22	2,085.49	94.80	1.99
Error	188	8,941.02	47.56	

** Significant at the .01 level of probability.



The lowest level of the ANRC Vitamin A Reference Standard resulted in a significantly higher ($P < 0.05$) plasma vitamin A concentration than did all other products at a corresponding level. At the other two corresponding levels, the ANRC Vitamin A Reference Standard resulted in a significantly higher ($P < 0.01$) plasma vitamin A concentration. At 1,298 IU per pound of feed, the plasma vitamin A concentration was significantly higher than with any other diet.

In most instances, as the dietary level of vitamin A activity increased, the liver vitamin A concentration also increased, but few significant differences in liver vitamin A concentration occurred (Tables 3-8 and 3-9). There was very little liver vitamin A storage at any of the feed levels from any of the products used in this experiment. However, the highest level of the ANRC Vitamin A Reference Standard resulted in significantly higher liver vitamin A concentration than all other treatments.

The effects of the test diets on survival are shown in Tables 3-10 and 3-11. There was a trend toward longer survival as the level of the vitamin A activity of the diet increased from each of the products tested. However, few significant differences were observed.

Table 3-8. Chick liver vitamin A concentrations

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† vit. A liver concentration (mcg/gm fresh basis)
Fermentation beta-carotene	514	.38 Dd
" " "	836	.57 CDd
" " "	1,433	1.14 BCbc
Crystalline beta-carotene	548	.50 CDd
" " "	876	.50 CDd
" " "	1,453	.65 CDcd
Synthetic beta-carotene	--††	.40 Dd
" " "	--	.47 CDd
" " "	--	1.34 Bb
ANRC Vitamin A Reference Standard	539	.47 CDd
" " " "	819	.86 BCDbcd
" " " "	1,298	3.32 Aa

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

†† Omitted due to incorrect value assigned to premix. See text for complete explanation.

Table 3-9. Analysis of variance of chick liver vitamin A concentrations

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	138	136.18		
Subclass	35	99.91	2.85	8.14**
Treatment	11	95.10	8.64	24.68**
Replication	2	1.07	.535	1.53
Total int.	22	3.74	.17	.49
Error	103	36.27	.35	

** Significant at the .01 level of probability.

Table 3-10. Survival of chicks placed on the basal ration

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† Survival days until death
Fermentation beta-carotene	514	26.25 ABCbcde
" " "	836	24.55 BCcde
" " "	1,433	29.33 ABCabcd
Crystalline beta-carotene	548	21.63 Cc
" " "	876	23.40 BCde
" " "	1,453	28.94 ABCabcd
Synthetic beta-carotene	--††	29.23 ABCabcd
" " "	--	27.83 ABCabcde
" " "	--	30.83 ABCabc
ANRC Vitamin A Reference Standard	539	24.79 BCcde
" " " "	819	32.50 ABab
" " " "	1,298	34.33 Aa

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

†† Omitted due to incorrect value assigned to premix. See text for complete explanation.

Table 3-11. Analysis of variance of survival

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	142	10,126		
Subclass	35	3,838	109.66	1.87**
Treatment	11	1,826	166	2.82**
Replication	2	672	336	5.72**
Total int.	22	1,340	60.90	1.04
Error	107	6,288	58.77	

** Significant at the .01 level of probability.

Experiment II:

Since the procedures in Experiment I were satisfactory, Experiment II was conducted with the changes previously outlined. The plasma vitamin A concentrations followed (now shown) an almost identical pattern to that shown in Figure I and reached a low of about five micrograms per 100 ml of plasma on the 14th day after the chicks had been placed on the basal ration.

The effects of the test diets on weight gain and feed efficiency, are shown in Tables 4-1 to 4-5.

The statistical analysis revealed that there were few significant differences in growth responses from any of the products or levels tested after two weeks on the test diets (Table 4-1). However, the lowest level of the crystalline beta-carotene did result in a somewhat smaller mean body weight although not significantly ($P < .01$) smaller than that produced on most of the other diets.

After four weeks on the test diets there were significant differences ($P < .01$) in several of the mean body weights (Table 4-3). The lowest level of the fermentation beta-carotene and the three lowest levels of the crystalline beta-carotene did not support the rate of weight gain obtained by the diet which supported the greatest weight gain (Basal + ANRC Vitamin A Reference Standard at 1,320 IU vitamin A per lb.). All of the other diets resulted in mean body weights which were not significantly different ($P < .01$).

Feed efficiency (Table 4-5) was quite similar for chicks on all the diets except the lowest level of the crystalline beta-carotene.

Table 4-1. Two-week chick weights

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† body wts. (gms)
Fermentation beta-carotene	765	427.3 ABabc
" " "	1,035	440.3 ABabc
" " "	1,565	435.4 ABabc
" " "	2,070	446.1 ABabc
Crystalline beta-carotene	810	406.8 Bc
" " "	1,090	429.6 ABabc
" " "	1,510	422.8 ABab
" " "	2,050	449.3 ABabc
ANRC Vitamin A Reference Standard	1,030	457.6 Aab
" " " " "	1,320	458.8 Aab
" " " " "	1,590	464.3 Aa
" " " " "	2,090	453.4 ABab

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 4-2. Analysis of variance of two-week chick weights

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	351	1,504,039	4,285	
Subclass	35	190,782	5,451	1.31
Treatments	11	95,481	8,680	2.09*
Replication	2	11,275	5,638	1.36
T X R (Int.)	22	84,026	3,819	.92
Error	316	1,313,257	4,156	

* Significant at the .05 level of probability.

Table 4-3. Four-week chick weights

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† body wts. (gms)
Fermentation beta-carotene	765	743.6 CDcd
" " "	1,035	788.5 ABCabc
" " "	1,565	807.9 ABCab
" " "	2,070	807.6 ABCab
Crystalline beta-carotene	810	707.2 Dd
" " "	1,090	747.4 CDcd
" " "	1,510	761.6 BCDbcd
" " "	2,050	833.2 ABa
ANRC Vitamin A Reference Standard	1,030	818.6 ABCab
" " " " "	1,320	846.0 Aa
" " " " "	1,590	814.9 ABCab
" " " " "	2,090	835.2 ABa

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 4-4. Analysis of variance of four-week chick weights

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	348	4,135,432		
Subclass	35	859,474	24,556	2.35**
Treatments	2	403,995	201,996	19.36**
Level	3	204,373	68,124	6.53**
Replication	2	39,799	19,900	1.91
Total int.	28	211,307	75,467	7.23**
Error	314	3,275,958	10,433	

** Significant at the .01 level of probability.

Table 4-5. Feed efficiency obtained during the four-week experimental period -- Experiment II

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Pounds of feed per pound of gain in body wt.
Fermentation beta-carotene	765	2.58
" " "	1,035	2.52
" " "	1,565	2.48
" " "	2,070	2.49
Crystalline beta-carotene	810	2.70
" " "	1,090	2.59
" " "	1,510	2.52
" " "	2,050	2.46
ANRC Vitamin A Reference Standard	1,030	2.47
" " " "	1,320	2.48
" " " "	1,590	2.52
" " " "	2,090	2.49

This diet resulted in a somewhat poorer feed efficiency which was closely associated with the slower rate of weight gain obtained.

The effects of the test diets on mean plasma vitamin A concentration of chicks that had been on the test diets four weeks are shown in Tables 4-6 and 4-7 and Figure II. Large variations in blood plasma vitamin A concentration were observed in chicks consuming the same diet.

The mean blood plasma vitamin A concentration increased when the feed level of vitamin A activity increased regardless of the product included in the feed. All diets resulted in raising the mean plasma vitamin A concentration well above the depleted concentration value.

The assayed value of vitamin A activity for the two beta-carotene products was quite similar at each level tested. At each of the comparable levels tested, the fermentation beta-carotene resulted in a significantly ($p < .01$) higher mean vitamin A plasma concentration than did the crystalline beta-carotene. As the feed level of the vitamin A activity increased, the efficiency of the fermentation beta-carotene was reduced in its ability to raise the blood concentration of vitamin A. This was not true of the crystalline beta-carotene which resulted in a nearly straight line increase in its ability to increase the blood plasma vitamin A concentration in relation to the feed level of vitamin A activity.

It was also observed that at all comparable levels, the ANRC Vitamin A Reference Standard resulted in higher, but not always significantly, vitamin A plasma concentrations than did either of the

Table 4-6. Chick plasma vitamin A concentrations after four weeks on the test diets

Treatment of basal ration	I.U. Vit. A/lb Assayed	Mean† plasma concentration (mcg Vit. A/100 ml)
Fermentation beta-carotene	765	28.6 Ggh
" " "	1,035	38.1 EFef
" " "	1,565	51.2 CDc
" " "	2,070	53.9 Cc
Crystalline beta-carotene	810	20.9 Hi
" " "	1,090	26.4 GHh
" " "	1,510	33.1 FGfg
" " "	2,050	44.8 DEd
ANRC Vitamin A Reference Standard	1,030	41.3 Ede
" " " " "	1,320	52.7 Cc
" " " " "	1,590	64.5 Bb
" " " " "	2,090	76.1 Aa

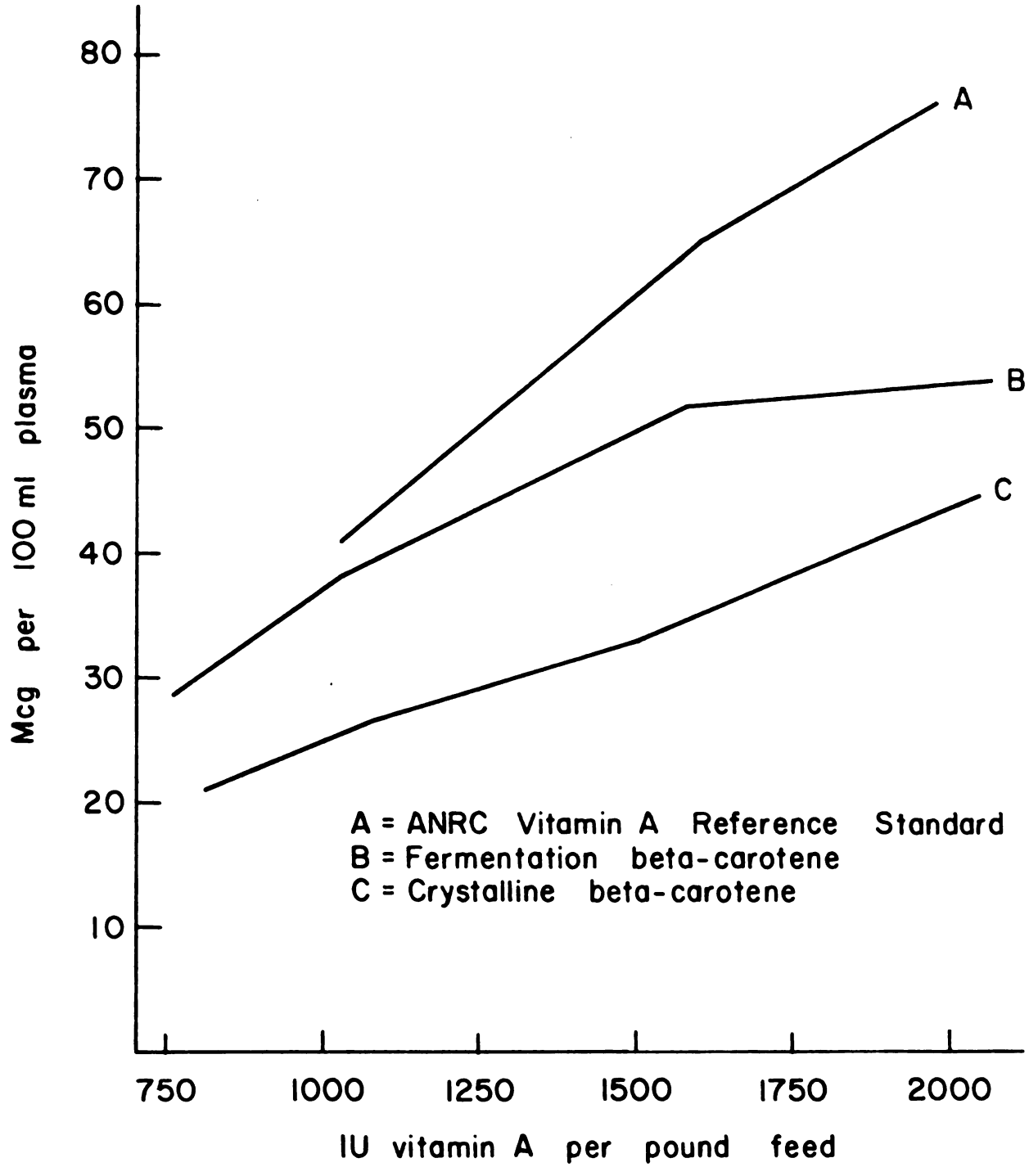
† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 4-7. Analysis of variance of chick plasma vitamin A concentrations

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	328	113,950		
Subclass	35	83,094	2,374	22.61**
Treatment	2	41,406	20,703	197.17**
Level	3	38,468	12,823	122.12**
Replication	2	197	98.5	.94
Total int.	28	3,023	108	1.02
Error	293	30,856	105	

** Significant at the .01 level of probability.

Figure 11. Mean plasma vitamin A concentration (chicks)



beta-carotene products. It was also noted that when the ANRC Vitamin A Reference Standard was increased from 1,590 IU to 2,090 IU per pound of feed, the vitamin A blood plasma concentration did not increase correspondingly.

The effects of the test diets on liver vitamin A concentration are shown in Tables 4-8 and 4-9 and Fig. III. There was a large variation in liver vitamin A concentration in chicks consuming the same diet. Vitamin A liver concentration occurred regardless of product at the lowest level of each product tested. The mean vitamin A liver concentration increased as the level of vitamin A activity in the feed increased stepwise from 800 to 2,195 IU/lb for all products tested. Large differences resulted between liver concentrations of vitamin A but the differences were not always statistically significant. At the lowest level of vitamin A activity in the feed, there was no significant difference in vitamin A liver concentration. As is evident from Table 4-9, at all of the other levels, the vitamin A liver concentration obtained was significantly higher (.01 level of probability) from the ANRC Vitamin A Reference Standard than from the other products. The next highest level (1,590 IU/lb of diet) produced significantly higher mean vitamin A liver concentration than all diets, except the highest feed level (2,090 IU) of the ANRC Vitamin A Reference Standard, which resulted in a mean liver vitamin A concentration significantly higher than all other diets.

At all of the comparable levels of vitamin A activity of the feed, the liver vitamin A concentration obtained was similar for the fermentation beta-carotene and the crystalline beta-carotene. However, at all

Table 4-8. Chick liver vitamin A concentrations after four weeks on the test diets

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† Vit.A liver concentration (mcg/gm fresh basis)	Total mcg per liver
Fermentation beta-carotene	765	1.22 Efg	24.50
" " "	1,035	1.47 DEfg	31.99
" " "	1,565	3.38 CDde	69.30
" " "	2,070	4.97 Cc	94.50
Crystalline beta-carotene	810	.79 Eg	14.56
" " "	1,090	1.08 Efg	22.30
" " "	1,510	1.84 DEfg	36.67
" " "	2,050	2.49 DEfg	50.65
ANRC Vitamin A Reference Standard	1,030	2.28 DEefg	50.40
" " " " "	1,320	4.66 Ccd	101.00
" " " " "	1,590	9.47 Bb	196.70
" " " " "	2,090	21.32 Aa	471.80

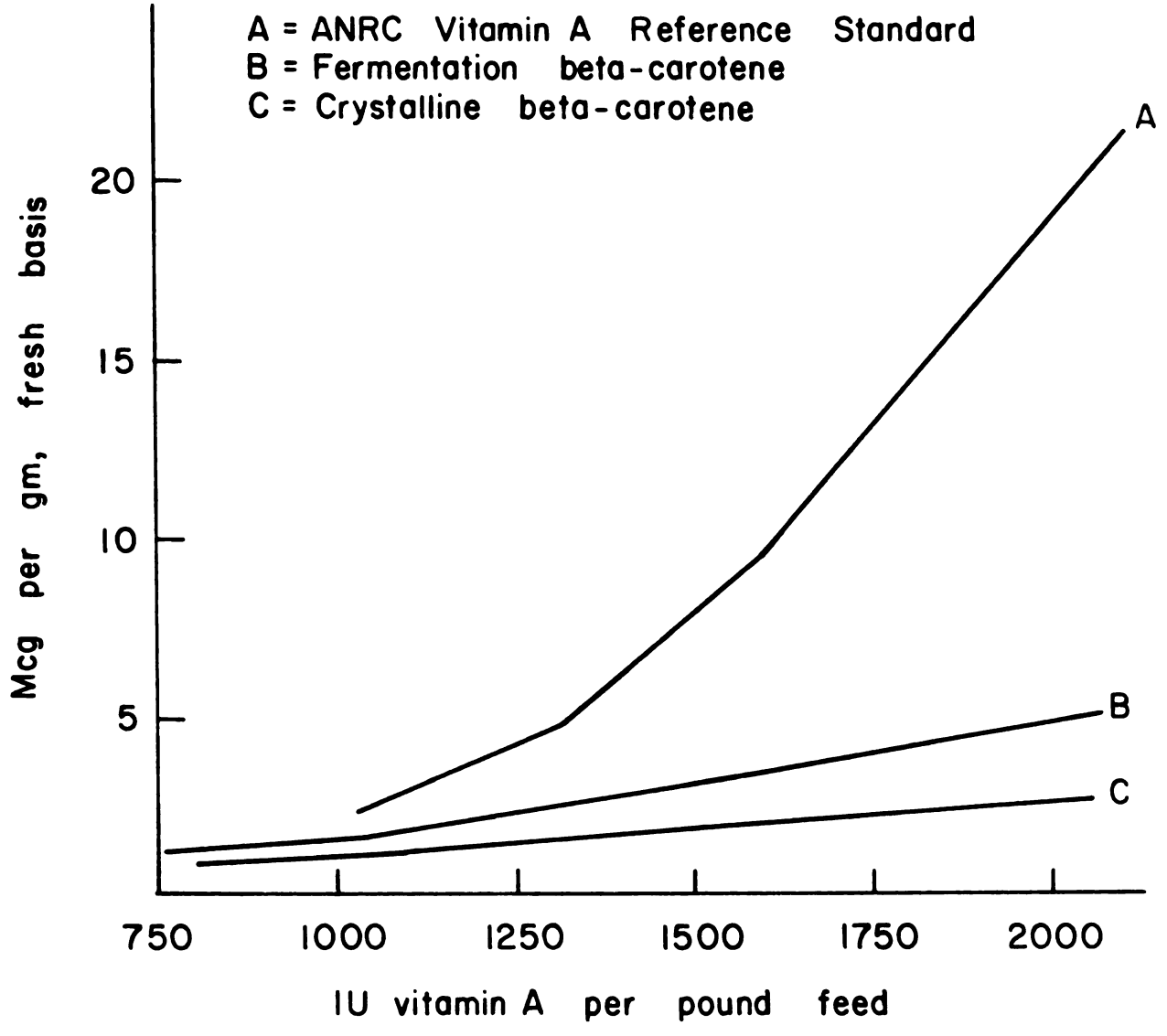
† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 4-9. Analysis of variance of chick liver vitamin A concentration

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	175	6,456.8		
Subclass	35	5,963.2	170	48.57**
Treatment	2	2,332.0	1,166	333.14**
Level	3	1,770.8	590	168.57**
Replication	2	30.9	15.5	4.43*
Total int.	28	1,829.5	65.4	18.68**
Error	140	493.6	3.5	

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.

Fig. [illegible]. Vitamin A liver concentration [illegible]



given levels, the fermentation beta-carotene resulted in a slightly higher, but not always significant, mean liver vitamin A concentration than did the crystalline beta-carotene. The lowest level (1,030 IU) of the ANRC Vitamin A resulted in a higher mean liver vitamin A concentration than did all levels of the crystalline beta-carotene tested and higher than all but the highest level (2,070) of the fermentation beta-carotene.

Using liver vitamin A concentration after four weeks on the test diets as the criteria, the relative biopotency of the fermentation beta-carotene and crystalline beta-carotene was different for each of the levels tested when compared with the ANRC Vitamin A Reference Standard.

Fermentation beta-carotene (IU)	1,035	1,510	2,070
Total liver vitamin A (% of ANRC)	63.5	35.2	20.0
Liver vitamin A concentration (% of ANRC)	64.5	35.7	23.3
Crystalline beta-carotene (IU)	1,090	1,590	2,050
Total liver vitamin A (% of ANRC)	44.2	18.6	10.7
Liver vitamin A concentration (% of ANRC)	47.4	19.4	11.7

It is obvious that as the vitamin A activity of the feed increased, the relative efficiency of conversion to liver vitamin A was decreased.

Tables 4-10 and 4-11 show the effects of placing the birds on the basal depletion diet after having been on the test diets four weeks. There was a trend toward longer survival as the vitamin A activity of the diet increased from each of the products tested. However, with the exception of the lowest level of the crystalline beta-carotene (810 IU/lb)

Table 4-10. Survival of chicks placed on the basal ration

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† days of survival
Fermentation beta-carotene	765	34.7 BCc
" " "	1,035	39.1 ABbc
" " "	1,565	45.7 ABabc
" " "	2,070	44.0 ABabc
Crystalline beta-carotene	810	25.0 Cd
" " "	1,090	39.2 ABbc
" " "	1,510	34.8 BCc
" " "	2,050	42.1 ABabc
ANRC Vitamin A Reference Standard	1,030	40.3 ABabc
" " " " "	1,320	48.1 ABab
" " " " "	1,590	51.4 Aa
" " " " "	2,090	48.0 ABabc

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 4-11. Analysis of variance of survival of chicks placed on the basal ration

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	163	34,477		
Subclass	35	13,136	375	2.27**
Treatment	2	3,832	1,916	11.61**
Level	3	3,393	1,131	6.85**
Replication	2	5	2.5	.015
Total int.	28	5,906	211	1.28
Error	129	21,341	165	

** Significant at the .01 level of probability.

which resulted in significantly shorter survival than all other diets (.05 level of probability), few significant differences in survival time were observed.

Experiment III:

In Tables 5-1 and 5-2 are shown the effects of the test diets on mean liver vitamin A concentration. Significant differences were observed between all diets. Using liver concentration of vitamin A as the criteria for comparison, the relative biopotency of the fermentation beta-carotene was 30.1 per cent (8,950 IU) and 21.7 per cent (21,350 IU) when compared to all the all-trans vitamin A palmitate. Using total liver vitamin A as the criteria, the relative biopotency of the fermentation beta-carotene was 48.6 per cent (8,950 IU) and 41.0 per cent (21,350 IU) when compared to the all-trans vitamin A palmitate.

Experiment IV:

The effects of the basal depletion diet on poult vitamin A plasma concentration and liver vitamin A concentration are shown in Fig. IV and Table 6-1. The decrease in plasma vitamin A concentration from approximately 140 mcg per 100 ml plasma was very steep and nearly in a straight line for about 30 days at which time the plasma vitamin A concentration did not drop any lower than about 12 mcg per 100 ml of plasma. At about 40 to 42 days the poults began to show typical signs of vitamin A avitaminosis as described by Moore (1957). No death loss (except those killed by securing blood samples) occurred up to 42 days.

Liver vitamin A concentration dropped from a mean of about 40 mcg/gm at day one to less than one mcg/gm at 42 days.

Table 5-1. Chick liver vitamin A concentration

Treatment of basal ration	I.U. Vit. A/lb. Assayed	Mean† Vit. A liver conc. (mcg/gm, fresh basis)	Total mcg per liver
Fermentation beta-carotene	8,950	18.49 Dd	113.4
" " "	21,350	37.60 Cc	233.2
PGB-250 dry vitamin A	10,400	61.40 Bb	390.0
" " " " "	21,700	173.20 Aa	952.0

† Any two means having the same letter are not significantly different; means not having the same letter are significantly different. Small letters indicate significance at the .05 level; large letters at the .01 level.

Table 5-2. Analysis of variance of chick liver vitamin A concentration

Source of variance	Degrees of freedom	Sum of squares	Mean square	F ratio
Total	159	712,573.7		
Subclass	15	580,085.6	36,672.4	42.03**
Treatments	1	317,980.2	217,980.2	345.63**
Level	1	170,380.8	170,380.8	185.19**
Replication	3	1,156.3	385.5	.42
Total int.	10	90,568.3	9,056.8	9.84**
Error	144	132,488.1	920.0	

** Significant at the .01 level of probability.

Figure 1. Plasma vitamin A concentration (pouits)

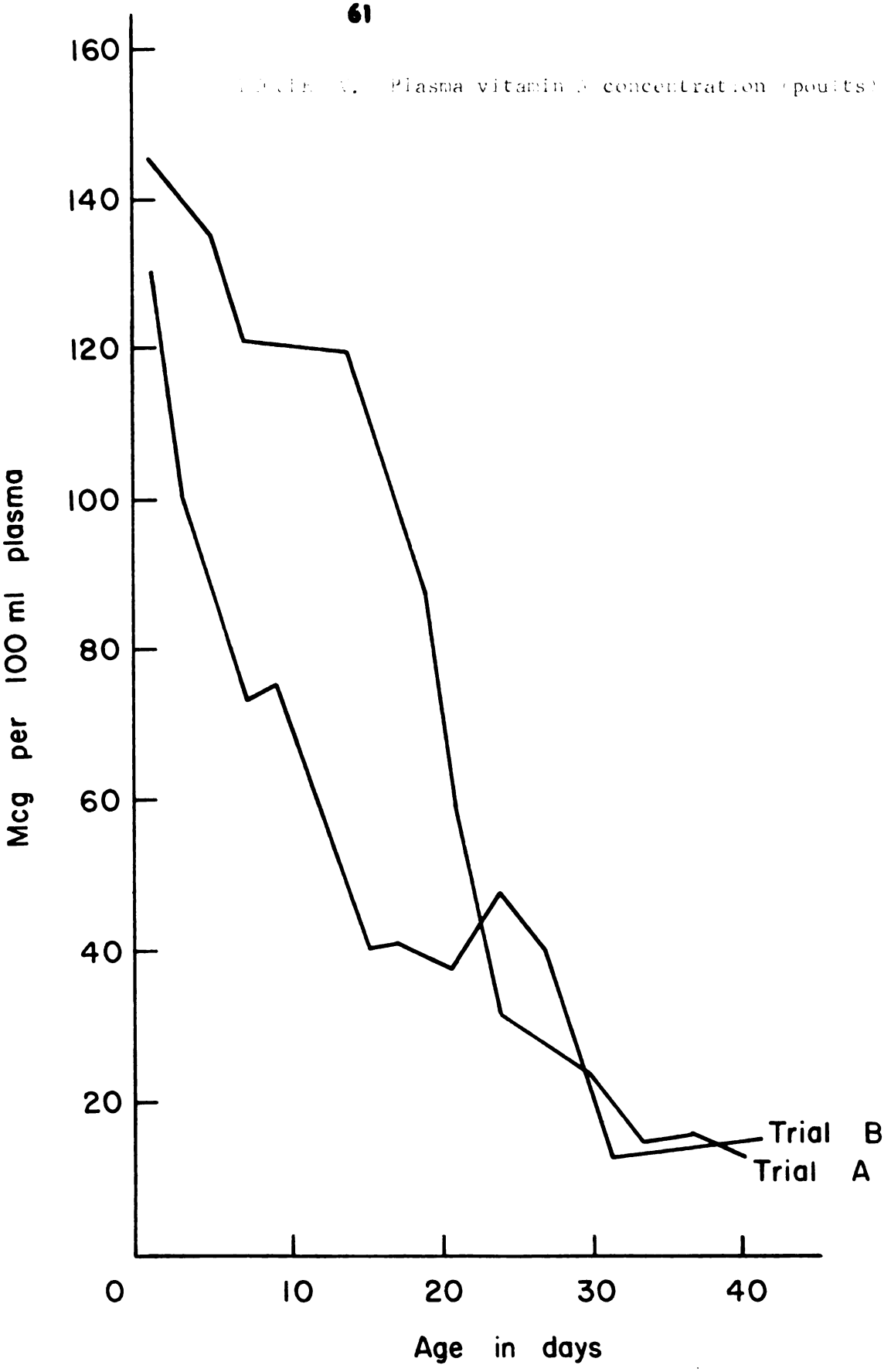


Table 6-1. Poult liver vitamin A concentration

Days of age	Mean Mcg/gm (fresh basis)
1	39.5
3	30.0
7	18.5
9	10.5
29	2.8
42	.39

DISCUSSION

Based upon a review of the literature, there is much controversy concerning the utilization of beta-carotene by poultry and other animals as a source of vitamin A. There also exists doubt as to the need for high levels of vitamin A either in the blood or in the liver. The relationship between dietary, blood and liver concentration is uncertain. Several workers have reported beneficial effects of using therapeutic levels of dietary vitamin A in alleviating certain disease conditions. Much of the information reported relative to the above-mentioned areas of investigation depended upon the criteria employed as measuring devices of the relative performance for vitamin A and/or beta-carotene. In addition, it became obvious that little information was available on the vitamin A reserve in newly-hatched turkey poults. Therefore, it seemed desirable to determine the utilization of a newly developed fermentation beta-carotene product by chicks and to investigate some of the relationships of vitamin A and beta-carotene in growing chicks and to investigate the body reserves of vitamin A in the turkey poult. If this new fermentation beta-carotene product could be incorporated into poultry rations as a source of vitamin A, it would possibly be economically advantageous to the poultry producer who in recent times has encountered a continuing economic squeeze between lower prices for his products and rising costs of production.

In Experiment I, the initial (day-old) plasma vitamin A concentration was higher than that reported by Squibb (1961) and lower than that reported by Castano *et al.* (1951), while the plasma vitamin A concentration following depletion was about equal to that reported by

Squibb (1961). These differences are probably due to the difference in the amount of parental carry-over, as shown by Squibb (1961). By depleting the chick of all or nearly all of its vitamin A reserves, a more critical evaluation of the test diets can be made, especially if the test period is of only short duration.

In this experiment, the dietary levels of vitamin A selected for all of the products (except the synthetic beta-carotene) were what have been considered below the minimum requirement, approximately the minimum requirement and above the minimum requirement, based on growth. However, at the two lowest levels neither the fermentation beta-carotene nor the crystalline beta-carotene supported growth comparable to the ANRC Vitamin A Reference Standard and only the fermentation beta-carotene supported growth comparable to the ANRC Reference Standard at the highest level. This would lead one to believe that at or below the minimum requirement of vitamin A which is required for growth by chicks, the Vitamin A Reference Standard was utilized more effectively than either the fermentation beta-carotene or the crystalline beta-carotene. Although the synthetic beta-carotene was fed at several times the intended levels, differences in growth rate occurred from this product and the highest dietary level produced the largest mean body weights in this trial.

It appeared that blood plasma vitamin A concentration was dependent on the length of time the chick was on the test diet, level of dietary supplementation, and the dietary source -- whether it was from the Vitamin A Reference Standard or the beta-carotene products. No

matter which product was the dietary source, blood plasma vitamin A concentration was very low unless optimum or near optimum growth was produced. In the case of the synthetic beta-carotene, even though very high levels of vitamin A activity were in the diet and excellent growth was produced, the vitamin A plasma concentrations were similar to the other beta-carotene products.

Vitamin A liver concentrations obtained in Experiment I were very low in all cases, except the highest dietary level of the ANRC Vitamin A Reference Standard which resulted in significantly larger vitamin A liver concentration than all other diets. This could very well be the case if one accepts the theory that in all but this high level of the ANRC Reference Standard the chick utilized all of the available vitamin A for metabolic processes. Very few significant differences in survival resulted from any of the diets even though significant differences existed in body reserves of vitamin A.

The inability of the synthetic beta-carotene to favorably affect any of the criteria, except growth, even though it was fed at several times the intended levels was probably due to the abundance of isomers of beta-carotene which were not of the all-trans type. The latter have poor biological availability as shown by Moore (1957).

The results obtained with the second group of birds fed the basal ration coincided very closely with those of the first experiment, indicating approximately the same parental carry-over.

In Experiment II, the dietary levels selected were all above what is generally accepted as the absolute minimum amount of vitamin A

activity to produce optimum growth; this accounts for the relatively few significant differences in mean body weights. However, in this experiment, as well as in Experiment I, the lowest level of the beta-carotene products did not support the rate of growth that was obtained in the case of the other diets. This again indicates that the lowest dietary level of beta-carotene used in this experiment (regardless of product) was not used as effectively for growth as the comparable dietary level of vitamin A. It was also apparent that the fermentation beta-carotene was at least equal to the crystalline beta-carotene in its ability to support growth of chicks in this experiment.

In Experiment II, the ANRC Vitamin A Reference Standard increased the vitamin A plasma concentration more than did either of the beta-carotene products at all comparative levels and would, therefore, appear more biologically available for inducing blood concentration of vitamin A. It also appeared that in the case of the fermentation beta-carotene, the highest level used in this test may have been approaching its upper limit in ability to influence vitamin A blood concentration. This did not appear to be true for either of the other products tested. At the lowest dietary level of all products tested, little vitamin A liver concentration was found. However, at each of the other dietary levels, the liver vitamin A concentration was significantly higher from the ANRC Vitamin A Reference Standard than from the other products tested and as the dietary level of the ANRC Reference Standard increased from 1,320 IU to 2,090 IU per pound of diet, a straight line increase in vitamin A liver storage occurred. As the level of both beta-carotene

products increased from about 1,000 IU per pound to about 2,050 IU per pound, their ability to produce vitamin A liver storage was reduced. Again, even though large differences existed in the bird's vitamin A reserves from the various diets, few significant differences resulted in survival of the birds placed on the basal ration after having been on the test diets.

In these first two experiments, similar responses were observed for all of the criteria used to evaluate the utilization of the fermentation beta-carotene and the relationships between vitamin A and beta-carotene. In both experiments, when the chicks were fed diets that contained very near or below the requirement of vitamin A needed for optimum growth, the ANRC Vitamin A Reference Standard was utilized more effectively than any of the beta-carotene products in promoting growth. However, when dietary levels were in excess of the known minimum requirement for growth, all products tended to support optimum growth. In both experiments it was obvious that optimum growth could occur without high plasma or liver concentrations of vitamin A. Although optimum growth was obtained without any appreciable vitamin A liver storage, this may not be a practice which is practical for the commercial poultryman. The chick can utilize the stored vitamin A and if little was available in the body reserves, the chick could suffer during periods when no dietary vitamin A was available or being ingested. This could be especially important in periods of disease, since the chick usually consumes less feed during sickness and vitamin A has been found to be effective in connection with several disease

conditions. It was also observed that even though known differences occurred in the body reserves of vitamin A few significant differences occurred in survival on the basal ration. This could be explained if it were determined that all of the body's reserves of vitamin A were not mobilized or were not available for use by the chick; however, this was not determined. It was also observed that the fermentation beta-carotene and the crystalline beta-carotene influenced the characteristics used for comparison quite similarly; however, both beta-carotene products were inferior to the ANRC Vitamin A Reference Standard in their ability to influence plasma and liver concentrations of vitamin A, while all products influenced survival on the basal ration in about the same manner.

In Experiment III, high dietary levels of vitamin A activity were compared for a short period. It appeared that dietary fermentation beta-carotene did not support the liver vitamin A concentrations obtained with comparable levels of dietary vitamin A. However, for liver vitamin A concentration, the fermentation beta-carotene was more effective in this experiment than in previous experiments when compared to vitamin A palmitate. This could have been influenced by the shorter depletion period, large liver size, and product differences.

The relative ineffectiveness of the dietary beta-carotene used in these experiments to induce plasma and liver concentrations of vitamin A comparable to that of dietary vitamin A is intriguing. Perhaps there are specific sites and/or enzymes within the intestine of the chick utilized for conversion of beta-carotene to vitamin A, and there are

only slightly more sites and/or enzymes for conversion than are necessary for optimum growth. This could explain the results shown in these experiments.

In Experiment IV, turkey poults had a high carry-over of vitamin A in the liver, lived for a much longer period on the vitamin A deficient basal ration than chicks, and the vitamin A plasma concentration did not drop as low after 42 days on the basal ration as in the case of chicks after only 14 days on the basal ration. After about 30 days on the basal ration, the poult's plasma reached its lowest concentration in vitamin A. However, vitamin A deficiency symptoms did not appear until 10-12 days later. It is possible that the turkey can live this additional time on the body reserves as there was apparently some liver storage (2.8 mcg per gram) when the plasma vitamin A concentration reached its lowest level.

The initial vitamin A concentration in the liver of these turkeys was much higher than the initial vitamin A liver concentration of chicks reported by Squibb (1961). The turkey poult may also have more parental vitamin A carry-over in the yolk due to size of the yolk and the possibility of a higher yolk concentration of vitamin A in turkey eggs.

CONCLUSIONS

Commercially obtained day-old Cobb's Strain White Rock cockerels were successfully grown at an optimum rate of growth and feed efficiency when fermentation beta-carotene supplied the only source of vitamin A activity.

For the criteria established for comparison, fermentation beta-carotene compared favorably with the commercial crystalline beta-carotene; neither beta-carotene product did as well as the ANRC Vitamin A Reference Standard in influencing plasma and liver concentrations of vitamin A.

In these experiments, optimum growth of broiler chicks was obtained without high levels of plasma and/or liver vitamin A concentration(s).

At levels of vitamin A activity which support optimum chick growth, beta-carotene is fully effective to the extent that .6 mcg of all-trans beta-carotene = 1 IU of vitamin A. When higher levels are fed to induce liver storage, beta-carotene was about 1/10 to 4/10 as effective as vitamin A.

Survival time of chicks, which had been on the test diets four weeks and then placed on the basal ration, was at best a poor measure to determine the effectiveness of a product to possess vitamin A activity.

In order to accurately discuss the vitamin A requirement of chicks, the requirement must be defined. There was a different requirement for vitamin A for growth, blood concentration, and liver concentration. There was a product biological availability difference.

There was a large variability in plasma vitamin A concentration and liver vitamin A concentration in chicks consuming the same diet

Turkey poults hatched from a breeding flock consuming a typical breeder ration lived on a ration devoid in vitamin A for a period in excess of 40 days.

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