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To the Graduate Council:

I am submitting herewith a dissertation written by Carroll Reece Douglas entitled "Selected Factors Affecting the Metabolism of Xanthophyll in Chickens." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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SELECTED FACTORS AFFECTING THE METABOLISM OF XANTHOPHYLL IN CHICKENS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Carroll Reece Douglas

March 1966

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INTRODUCTION

Consumer demands for chickens with deep yellow skin color and for eggs with moderately yellow yolks have been evident for many years. The degree of pigmentation desired varies considerably according to locations throughout the United States and the world. The use of egg yolks with deep yellow color for specialized foods such as noodles has made the production of such eggs an important part of the poultry industry. Pigmentation is often used in consumer appeal as an attempt to halt the decline in per capita egg consumption and to increase the consumption of broilers.

There are problems involved in the production of highly pigmented poultry products. With the advent of high energy-low fiber feeds for both broilers and layers, there has been an interest in the reduction of certain high fiber containing ingredients which may be high in xanthophyll, and, in certain geographical areas, an interest in the use of larger amounts of low xanthophyll containing grains.

One species of coccidia, Eimeria maxima, while having little effect on mortality in chickens, is especially troublesome due to its depigmenting effect. The main emphasis in the control of coccidiosis with coccidostats has been against more pathogenic species of Eimeria than against E. maxima. Therefore, depigmentation continues to be a problem in the production of broilers.

While the chicken has no known nutritional or physiological requirements for the xanthophyll pigments, nor can it synthesize these pigments within its body, a greater understanding of factors influencing xanthophyll metabolism would be highly beneficial to the poultry industry.

The objectives of the research reported herein were:

(1) To study the effects of selected feedstuffs and non-nutritive additives upon xanthophyll metabolism, upon skin and shank pigmentation of young chicks, live and dressed broilers and roasters, and deposition and loss of pigmentation of hens during production.

(2) To study the effect of feed intake and environmental temperature upon pigmentation and blood xanthophyll levels of chicks.

(3) To study the effects of coccidia upon xanthophyll metabolism.

REVIEW OF LITERATURE

Chemistry of Xanthophylls

The definition of carotenoids, which has been accepted by the "Union internationale de Chimie," states that "Carotenoids are yellow to red pigments of aliphatic or alicyclic structure, composed of isoprene units (usually eight) linked so that the two methyl groups nearest the centre of the molecule are in positions 1:6 whilst all other lateral methyl groups are in positions 1:5, the series of conjugated double bonds constitutes the chromophoric system of the carotenoids." (Goodwin, 1954). Fruton and Simmonds (1958) more simply describe carotenoids as light yellow to purple pigments found among the unsaponifiable lipids of nearly all higher plants, many microorganisms and many animals. Carotenoids found in nature may be considered derivatives of the red pigment lycopene which has the empirical formula $C_{40}H_{56}$.

Wagner and Mitchell (1960) divide the carotenoids into two groups: (1) carotenes, which are simple polyene hydrocarbons; and (2) xanthophylls, which are the oxidized forms of the carotenes. Goodwin (1950) states that animals may be classified into four groups according to their ability to absorb and to store the several carotenoids as follows: (1) those capable of storing carotenes and xanthophylls of which man is an example; (2) those storing carotenes preferentially such as the cow;

(3) those storing xanthophyll preferentially such as the chick; and (4) those which store neither carotenes nor xanthophylls such as the pig.

This preferential absorption was noted by Palmer (1915) when he reported that the natural pigment characterizing the egg yolk, body fat, and blood serum of the hen is physiologically identical with the carotene and xanthophyll pigments of plants with xanthophyll being present in far greater proportion. When hens were fed diets in which the pigment was carotene with no xanthophyll, there was no appreciable influence upon the amount of pigment carried by the blood serum and deposited in the egg yolk. The author pointed out the difference in utilization of plant carotene and xanthophyll by the cow, where carotene was the predominating pigment found in milk fat, body fat, and blood serum.

Palmer (1915) recognized the value of yellow corn as being particularly suited for producing yellow egg yolks. The prinicple xanthophyll in yellow corn is zeaxanthin (3,3'-dioxy-beta carotene); however, yellow corn also contains a considerable amount of cryptoxanthin (3-oxy-beta carotene) according to Williams et al. (1963). The other principal natural sources of xanthophyll long used in poultry feeds are corn gluten meal and dehydrated alfalfa meal. Using column chromatography, Bickoff et al. (1954a) found a total of 40 xanthophyll bands in dehydrated alfalfa meal. Lutein (3,3'-dioxy-alpha carotene) was found to be predominant, with 87

percent of the total being comprised of lutein, cryptoxanthin, zeaxanthin, violaxanthin and neoxanthin.

Scott and Norris (1959) stated that the vitamin A-inactive xanthophylls, mainly lutein and zeaxanthin, together with the vitamin A precursor, cryptoxanthin, are largely responsible for the pigmentation of the skin and shanks of chickens, the color in the iris of the eye, and the yellow color of the yolk. Kuhn et al. (1931) showed that yolk pigments of hens on common rations were about 70 percent lutein, while the remainder was principally zeaxanthin. These were stored as the free form with about 8 percent esterified.

Metabolism of Xanthophylls

An understanding of xanthophyll metabolism in the chicken is incomplete. Palmer and Kempster (1919a) set out to establish whether plant pigments were entirely dispensable to the chicken. Eggs from chickens grown on a xanthophyll-free diet were set, and the chicks hatched were vigorous and normal in every way except for the complete absence of pigment in the shanks, beaks and skin.

A study by Palmer (1915) regarding transportation of xanthophyll in the chicken failed to show conclusive evidence of the transportation of xanthophyll by means of blood albumin. The author did report, however, that conclusive evidence was obtained showing that the globulin fraction of the serum was free from the pigments.

Ganguly *et al.* (1959), in an intensive study of the transport of carotenoids across the intestines of rats and chickens, suggested the possibility that the species differences in absorption and deposition of different carotenoids might be due to the presence of specific receptor proteins in animal tissue. Experiments in which chicks were first starved for 24 hours, dosed with 2-milliliter portions of oily solutions of lutein and lycopene, and killed for removal and processing of the intestine, indicated that carotenoids were thrown out of fat solution during absorption. It was suggested that intestinal cells of the chicken contain stereospecific lipoproteins capable of combining specifically with lutein at the molecular level, thereby allowing it to enter the animal. Investigation of the intestine showed lutein to be present in large concentration both in the muscle and in the mucosa. Within the mucosal cells, it was concentrated in the mitochondrial and microsomal particles. The lutein of the muscles was in the free form without any appreciable change. They further suggest that the absorption of lutein might be due to the presence of hydroxyl groups offering anchorage, and that it is possible that a lipoprotein stereospecific for lutein exists in dynamic equilibrium among various tissues of chickens and helps in the transport of lutein across the mucosal cells and in its distribution among tissues.

Palmer and Kempster (1919b) concluded that xanthophyll

in the epidermis of earlobes, beak, and shanks of Leghorn and American breeds of fowl was largely in granular form with little or no fat associated with it. By using a series of stained sections of foot and shank skin, they found xanthophyll to be located chiefly in the rete of Malpighi with some diffusion of pigment through the corium. Granular masses also were seen lining the blood capiallaries in the lower corium layers. Stained sections of skin from birds which were fed a xanthophyll-free diet showed first a disappearance of pigment from the corium, then a disappearance from the outer layer of epidermis which gradually extended into the rete of Malpighi, with the last to disappear being that at the base of the Malpighian layer. The authors interpreted this observation to mean that, when a bird is fed a xanthophyll-free diet, the pigment deposited in the outer layer of the epidermis either wears off by means of normal replacement of the outer cells from those lower down, or is oxidized because of the closer contact with air. Possibly both factors are important, because the adipose tissue still retained its normal color after the visible skin parts lost their pigment completely.

Loss of pigment in the visible areas of the body is also used as an indication of the past egg production of a laying hen. This relationship was first reported in scientific literature by Blakeslee and Warner (1915). Using hens in the egg-laying test at Storrs, Connecticut, they found that the amount of yellow in the earlobes increased consistently with the number of

days since cessation of laying. Their conclusion was that laying removed yellow pigment with the yolks more rapidly than it could be replaced by normal metabolism, and in consequence, the earlobes, beak and shanks became pale by subtractions of pigment. Palmer and Kempster (1919b) agreed with this earlier work but also concluded that no matter how large an excess of pigment was fed, it was impossible to restore xanthophyll to the skin. These workers felt that this fading of pigmented areas was not an index of heavy egg laying but of continuous laying only.

Wilson (1956) found that non-laying hens had a higher carotenoid pigment level in their blood serum than did laying hens and suggested that this could be used as a method of determining non-layers. Serum pigment of birds increased rapidly until the sixth day of pause when it reached a peak. Williams et al. (1963) reported that hens laying at a high rate deposit fewer carotenoids in the egg yolk than do hens which lay at a low rate.

Much of the experimental work concerned with pigmentation in broilers has been related to pigmentation at the end of a treatment period. Much of the data concern chicks four or eight weeks of age, with pigmentation scores given at the end of the trial without regard to the pigmentation of the chicks when they were first hatched or the pigmentation pattern during the treatment period. Mann (1946), in studies with very young chicks, made the statement, "It is a well-known fact that while chicks at hatching may have well-pigmented legs, after the first week the

pigment tends to fade and thereafter, depending upon the diet, continues to fade until pigmentation reaches a low point at about the fourth week, when, under favorable circumstances, the color gradually becomes restored." He used diets containing 55 percent yellow corn and green feed in the form of spring cabbage to supply the source of carotenoids. His analytical procedure for measuring xanthophyll content of the chick was to saponify the entire carcass for extraction and evaluation. While his data did show that chick xanthophyll content dropped during the first two or three weeks, there was considerable fluctuation in xanthophyll content during the weeks following.

Davis and Kratzer (1958) studied serum xanthophyll levels in chicks and concluded that serum xanthophyll for the first week after hatch was probably supplied by the yolk absorbed by the chick. They found that when chicks were fed a non-pigmented diet for one week, it took approximately four days after the chicks were placed on a normal chick diet for the blood xanthophyll to rise from a level of 7 to a peak of 15 micrograms per milliliter of serum where it remained through the experimental period of fourteen days

Quantitative Determination of Xanthophylls

Quantitative determination of xanthophyll involves: (1) assay of xanthophyll content of feed ingredients; (2) objective determination of tissue xanthophyll content; and (3) sub-

jective or visual measurement of the xanthophyll content of egg yolk, and of skin, shank and other exposed pigmented areas. Ewing (1963) points out that laboratories do not necessarily agree on the value of the same sample of feed ingredient because of lack of any official method of assay. The method of Bickoff et al. (1954b) for xanthophyll determinations in dehydrated alfalfa has been used by many workers. A modification of this method was used by Mitchell (1961) for xanthophyll determination of corn, corn gluten meal, and alfalfa meal. Madiedo et al. (1964) used the method of Bickoff et al. (1954b) for alfalfa meal and lake weed xanthophyll determinations. However, they modified the saponification and extraction procedure for measurements of corn, algae meal and marigold petals. These authors point out that differences in assay procedure could account for differences in biological availability of feed ingredients as reported by different workers.

There appears to be more agreement on methods of xanthophyll tissue analysis. There is a method for determination of xanthophyll in egg yolk based on carotene equivalents (A.O.A.C., 1960). Kahlenberg (1949) described a method of measuring egg yolk color called the National Egg Producers Association (N.E.P.A.) method. The use of N.E.P.A. numbers for scoring egg yolk color has been widely accepted especially for industrial use as well as in research (Day et al., 1963, and Kingan and Sullivan, 1964). The method described by Wilson (1956) has been used extensively for

determination of the xanthophyll content of the blood, while the method of Wilgus (1954) is often used to determine the xanthophyll content of toe web tissue.

More methods have been described and used for subjective, visual measurement of tissue than for any of the determinations by chemical means. Blakeslee and Warner (1915) described the use of a simple toy, the Bradley color top, for visually determining the amount of yellow in the earlobes of hens. A more complete description of the Bradley color top was given by Palmer and Kempster (1919a). Egg yolks were mixed, spread out in a 5-centimeter porcelain crucible cover, and matched to the color of paper disks on the spinning top while both yolk and top were resting on a white background.

Hammond and Harshaw (1941) scored shank and skin pigmentation of chicks by assigning numerical values as follows: no yellow pigment 0, very slight yellow 1, slightly yellow 2, medium yellow 3, deep yellow 4, very deep yellow 5, very slight orange 6, slightly orange 7, and medium orange 8. Marusich *et al.* (1960) matched the color of egg yolks to a set of 15 sodium dichromate solutions of increasing concentration. Waldroup *et al.* (1960) obtained standards for use in studying broiler pigmentation from each experiment reported by selecting birds which ranged from least to greatest amount of pigmentation. A specially prepared color chart was the description given by Zervas *et al.* (1962) for the standard used in scoring shank pigmentation of

broilers. Egg yolk color scores were determined by use of "Fletcher Rings" by Summers et al. (1963). Milligan and Winn (1964) reported the use of charts developed by the Ralston Purina Company and a color scale by Hoffmann-La Roche, Inc., in rapid pigmentation scoring of the shank and foot pad of broilers.

A color rotor was developed by Heiman and Carver (1935) for visually scoring egg yolk. Although it was developed some 30 years ago, the rotor has been widely used not only for yolk color determination but also for scoring skin and shank pigmentation (Culton and Bird, 1941; Bird, 1943; Brambila et al., 1963; Barnett and Stephens, 1963; and Madiedo et al., 1964). Comparison of data on visual pigmantation scores as reported by various research workers has been difficult due to the use of different standards.

A limited number of reports have been published concerning the relationship between shank and skin color or between levels of xanthophyll in the blood and shank color. Culton and Bird (1941) found skin pigmentation difficult to measure. A limited number of birds were killed and plucked for observations of skin pigmentation. Although no statistical results were reported, these authors observed that for birds with relatively high pigmentation scores of the shank, pigmentation of the skin was proportional to that of the shank, and that for birds with relatively low pigmentation scores of the shank, pigmantation of the skin was negligible. Observations of shank and skin color

were made by Hammond and Harshaw (1941) on birds six and eight weeks of age using numerical values from 0 to 8. Average scores of shanks ranged from 0.40 to 4.03 while scores of skin averaged 0.00 to 2.63. In general there was positive relationship with average scores of skin always falling below average scores of shanks for the same group of birds. No statistical correlation was reported nor was it reported if scores of skin were of live or dressed birds. Using standard birds which were selected for a range in pigment for scoring shanks and skin, Waldroup et al. (1960) found that with an increase in average scores of shanks of eight-week old broilers, there was a relative increase in average scores of skin.

The relationship between levels of xanthophyll in the blood and pigmentation scores of shanks of chicks was studied by Davis and Kratzer (1958). They concluded that the xanthophyll content of blood after one week of experimental feeding could be used to predict the shank pigmentation of these chicks after two or three weeks of experimental feeding.

Factors Affecting Pigmentation

While the degree of pigmentation in eggs or tissues of chickens is predictable by the level of xanthophyll in the feed, there are times when other factors have a greater influence than dietary xanthophyll levels. A review of these factors was given by Mitchell (1961). Only a limited number of factors

affecting xanthophyll pigmentation will be reviewed here.

An important factor in pigmentation is biological availability of the various natural sources of xanthophyll. Ratcliff et al. (1959) found the xanthophyll in alfalfa meal (20 percent protein) to be 46.5 and in corn gluten meal to be 30.7 percent as available as that in yellow corn for pigmentation of chicks when each was used to provide comparable dietary xanthophyll levels. Ratcliff et al. (1962), however, found xanthophyll in alfalfa meal to be 73.8 percent as available as that in yellow corn for pigmentation. Xanthophyll found in clover meal was also compared to that in corn and found to be 86.4 percent as available in pigmentation value. Williams et al. (1963) reported that the carotenoids of yellow corn appeared to be deposited more efficiently in the egg yolk than the carotenoids of alfalfa meal or corn gluten meal. They suggested that this difference may have been due to the cryptoxanthin content of yellow corn rather than to any unique property of the zeaxanthin of yellow corn. They also found that when zeaxanthin and lutein were each fed in a purified form, there was no apparent difference in efficiency of utilization. Peterson et al. (1939), however, had studied the efficiency of transfer of purified carotenoids to the egg yolk when carotenoid-low basal diets were used. These workers found zeaxanthin, lutein and cryptoxanthin to be transferred with efficiencies of 24.35 percent, 11.45 percent and 0 percent, respectively. Deposition of cryptoxanthin, however, was

observed when diets high in yellow corn was fed. Day and Williams (1958) found that lower levels of dietary xanthophylls were more efficiently utilized for pigmentation than higher levels.

In recent years many dietary sources of xanthophyll have been investigated. Brambila *et al.* (1963) reported that Aztec marigold petals (*Tagetes erecta*) produced skin color in broilers and egg yolk color equal to that produced by alfalfa meal when used to supply equivalent xanthophyll levels. The addition of brown seaweeds (*Fucus vesiculosus* and *Fucus serratus*) to basal diets was reported by Jenson (1963) to have resulted in a 7.5 to 10-fold increase in carotenoid content of yolks.

Grau and Klein (1957) found that sewage-grown algae meals (predominantely *Chlorella* and *Scenedesmus* species) contained significant amounts of xanthophyll pigments which are available to the chick for production of yellow shanks and skin. Madiedo *et al.* (1964) found algae meal to be equivalent to alfalfa meal for pigmentation.

Marusich *et al.* (1960) studied several xanthophylls including beta-apo-8'-carotenal and canthaxanthin, which are naturally occurring xanthophylls, but which have been prepared in quantity by chemical synthesis. They found beta-apo-8'-carotenal to be the preferred carotenoid for egg yolk pigmentation. Canthaxanthin was successfully used by Camp *et al.* (1963) in finisher feeds for broilers. Increased pigmentation in skin and shanks was noted when canthaxanthin was added to diets containing yellow corn or alfalfa meal. Cottier *et al.* (1965) found that

peanut vine meal harvested just prior to digging of the nuts compared favorably with alfalfa meal in pigmenting value when fed to broilers at the 3 percent level. New strains of yellow corn have been developed which contain up to 18 milligrams of xanthophyll per pound or about twice the normal level usually occurring in corn according to Ratcliff et al. (1962).

The addition of animal fat to poultry diets has given variable effects upon pigmentatio n. Day and Williams (1958) reported that 5 percent stabilized beef tallow significantly increased the utilization of xanthophyll when it replaced an equal amount of white corn in the broiler rations. However, Ratcliff et al. (1959) found that the addition of 4 percent animal fat did not significantly improve pigmentation in broiler. Donaldson and Gordon (1960) reported that egg yolk color was not affected by the addition of 3 percent stabilized animal fat.

Menadione sodium bisulfite was reported by Griminger and Fisher (1960) to increase shank pigmentation when added to the diet of chicks, while Smidt et al. (1965) reported no effect on the utilization of xanthophyll when menadione was added to the diet of chicks.

The antioxidant, ethoxyquin, has been reported by Waldoup et al. (1960) and Ratcliff et al. (1961) to increase significantly the deposition of pigment in broilers when added at the level of 0.0125 percent. Madiedo and Sunde (1964) found that ethoxyquin increased pigmentation of egg yolks when added at 0.01 or 0.10 percent levels to layer diets. These workers

as well as Waldroup *et al.* (1960) suggested that ethoxyquin may act by improving the stability of xanthophyll in the digestive tract and blood stream or in feed during storage. Atkinson *et al.* (1963) also suggested that ethoxyquin appeared to function as an *in vitro* antioxidant in protecting and maintaining liver vitamin A stores.

Several feedstuffs have been reported to inhibit the deposition of xanthophyll in chicks. Culton and Bird (1941) reported that meat scraps, fish meal and soybean oil meal reduced pigmentation in growing chicks. Variations were found in the inhibitory effects between different lots of menhaden fish meal and of soybean oil meal. Hammond *et al.* (1942) found that 3 percent fortified cod liver oil greatly reduced the transfer of pigment from the diet through the egg to the chick's shank. Meat and bone meal was found by Bird (1943) to have a pigment depressing effect when fed to chicks 6 to 14 weeks of age. A slightly lower pigmentation score was noted by Hardin *et al.* (1964) in groups of broilers fed diets containing fish oil.

Vitamin A at excessive levels has been found to affect skin and yolk pigmentation adversly. Dua and Day (1964) reported that pigmentation was reduced in broilers when 8,000; 12,000; and 90,000 I.U. of vitamin A per pound was added to the diet. Total carotenoids of serum, liver and toe web skin were highly significantly depressed in broiler chicks fed levels of 158,400 and 475,200 I.U. of vitamin A palmitate per kilogram of diet as reported by Dua *et al.* (1965). Feeding diets containing

100,000 and 125,000 I.U. of vitamin A per kilogram of diet were found by Madiedo and Sunde (1964) to produce light colored yolks. Certain hens, however, were able to resist the depigmenting effect of vitamin A.

Many diseases have been associated with reducing the deposition of xanthophyll pigments in chickens. The literature is lacking in scientific experiments which show proof of this relationship. Bird (1953), in reporting on the problem of pigmentation in broilers, indicated that it is well known that an outbreak of coccidiosis very quickly drains pigment from the birds. He also related that respiratory diseases adversely affect pigmentation. Bleaching of the shanks is one of the early signs of the presence of coccidiosis according to Collins et al. (1955). These authors also state that respiratory diseases modify the degree of shank pigmentation in the growing chicken. Coccidiosis has been accused in general of causing decreased pigment utilization by Fritz et al. (1957), Ratcliff et al. (1961), Fritz (1962), Day et al. (1963), and Couch et al. (1963).

Squibb et al. (1955) found that total serum carotenoids were reduced significantly in three-week old chicks infected with cholera.

A study of one species of coccidia, E. maxima, was conducted by Mitchell et al. (1961). These workers reported that depigmentation due to infection of chicks with E. maxima

and lowered blood serum xanthophyll levels were not prevented by the addition of menadione sodium bisulfate or by feeding large amounts of xanthophyll concentrate. This study is the only reported data available where controlled infections were shown to cause depigmentation. Stephens (1965), however, reported that the percent light transmittance of blood plasma of chicks infected with E. necatrix was increased to 200 percent of that of non-infected chicks on the sixth day after inoculation. This was believed to have been due to an inability of chicks to absorb carotenoid pigments from the intestine during infection.

Barnett and Stphens (1963) examined the intestinal content of hens which had laid eggs with pale yolks and hens which had laid eggs with dark yolks and found that individuals producing pale yolk eggs tended to have more coccidal oocysts. Stephens (1964) reported that most groups of bacteria were increased in number in the intestine during an acute phase of coccidal infection. Further work by Turk and Stephens (1965) with cecal coccidiosis caused by E. tenella showed that in four-week old chicks inoculated with 75,000 oocysts, radiozine absorption from the intestine into the bloodstream was decreased

Published reports of scientific research on the effects of feed intake upon pigmentation are also lacking. The part which feed intake plays in depigmentation due to disease has not been determined. Reduction in consumption has been suggested in general terms by Fritz et al. (1957) and Smidt et al. (1965) to have an adverse effect on pigmentation. Although

the authors did not indicate that feed intake was a factor, Milligan and Winn (1964) found chick skin pigmentation to be adversely affected by temperatures of 80° and 100° F. when compared to pigmentation of chicks grown in rooms with temperatures of 70, 60, 53, and 46° F. Kingan and Sullivan (1964) reported that egg yolk color tended to decline with onset of warm weather and that this decline seemed to be correlated with decreased feed intake and possible greater breakdown of dietary xanthophylls by auto-oxidation. Feed consumption was found to be significantly decreased when hens were held at a temperature of 23.8° C. as compared to 12.6° C.

EXPERIMENTAL PROCEDURE

General

Chick battery studies. The crossbred broiler-type (Vantress x Arbor Acre 50) chicks used in some of these experiments were obtained from a local commercial hatchery. For other experiments, Single Comb White Leghorn male chicks were obtained from the University of Tennessee Station stock, and Single Comb White Leghorn-type male chicks were obtained from the Tennessee Random Sample Laying Test or from a local commercial hatchery.

At one day of age, the chicks were sexed and vaccinated for infectious bronchitis and Newcastle disease with a combination vaccine which was administered by the intraocular method. Chicks used in Experiments 7 and 11 were also vaccinated by the same method for avian nephritis-nephrosis (gumboro disease). Chicks were wing banded at one day of age for identification.

All chicks battery brooders were electrically heated and had raised wire floors. The temperature of each brooder was thermostatically adjusted to operate at approximately 95° F. Brooder temperatures were lowered five degrees each week until they reached room temperature. Room temperature was maintained by electric wall heaters at approximately 80° F. for the first week and 70-75° F. for the last three weeks, except in Experiment 5, which was designed to study the effects of excessive temperatures.

Feed and tap water were available to the chicks at all times. Water was placed in the troughs one day prior to the start of the experiment so that it could adjust to room temperature.

Chicks to be kept beyond four weeks of age were moved to growing batteries equipped with raised wire floors but without heating elements. The room temperature was approximately 70° F.

Broiler and roaster floor studies. Crossbred broiler-type chicks obtained from a local commercial hatchery were sexed, vaccinated for infectious bronchitis and Newcastle disease by the intraocular method and wing banded at one day of age. They were randomly assigned to floor pens equipped with trough type feeders and chick water founts. Wood shavings were used as litter. Heat was supplied by infra red heat lamps. Feed and tap water were available to the chicks at all times.

The broiler experiment was terminated when the chicks were 8 weeks of age and the roaster experiment when the birds were 16 weeks of age.

Hen studies. All hens used in these experiments were Single Comb White Leghorns from the University of Tennessee Station stock.

Determination of xanthophyll content of feedstuffs. A modification of the method described by Bickoff et al.(1954b) was used to determine the xanthophyll content of the alfalfa meal, corn, corn gluten meal and certain complete feeds.

This method involved extraction of carotenoids with acetone, separation of xanthophyll from carotene by column chromatography and quantitative measurement on a spectrophotometer.

The method used herein differed from the method of Bickoff et al. (1954b) in that a 9 millimeter diameter tube was packed with filter-aid and Sea Sorb 43 (activated magnesium oxide manufactured by Fisher Scientific Company). Positive air pressure was applied to the tube to facilitate moving the eluant through. The optical density of the xanthophyll fraction was determined at 445 millimicrons on a Coleman No. 11A spectrophotometer. The xanthophyll concentration was calculated with the following procedure given by Moster and Quackenbush (1952). Optical density times milliliters of xanthophyll eluant divided by the grams of sample times 231 equals the milligrams of xanthophyll per gram of sample.

Since the objectives of the experiments reported herein did not necessitate the use of exact levels of dietary xanthophyll, calculated dietary levels of xanthophyll were obtained by using values for yellow corn and alfalfa meal as reported by Mitchell (1961) and by using information obtained from the manufacturer (Corn Products Co., New York) for xanthophyll values of corn gluten meal. These values were 7, 125, and 150 milligrams of xanthophyll per pound, respectively, for yellow corn, alfalfa meal (17 percent protein), and corn gluten meal (60 percent protein). Samples were analyzed periodically for verification,

Calculation of dietary energy and protein levels were based on average analyses of feedstuffs according to Hubbell (1962).

Determination of blood xanthophyll content. The blood used for xanthophyll determination was obtained either from the wing vein or by heart puncture. The method of Wilson (1956) was used to extract xanthophyll from blood serum. In preparing the blood for analysis, 4 milliliters of whole blood were allowed to clot on a slant while remaining at room temperature for three to four hours. It was then refrigerated overnight.

The procedure required 1 milliliter of serum to be placed in 15 milliliters of acetone which precipitated the proteins. The acetone extract was filtered through a No. 2 or No. 42 Whatman filter paper under suction and percent transmittance of the filtrate determined with a Coleman No. 11A spectrophotometer at 445 millimicrons. The xanthophyll content, expressed in micrograms per milliliter of serum as beta-carotene equivalents, was then determined from a standard curve made according to Munsey (1938).

Determination of visual pigmentation scores. The 1961 "Roche Improved Yolk Colour Fan" obtained from Hoffmann-La Roche Nutley, New Jersey, was used as the standard for determining visual pigmentation scores. Scores were obtained by matching the ventral side of the toe web area of the chick's foot to one of the ten color standards. The toe web area was prepared for scoring

by pressing the bird's foot against a sponge wet with cold tap water and by drying with a paper towel. Scoring of skin pigmentation was accomplished by matching the pigmentation of the skin along the breast feather tract of dressed, chilled birds with the standard. A sub-scald was used for processing in order to maintain the pigmented epidermal layer of the skin.

All scoring was done in rooms with covered windows for exclusion of bright, changeable sunlight. The standard was placed on a piece of black plastic for the background and a constant source of light was supplied by a fluorescent desk lamp with two 15-watt, 18-inch daylight bulbs covered with double strength frosted glass. The light was set about 18 inches above the standard.

Methods of data analysis. The data were statistically analyzed by the analysis of variance procedure according to Snedecor (1956), and significant treatment mean differences were determined by Duncan's Multiple Range Test (1955). Simple correlations were determined between blood xanthophyll levels and foot pigmentation and between foot pigmentation of birds when alive and skin pigmentation of the same bird when killed and dressed with the procedure given by Snedecor (1956).

Experiment 1

Experiment 1 was designed to study the effects of various ingredients, singly or in combination, upon pigmentation and the blood xanthophyll level of chicks. Composition of the diets

is shown in Table I. All diets shown in Table I were used in Experiment 1 except diet 8. Calculated analyses of micro-ingredient premixes are given in Table II. Canthaxanthin was used as a source of xanthophyll concentrate. Stabilized, fancy bleachable fat was added to diets containing natural sources of xanthophyll and/or a canthaxanthin. The effect of added fat was studied by calculating diets 6 and 7 to be iso-caloric, iso-nitrogenous and the same in xanthophyll content, but differing in that diet 7 contained 3.7 percent added fat while diet 6 contained none. This experiment was also designed to test the use of the Roche Colour Fan as a standard for broiler pigmentation studies.

Five male and five female crossbred broiler-type chicks were randomly assigned to each of three replicate pens of each dietary treatment. Blood xanthophyll levels of two male and two female chicks from each dietary treatment in a fourth replicate group were determined at the end of each week. All birds were scored for foot pigmentation at one day of age and weekly thereafter through the fourth week. Body weights were taken at one day, one week, three weeks and four weeks of age. Feed consumption by pens was measured when the chicks were four weeks of age at which time the experiment was terminated.

Experiment 2

This experiment, consisting of two trials, was designed to study the effect of the xanthophyll content of the breeder

TABLE I

COMPOSITION AND CALCULATED ANALYSES OF DIETS--EXPERIMENTS 1, 2, 8, 9, 10, AND 11

Ingredients	Diet number							
	1	2*	3	4	5	6	7	8
	percentage							
Yellow corn	---	56.70	51.10	54.80	53.00	46.80	46.80	55.80
White corn	56.70	---	---	---	---	9.90	---	---
Fat, stabilized, fancy bleachable	---	---	3.70	---	3.70	---	3.70	---
Soybean oil meal (50% protein)	37.40	37.40	33.80	33.80	37.40	37.40	37.40	25.80
Fish meal (60% protein)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Alfalfa meal (17% protein)	---	---	2.50	2.50	---	---	---	2.50
Corn gluten meal (60% protein)	---	---	3.00	3.00	---	---	---	10.00
Oat mill by-product feed	---	---	---	---	---	---	6.20	---
Defluorinated rock phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
$MnSO_4$ supplement (75%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Micro-ingredient premix 1**	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Canthaxanthin, (9.7%)	---	---	---	---	2.06g.	2.06g.	2.06g.	---
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses								
Productive energy, Cal./lb.	910	910	973	908	975	910	910	949
Crude protein, %	24.99	24.99	24.93	25.25	24.66	24.99	24.34	25.54
Xanthophyll, mg./lb.	---	3.97	11.20	11.46	5.71	5.28	5.28	22.00

*Diets 2A, 2B, and 2C were formulated by adding 1.03, 2.06, and 3.09 grams of canthaxanthin (7%) to 100 pounds of diet 2 and were calculated to contain respectively 4.97, 5.97, and 6.97 milligrams of xanthophyll per pound.

**Calculated analysis given in Table II.

TABLE II

CALCULATED ANALYSES OF MICRO-INGREDIENT PREMIXES

Vitamin, mineral or non-nutritive additive	Premix number 1	2	3	4
	Pounds of premix*			
	0.6	0.5	0.25	0.02
Vitamin A, IU	189,167	111,823	400,000	
Vitamin B_{12}, mg.	0.498	0.295	0.300	
Vitamin D_3, ICU	34,000	134,187	75,000	
Vitamin E, IU			200	
Riboflavin, mg.	215	201	150	
Niacin, g.	1.828	1.097	0.500	
Pantothenic acid, mg.	318	216	200	
Choline, g.	18.42	20.00	15.00	
Aureomycin, mg.	540			
Arsanilic acid, g.	3.786			
Manadione sodium bisulfite, mg.			100	
Folic acid, mg.			15	
Ethoxyquin, mg.			2,850	
Manganese, g.				2.720
Zinc, g.				2.04
Iron, mg.				907.5
Copper, mg.				112.5
Iodine, mg.				56.5
Cobalt, mg.				22.5
Calcium, mg.				608-907.5

*Amount of each premix added per 100 pounds of complete diet.

diet upon pigmentation of the day old chick and upon subsequent pigmentation of these chicks when fed diets of varying xanthophyll content.

Single Comb White Leghorn hens which had been in production for ten months were fed breeder diets 9, 10, and 11 as shown in Table III. The desired xanthophyll levels of the diets were obtained by varying the amount of yellow corn, alfalfa meal and corn gluten meal. Calculated xanthophyll levels of 0, 10.67 and 21.29 milligrams per pound of diet were employed.

After the hens had received the experimental breeder diets for eighteen days, eggs were saved for hatching. Chicks which were hatched were used in two battery trials, each lasting four weeks. Visual foot pigmentation scores were determined when the chicks were one day of age and at weekly intervals thereafter through four weeks. Individual body weights were obtained when chicks were one day, two weeks and four weeks of age. Feed consumption was determined at the end of each trial.

Trial 1. Chicks from each of the three breeder groups were fed diets 1, 4 or 8 (Table I). The chick diets were calculated to contain 0, 11.46 or 22.00 milligrams of xanthophyll per pound supplied by natural feed ingredients. Duplicate pens of six male and six female chicks were fed each chick diet.

Trial 2. This trial was initiated one week after the start of Trial 1. Chicks from breeder groups receiving diets containing 0 and 21.29 milligrams of xanthophyll per pound were used. As in Trial 1 all three chick diets were fed to duplicate pens

TABLE III

COMPOSITION AND CALCULATED ANALYSES OF DIETS--EXPERIMENT 2

Ingredients	Diet number		
	9	10	11
	percentage		
Yellow corn	---	63.20	64.90
White corn	67.20	---	---
Soybean oil meal (50% protein)	19.20	18.20	9.50
Fish meal (60% protein)	2.50	2.50	2.50
Alfalfa meal (17% protein)	---	5.00	5.00
Corn gluten meal (60% protein)	---	---	7.00
Defluorinated rock phosphate	1.48	1.48	1.48
Limestone	8.60	8.60	8.60
Salt	0.50	0.50	0.50
$MnSO_4$ supplement (75%)	0.02	0.02	0.02
Micro-ingredient premix 2*	0.50	0.50	0.50
Menadione sodium bisulfite	45 mg.	---	---
Total	100.00	100.00	100.00
Calculated analyses			
Productive energy, Cal./lb.	917	880	927
Crude protein, %	17.01	17.01	17.01
Xanthophyll, mg./lb.	---	10.67	21.29

*Calculated analysis given in Table II.

of six male and six female chicks.

Extra pens of chicks were used for blood xanthophyll determinations. Chicks from the breeder group receiving the xanthophyll-free diet were fed each of the three chick diets. Chicks from the breeder group receiving the diet containing 10.67 milligrams of xanthophyll per pound were fed the chick diet containing 11.46 milligrams of xanthophyll per pound. Blood xanthophyll levels were determined when the chicks were one day of age and at weekly intervals thereafter through four weeks.

Experiment 3

Crossbred broiler-type chicks were used to study the effects, upon foot pad and skin pigmentation, of starter and finisher diets containing as variables, soybean oil meal, peanut meal, stabilized fat and peanut oil, and to determine the correlation between foot pad pigmentation scores of live birds and skin pigmentation scores of dressed carcasses. Diets 12, 13, 14, 12A, 13A, and 14A shown in Table IV were used in Experiment 3. Diet 12 contained 30 percent soybean oil meal and 3 percent fat; diet 13 contained 30 percent peanut meal and 3 percent fat; diet 14 contained 30 percent peanut meal and 3 percent peanut oil, Diets 12A and 13A were formulated by adding 10 pounds of fat to 100 pounds each of diets 12 and 13. Diet 14A was formulated by adding 10 pounds of peanut oil to 100 pounds of diet 14. Diets 12, 13, and 14 were starter diets while diets 12A, 13A and 14A

TABLE IV

COMPOSITION AND CALCULATED ANALYSES OF DIETS--EXPERIMENTS 3 AND 4

Ingredients	Diet number					
	12*	13	14	15	16	17
	percentage					
Yellow corn	53.05	51.95	51.95	74.65	79.15	84.15
Fat, stabilized, fancy bleachable	3.00	3.00	---	2.00	2.00	2.00
Peanut oil	---	---	3.00	---	---	---
Soybean oil meal (50% protein)	30.00	---	---	15.00	10.50	5.50
Peanut meal (50% protein)	---	30.00	30.00	---	---	---
Corn gluten meal (60% protein)	3.00	3.00	3.00	---	---	---
Fish meal (66% protein)	5.00	5.00	5.00	2.50	2.50	2.50
Alfalfa meal (17% protein)	2.50	2.50	2.50	2.50	2.50	2.50
Defluorinated rock phosphate	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.48	0.48	0.48	0.48	0.48	0.48
$MnSO_4$ supplement (75%)	0.02	0.02	0.02	0.02	0.02	0.02
Micro-ingredient premix 1**	0.60	0.60	0.60	0.60	0.60	0.60
Dl-methionine	0.10	0.40	0.40	---	---	---
L-lysine	---	0.80	0.80	---	---	---
Amprol plus (25%)***	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00

TABLE IV (Continued)

Ingredients	Diet number					
	12*	13	14	15	16	17
	percentage					
Calculated analyses						
Productive energy, Cal./lb.	1014	1007	1007	1040	1056	1089
Crude protein, %	25.30	25.90	25.90	16.00	14.10	12.08
Xanthophyll, mg./lb.	11.96	11.89	11.89	8.35	8.66	9.01

*Diets 12A and 13A were formulated by adding 10 pounds of fat to 100 pounds of diets 12 and 13; diet 14A was formulated by adding 10 pounds of peanut oil to 100 pounds of diet 14. The protein content was reduced by 2.3 percentage points, while energy was increased 171 Calories per pound in all diets by the addition of fat or peanut oil. The calculated xanthophyll content of diet 12A, 13A, and 14A, respectively, was 10.87, 10.81, and 10.81 milligrams per pound.

**Micro-ingredient premix 1 (Table II) without arsanilic acid used in diets 12, 13 and 14.

***A coccidiostat containing 25 percent amprolium and 0.8 percent ethopabate manufactured by Merck and Co., Rahway, New Jersey.

were finisher diets.

Combinations of these diets were employed to give six treatments as follows; (1) 12-12A (diet 12 from zero to five weeks of age, diet 12A from five to eight weeks of age); (2) 13-13A; (3) 14-14A; (4) 12-12; (5) 13-13; and (6) 14-14. Fifty chicks of each sex were randomly assigned to each of six floor pens representing the six treatments.

The experiment was terminated when the chicks were eight weeks of age. A random sample of 15 birds of each sex per pen was selected for further processing. Foot pad pigmentation scores of the live birds as well as skin pigmentation scores of the dressed, chilled carcasses were obtained for correlation determinations.

Experiment 4

The purpose of Experiment 4 was to study the effect of varying the level of protein in the diets of roasters from the twelfth through the sixteenth week of age upon pigmentation and to determine the correlation between foot pigmentation of roasters when alive and skin pigmentation after the roasters had been dressed and chilled.

Crossbred broiler-type chicks, which had been used in a previous experiment, were randomly assigned to 10 floor pens. The diets used in Experiment 4 were diets 15, 16 and 17 shown in Table IV. These diets were calculated to contain 16.00, 14.10 and 12.08 percent protein, respectively, by varying the amounts of yellow corn and soybean oil meal. Diet 15 was fed to all

birds from eight to twelve weeks of age. At this time duplicate pens of 40 females were given diets according to the following plan hereinafter referred to as treatmens: (1) 16-12 (16 percent protein from twelve to fourteen weeks of age, 12 percent protein from fourteen to sixteen weeks of age); (2) 16-14; (3) 16-16; (4) 14-12; and (5) 14-14. Only female roasters were used for the pigmentation study.

Pigmentation scores of the feet of live roasters were obtained at 12, 14, and 16 weeks from ten females from each pen. These roasters were processed at 16 weeks at which time foot and skin pigmentation scores were obtained from each dressed, chilled carcass.

Experiment 5

This experiment was designed to study the effect of feeding diets containing 0, 2, 4 or 6 percent dried delactosed whey upon pigmentation of chicks kept in rooms with temperatures of 95° F. and "normal" temperatures (80° F. for the first week and 70-75° F. for the last three weeks). Brooder temperatures for the hot room were maintained at 95° F, while those for the normal temperature rooms were lowered each week as described under general procedure.

Each of the experimental diets (diets 18, 19, 20 and 21, Table V) was fed to two pens of five male and five female crossbred broiler-type chicks. These diets were formulated to be approximately isocaloric and isonitrogenous. The diets

TABLE V

COMPOSITION AND CALCULATED ANALYSES OF DIETS--EXPERIMENTS 5 AND 7

Ingredients	Diet number						
	18	19	20	21	22	23	24
	percentage						
Yellow corn	51.65	50.05	48.45	46.85	58.44	58.77	51.57
White corn	---	---	---	---	---	0.27	8.00
Fat, stabilized, fancy bleachable	3.70	3.70	3.70	3.70	---	---	---
Soybean oil meal (50% protein)	33.80	33.40	33.00	32.60	33.14	34.00	34.51
Corn gluten meal (60% protein)	3.00	3.00	3.00	3.00	---	---	---
Fish meal (60% protein)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Alfalfa meal (17% protein)	2.50	2.50	2.50	2.50	2.50	1.04	---
Whey, dried delactosed (16% protein)	---	2.00	4.00	6.00	---	---	---
Defluorinated rock phosphate	1.60	1.60	1.60	1.60	1.50	1.50	1.50
Limestone	0.60	0.60	0.60	0.60	1.20	1.20	1.20
Salt	0.48	0.48	0.48	0.48	0.50	0.50	0.50
$MnSO_4$ supplement (75%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Micro-ingredient premix 1*	0.60	0.60	0.60	0.60	0.60	0.60	0.60
DL-methionine	0.05	0.05	0.05	0.05	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses							
Productive energy, Cal./lb.	979	972	964	956	909	918	924
Crude protein, %	24.92	24.90	24.88	24.86	23.33	23.57	23.70
Xanthophyll, mg./lb.	11.24	11.13	11.02	10.90	7.22	5.41	3.61

*Micro-ingredient premix 1 (Table II) without arsanilic acid used in diets 18, 19, 20 and 21.

with 0, 2, 4 or 6 percent dried whey were calculated to contain 11.24, 11.13, 11.02, and 10.90 milligrams of xanthophyll per pound, repsectively.(Diets 18, 19, 20, and 21, Table V).

Foot pigmentation was determined when the chicks were one day of age and at weekly intervals thereafter until the chicks were four weeks of age at which time the experiment was terminated. Feed consumption was measured at the end of two and four weeks.

Experiment 6

Single Comb White Leghorn pullets which were 25 weeks of age were studied in an attempt to determine the effect of feeding diets containing various sources and levels of xanthophylls upon pigmentation of laying birds.

Observations were made on pigmentation of beak, eye ring and vent of birds from selected treatments involving a larger study. Eighteen groups of six pullets each were placed in individual laying cages when they were 25 weeks of age. Composition of the diets used is shown in Table VI. Nine of the eighteen treatments were involved in a cooperative study with the Animal Nutrition Research Council (ANRC), Hoffmann-La Roche, and other experiment stations. Birds involved in the ANRC portion of this study were fed diets comparable to those used by other cooperators. The pullets in these nine groups were maintained on the ANRC basal diet (diet 25) for four weeks in order to reduce markedly their body stores of xanthophyll.

TABLE VI

COMPOSITION AND CALCULATED ANALYSES OF DIETS--EXPERIMENT 6

Ingredients	Diet number									
	25*	26	27	28	29	30**	31	32	33	34
	percentage									
Yellow corn	---	---	---	---	---	---	---	---	---	66.98
White corn	53.68	53.13	52.58	52.03	51.48	53.43	55.43	56.43	55.43	---
Fat, stabilized, fancy bleachable	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	---
Soybean oil meal (50% protein)	20.00	18.75	17.50	16.25	15.00	20.00	12.00	5.00	---	17.00
Soybean mill feed	12.00	9.00	6.00	3.00	---	12.00	12.00	12.00	12.00	---
Corn gluten meal (60% protein)	---	---	---	---	---	---	6.00	12.00	18.00	---
Fish meal (60% protein)	---	---	---	---	---	2.00	2.00	2.00	2.00	2.50
Fish solubles	2.00	2.00	2.00	2.00	2.00	---	---	---	---	---
Alfalfa meal (17% protein)	---	5.00	10.00	15.00	20.00	---	---	---	---	5.00
Defluorinated rock phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.50
Limestone	6.50	6.30	6.10	5.90	5.70	6.50	6.50	6.50	6.50	6.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
$MnSO_4$ supplement (75%)	---	---	---	---	---	0.02	0.02	0.02	0.02	0.02
DL-methionine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	---
Menadione sodium bisulfite	---	---	---	---	---	45 mg.	45 mg.	45 mg.	45 mg.	---
Micro-ingredient premix 2***	---	---	---	---	---	0.50	0.50	0.50	0.50	0.50
Micro-ingredient premix 3***	0.25	0.25	0.25	0.25	0.25	---	---	---	---	---
Micro-ingredient premix 4***	0.02	0.02	0.02	0.02	0.02	---	---	---	---	---
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

TABLE VI (Continued)

Ingredients	Diet number									
	25*	26	27	28	29	30**	31	32	33	34
	percentage									
Calculated analyses										
Productive energy, Cal./lb.	865	859	854	849	843	871	912	947	974	915
Crude protein, %	16.85	16.68	16.49	16.30	16.12	17.39	17.17	17.35	18.37	16.74
Xanthophyll, mg./lb.	---	7.50	15.00	22.50	30.00	---	7.50	15.00	22.50	10.43

*Diets 25A, 25B, 25C, and 25D were formulated by adding 7.5, 15, 22.5, and 30 grams of beta-apo-8'-carotenal (10%) to 100 pounds of diet 25.

**Diets 30A, 30B, 30C, and 30D were formulated by adding 61.4, 122.8, 184.2, and 245.6 grams of Florafil to 100 pounds of diet 30. (Florafil is dried marigold petal meal containing 5.5 mg. xanthophyll per pound, manufactured by Dawes Laboratories, Inc., Chicago, Ill.)

***Calculated analyses given in Table II.

At the end of four weeks diets of four of the groups of pullets which had been fed the ANRC basal diet were supplemented with 7.5, 15, 22.5, or 30 milligrams of beta-apo-8'-carotenal per pound of diet (diets 25A, 25B, 25C, and 25D), and diets of four groups were supplemented with the same levels of xanthophyll indicated above but supplied by the assayed sample of alfalfa meal (diets 26, 27, 28, and 29). The ninth group was maintained on the ANRC basal diet (diet 25).

The remaining nine of the eighteen treatments were used to extend the ANRC portion of the study. Eight of these groups were fed the negative basal diet (diet 30). The ninth was fed diet 34, which contained 10.43 milligrams of xanthophyll per pound, to serve as a positive control.

At the end of the four-week depletion period, four of the groups which had received the negative basal diet were given diets supplemented with levels of xanthophyll indicated above but supplied by dried marigold petal meal (diets 30A, 30B, 30C, and 30D), and three groups were fed diets supplied with xanthophyll from corn gluten meal (diets 31, 32 and 33). The eighth group was maintained on the negative basal diet (diet 30).

The one group which received diet 34 during the depletion period was continued on this same diet. The supplementation period of feeding continued four weeks during which time individual daily egg production records were maintained.

Blood xanthophyll levels of six pullets receiving the basal diet 25 and of six pullets receiving diet 34 were determined

following the four-week depletion period. Observations of pigmentation in the eye ring and beak were made at the end of the supplemental feeding period.

Experiment 7

The purpose of this experiment was to determine the effect of limiting xanthophyll intake upon blood xanthophyll level and foot pigmentation of chicks by: (1) limiting the total feed intake; and (2) limiting the amount of xanthophyll in the diet.

The crossbred broiler-type chicks were grown to five weeks of age in battery brooders and fed a diet calculated to contain 23.45 percent crude protein, and 968 Calories of productive energy and 7.22 milligrams of xanthophyll per pound of feed.

At five weeks of age, the chicks were individually weighed. Three males and four females were assigned to each pen on an equal pen weight basis. Diets 22, 23, and 24 used in this experiment are given in Table V. The calculated dietary xanthophyll content (7.22, 5.41 or 3.61 milligrams per pound) was obtained by varying the amount of white corn, yellow corn and alfalfa meal. The diets were kept approximately isonitrogenous by varying the amount of soybean oil meal.

Three replicate pens of chicks were subjected to each of the following treatments: (1) 7.22 mg. xanthophyll diet - <u>ad libitum</u>; (2) 7.22 mg. xanthophyll diet - 75 percent of

amount consumed by chicks on treatment 1; (3) 7.22 mg. xanthophyll diet - 50 percent of amount consumed by chicks on treatment 1; (4) 4.12 mg. xanthophyll diet - 100 percent of amount consumed by chicks on treatment 1; and (5) 3.61 mg. xanthophyll diet - 100 percent of amount consumed by chicks on treatment 1. Feed restrictions were based each day on the amount eaten by birds in the full fed pen eating the least amount of feed on the previous day.

Individual body weights and foot pigmentation scores were obtained prior to the start of the experiment and at one, two and three weeks of the experimental period. Blood xanthophyll levels were determined at these times on two birds of each sex per pen. The experiment was terminated when the chicks were eight weeks of age.

Experiment 8

Experiment 8 was designed to determine the influence of feed intake upon the blood xanthophyll content and foot pigmentation of chicks parasitized with E. maxima.

Three replicate battery pens of five male and five female crossbred broiler-type chicks were used in each of four experimental treatments following a four-week pre-experimental growth period. The pre-experimental period began when the chicks were one day of age. All birds received the same broiler diet (Table I, diet 4), calculated to contain 25.25 percent protein, and 908

Calories of productive energy and 11.46 milligrams of xanthophyll per pound for the four-week pre-experimental period and for the three-week experimental period. The following treatments were used: (1) non-parasitized control - feed ad libitum; (2) non-parasitized - 75 percent of amount of feed consumed by chicks on treatment 1; (3) parasitized - feed ad libitum; and (4) non-parasitized - 100 percent of amount of feed consumed by chicks on treatment 3. Each parasitized bird was given 20,000 sporulated oocysts per os. Birds were assigned to each pen on an equal pen weight basis.

Individual body weights and foot pigmentation scores were obtained on birds when they were four, five, six and seven weeks of age. At these times blood xanthophyll levels were determined. Blood was secured from two birds of each sex from each pen.

Experiment 9

The purpose of Experiment 9 was to study the effect of feeding diets containing various levels of xanthophyll and the effect of various coccidiostats in a basal diet upon pigmentation and blood xanthophyll level of chicks parasitized with E. maximum.

Composition of the diets shown in Table 1. Diet 1 containing no xanthophyll was used as the negative control. Calculated xanthophyll levels of 3.97, 5.97 and 11.46 milligrams per pound of diet were obtained in the other diets by substituting yellow

corn for white corn, by adding canthaxanthin, and by adding alfalfa meal and corn gluten meal (diets 2, 2B, and 4). Four additional diets were fed. These were formulated by adding 0.1 percent Amprol (25 percent amprolium), 0.1 percent Amprol Plus (25 percent amprolium and 0.8 percent ethopabate), 0.05 percent Zoamix (25 percent Zoalene), or 0.1 percent Unistat (25 percent 3-5-dinitrobenzamide, 30 percent acetyl-para-nitrophenyl-sulfanilamide and 5 percent 3-nitro-4-hydroxyphenylarsonic acid) to diet 4. (Amprol and Amprol Plus are products of Merck and Co., Inc., Rahway, New Jersey; Zoamix is a product of The Dow Chemical Co., Midland, Michigan; and Unistat is a product of Dr. Salsbury's Laboratories, Charles City, Iowa).

Crossbred broiler-type chicks were grown to three weeks of age in battery brooders at which time all birds were weighed and scored for pigmentation. Birds weighing less than 250 grams each and those scoring less than four on the Roche Color Fan were rejected. From the remaining birds, six males and four females were placed in each of 32 pens so that average pigmentation scores were approximately equal amont pens. Each diet was fed to four replicate pens. After one week, birds in two pens fed each diet were given 10,000 sporulated oocytst of E. maxima per os. Foot pigmentation scores were obtained at this time as well as one, two and three weeks post inoculation. Blood xanthophyll levels were determined on two birds of each sex per pen at these times. Feed consumption per pen was determined three weeks

post inoculation at which time the experiment was terminated.

Experiment 10

This experiment was designed to study the effect of various species of coccidia upon pigmentation of six week old chicks.

Single Comb White Leghorn pullets from Station stock were grown to six weeks of age on a diet containing approximately 11.46 milligrams of xanthophyll per pound (Table I, diet 4), at which time 12 birds were assigend to each of sixteen pens. Four replicate pens were assigned to each treatment which were as follows: (1) 60,000 oocysts of E. maxima per bird; (2) 45,000 oocysts of E. necatrix per bird; (3) 25,000 oocysts of E. tenella per bird; and (4) non-inoculated controls. The oocysts were administered per os. All birds continued to receive the same diet containing approximately 11.46 milligrams xanthophyll per pound. Foot pigmentation scores of each bird were obtained on the third day following inoculation and weekly thereafter for three weeks at which time the experiment was terminated.

Experiment 11

This experiment was designed to study the effect of intramuscular injections of lutein upon blood xanthophyll levels of chicks parasitized with E. maxima.

Single Comb White Leghorn males obtained from the Tennessee Random Sample Laying Test were grown to seven weeks

of age on a xanthophyll-free diet (Table I, diet 1). At this time six birds were assigned to each of 12 pens. Pen assignments were made so that average body weights of the birds in each pen would be approximately equal.

Three experimental diets were employed. Diet 1 was formulated to contain no xanthophyll. Diet 4 was formulated to contain 11.46 milligrams of xanthophyll per pound obtained from yellow corn, alfalfa meal and corn gluten meal (Table I). The third experimental diet was formulated by adding 11.46 milligrams of lutein obtained from lutein concentrate (4 grams of lutein per pound) to diet 1. A factorial design was employed by use of three diets, parasitized and non-parasitized chicks, and injected and non-injected chicks.

Four pens of chicks were fed each diet with each chick in two of the replicate pens being given 20,000 E. maxima sporulated oocysts per os. On the third and fourth days post inoculation with E. maxima, one half of the birds in each pen were injected intramuscularly into the breast with two and one half milligrams of lutein. This was prepared by mixing the lutein concentrate containing 4 grams per pound with a mixture containing equal quantities of Wesson oil and Tween-80 (Polyoxyethylene sorbitan monooleate manufactured by Atlas Powder Co., Wilmington, Delaware) in proportions to give two and one half milligrams of lutein per milliliter. On the seventh day post inoculation, blood xanthophyll levels were determined on all birds. At this time feed consumption per pen was determined.

RESULTS AND DISCUSSION

Experiment 1

A summary of foot pigmentation scores is given in Table VII. Scores were determined when chicks were one day of age and weekly thereafter until chicks were four weeks of age. Statistical analyses were determined on one-day scores and four-week scores. The average scores of chicks at one day of age did not differ significantly ($P < .05$) among dietary treatments. This would have been expected since the chicks had been assigned to dietary treatments at random. However, since there was considerable variation in pigmentation between individuals at one day of age, the changes in scores between one day and four weeks were also subjected to statistical analysis as shown in Table VII. Interpretation of statistical analysis of the four-week scores and interpretation of analysis of the score changed from one to four weeks could lead to conflicting conclusions. These conflicts will be pointed out in the discussion.

Chicks fed the xanthophyll-free diet were significantly ($P < .01$) less pigmented than chicks on any other diet at four weeks of age. This was also true when score changes from one day to four weeks were compared.

Based upon average scores secured at the end of four weeks, the addition of canthaxanthin at 1.00, 2.00 or 3.00

TABLE VII

AVERAGE FOOT PIGMENTATION SCORES AND XANTHOPHYLL INTAKE PER BIRD --EXPERIMENT 1

Diet No.	Mg. xant. per lb. diet	Type of diet	Scores*						Milligrams xanthophyll intake/bird 1 day to 4 weeks
			1 day**	1 wk.	2 wks.	3 wks.	4 wks.***	Change 1 day to 4 wks.****	
1	0		5.8	4.8	2.2	1.2	0.3^{A}	$^{a}-5.5^{A}$	0
2	3.97	Yellow corn	4.7	5.6	5.4	4.5	4.1^{B}	$^{b}-0.6^{B}$	6.40
2A	3.97 1.00	Yellow corn Canthaxanthin	5.4	5.2	5.5	5.0	5.0^{C}	$^{b}-0.4^{B,C}$	8.30
2B	3.97 2.00	Yellow corn Canthaxanthin	5.7	5.2	5.0	5.4	5.2^{C}	$^{b}-0.5^{B}$	9.97
2C	3.97 3.00	Yellow corn Canthaxanthin	6.3	6.0	5.7	6.0	6.4^{D}	$^{b,c}0.1^{B,C,D}$	11.24
3	11.20	Yellow corn, alfalfa meal corn gluten meal, 3.7% added fat	5.7	5.7	5.3	5.1	5.3^{C}	$^{b}-0.4^{B,C,}$	18.78
4	11.46	Yellow corn, alfalfa meal corn gluten meal	5.3	5.7	5.5	5.3	5.2^{C}	$^{b}-0.1^{B,C,D}$	19.10

TABLE VII (Continued)

Diet No.	Mg. xant. per lb. diet	Type of diet	Scores* 1 day**	1 wk.	2 wks.	3 wks.	4 wks.***	Change 1 day to 4 wks.****	Milligrams xanthophyll intake/bird 1 day to 4 weeks
5	5.71	(Same as diet 2B with 3.7% added fat)	5.7	6.1	6.3	6.1	6.8^{D}	$^{d}1.1^{D}$	9.82
6	3.28 2.00	Yellow corn Canthaxanthin	5.4	5.7	5.4	5.5	5.4^{C}	$^{b,c}0.0^{B,C,D}$	8.87
7	5.28	(Same as diet 6 with 3.7% added fat)	5.5	5.9	6.0	6.0	6.3^{D}	$^{c,d}0.8^{C,D}$	9.27

*Figures given represent mean scores of three replicate pens of five males and five female chicks.

**Dietary treatment means do not differ significantly ($P < .05$).

***Means bearing the same capital letter superscripts do not differ significantly ($P < .01$). Means which did not differ at the 1 percent did not differ at the 5 percent level of probability.

****Means bearing the same capital letter superscript do not differ significantly ($P < .01$). Means bearing the same small letter superscript (preceeding the mean) do not differ significantly ($P < .05$).

milligrams per pound of diet to the diet containing 3.97 milligrams of xanthophyll per pound resulted in a significant improvement in pigmentation at four weeks with the highest level producing scores significantly higher than the two lower levels ($P < .01$). However, the analysis of change in scores showed no significant differences due to the addition of either level of canthaxanthin to the basal yellow corn diet containing 3.97 milligrams of xanthophyll per pound.

Canthaxanthin did increase pigmentation in skin and shanks of eight-week old broiler chicks when added to finisher diets containing yellow corn and alfalfa meal (Camp et al., 1963). The canthaxanthin was added at levels of 4 and 12 milligrams per pound and the diets were fed to chicks from five to eight weeks of age. Rauch (1965) reported that the addition of 2.72 or 5.45 milligrams of canthaxanthin per pound of diet resulted in an increase in pigmentation of chicks if the diet also contained pigments of natural origin. However, when canthaxanthin was added to diets containing no pigments of natural origin, shanks of the chicks developed a red tone. This same observation has been made by the author in preliminary studies. In view of reports of other workers, the use of pigmentation scores at four weeks is possibly more accurate measure of the effect of diet upon pigmentation than change in score from one to four weeks in Experiment 1.

The weekly variation in average pigmentation scores of chicks fed diet 2 may also be an indication that these chicks

may have contained within their bodies enough xanthophyll to have caused the increase in pigmentation observed during weeks 1 and 2. This would support the theory of Davis and Kratzer (1958) who suggested that serum xanthophyll for the first week after hatch was probably supplied by the yolk present in young chicks. It is possible that xanthophyll contained in the chick's body at hatching may overide any dietary effect for the first few days.

The effect of adding fat to the diet upon pigmentation is shown by comparing diet 5 containing 5.71 milligrams of xanthophyll per pound and 3.7 percent added fat with diet 2B containing 5.97 milligrams of xanthophyll per pound from the same sources but without added fat; by comparing diet 7 which contains 5.28 milligrams of xanthophyll per pound and 3.7 percent added fat with diet 6 which contains no added fat but which is iso-caloric and contains the same xanthophyll content as diet 7; and by comparing diet 3 containing 11.20 milligrams of xanthophyll per pound and 3.7 percent added fat with diet 4 containing 11.46 milligrams of xanthophyll per pound without added fat. When average scores at four weeks of age are used for analysis, the addition of fat as outlined in the first two mentioned sets of comparisons resulted in significantly ($P<.01$) higher scores, while in the latter comparison, diets containing 11.20 and 11.46 milligrams of xanthophyll per pound produced no significant difference in average scores although added fat was used in the diet containing 11.20 milligram per

pound. When these same comparisons were made using change in score from one day to four weeks, a significant difference ($P<.01$) was found in the comparison of diet 5 with diet 2B in favor of diet 5 which contained added fat. The other two sets of comparisons showed no significant influence of added fat upon pigmentation.

Foot pigmentation scores of four-week old chicks fed diets containing 11.20 (diet 3) or 11.46 (diet 4) milligrams of xanthophyll per pound supplied by yellow corn, alfalfa meal and canthaxanthin were not significantly different from scores of chicks fed diets containing 4.97 (diet 2A), 5.97 (diet 2B) or 5.28 (diet 6) milligrams of xanthophyll per pound from yellow corn and canthaxanthin. Furthermore, the feeding of diets 2C, 5, and 7 containing 6.97, 5.71 and 5.28 milligrams of xanthophyll per pound, respectively, all produced pigmentation scores which were significantly ($P<.01$) higher at four weeks than scores of chicks fed the diets containing 11.20 and 11.46 milligrams per pound. Similar conclusions may be drawn from comparisons of change in scores except that feeding diet 5 resulted in a significantly greater increase in pigmentation than feeding diet 2C. These results agree with those reported by Mitchell (1961) who found that increasing the dietary xanthophyll level from 6.37 to 10.00 milligrams per pound was without a significant effect on pigmentation of four week old chicks.

Pigmentation scores at four weeks of age were found to be significantly ($P<.01$) higher for males, which averaged 5.22,

than for females, which averaged 4.80.

The average calculated xanthophyll intake per bird is given in Table VII. The intake per bird paralleled closely the xanthophyll content of the feed due to the fact that feed intake per bird was similar regardless of the diet fed. Although chicks fed diets 3 and 4 consumed 18.78 and 19.10 milligrams of xanthophyll per chick, their scores at four weeks of age did not differ from scores of chicks consuming only one-half as much, indicating that the xanthophyll from alfalfa meal and corn gluten meal may be low in availability as has been suggested by Ratcliff *et al.* (1959) and Williams *et al.* (1963).

Blood xanthophyll levels of two male and two female chicks from a fourth replicate fed each of the experimental diets were determined and compared individually to foot pigmentation scores when the birds were two, three and four weeks of age. A significant ($P < .01$) correlation was found between blood xanthophyll level and pigmentation score of birds at each age. The correlation r values were 0.645, 0.664, and 0.796 representing chicks at two, three, and four weeks of age, respectively. A summary of scores and blood xanthophyll values is given in Table VIII.

No significant differences in average body weights of chicks at four weeks of age were found between chicks fed the various diets.

TABLE VIII

AVERAGE FOOT PIGMENTATION SCORES AND BLOOD XANTHOPHYLL LEVELS*
EXPERIMENT 1

Diet No.	2 weeks		3 weeks		4 weeks	
	Score	Blood xant. mcg./ml. serum	Score	Blood xant. mcg./ml. serum	Score	Blood xant. mcg./ml. serum
1	1.3	3.95	1.0	0.86	0.5	1.02
2	7.0	12.78	4.5	6.50	4.0	5.31
2A	5.3	6.72	5.2	7.34	4.7	4.85
2B	7.8	11.02	5.5	6.62	6.5	6.32
2C	6.0	9.04	7.8	7.84	6.5	5.20
3	6.0	11.00	5.0	10.98	6.3	8.88
4	6.0	9.86	5.5	8.32	5.7	7.89
5	6.0	12.21	4.8	9.36	6.2	7.94
6	6.0	7.64	5.5	5.60	4.3	5.12
7	5.8	8.16	6.8	11.78	5.5	8.32

*Figures given represent average scores and average micrograms of xanthophyll per milliliter serum of two males and two females per diet.

Experiment 2

Trial 1. A summary of average foot pigmentation scores of chicks in Trial 1 is shown in Table IX. Statistical analysis of data secured from all chicks hatched from eggs of hens receiving the three breeder diets revealed a significant ($P < .01$) improvement in pigmentation of day old chicks with the addition of each level of xanthophyll. Average scores were 0.0, 7.4, and 7.9 for all chicks representing, respectively, breeder diets containing 0, 10.67 and 21.29 milligrams of xanthophyll per pound. Values given in Table IX represent only values for chicks used in that portion of the experiment.

At hatching time, chicks from breeder hens which were fed the xanthophyll-free diet showed almost no visible pigment. Most scores were below one of the Roche Color Fan standard. When these chicks were fed diets lacking xanthophyll, they remained void of pigment. However, when depleted chicks were fed diets containing 10.67 or 21.29 milligrams of xanthophyll per pound, within one week pigment had been deposited in the bird.

The four-week pigmentation scores were almost entirely dependent upon the xanthophyll content of the chick diet and not upon the diet of the dam. These scores fell into three distinct groups with each group differing significantly ($P < .05$) from the other but not differing significantly within the groups. When tested at the 1 percent level of probability, certain differences were non-significant. Within the groups of chicks

TABLE IX

AVERAGE FOOT PIGMENTATION SCORES*
EXPERIMENT 2--TRIAL 1

Treatment		Age				
Xant. level breeder diet	Xant. level chick diet	1 day	1 wk.	2 wks.	3 wks.	4 wks**
0	0	0.0	0.0	0.0	0.0	$^{a}0.0^{A}$
10.67	0	7.7	4.5	2.0	0.5	$^{a}0.1^{A}$
21.29	0	8.1	6.2	3.5	1.4	$^{a}0.2^{A}$
0	11.46	0.0	2.4	5.4	5.6	$^{b}6.2^{B,C}$
10.67	11.46	7.5	7.3	8.1	7.0	$^{b}6.2^{B,C}$
21.29	11.46	7.5	6.6	7.7	6.6	$^{b}6.0^{B}$
0	22.00	0.1	4.2	7.7	7.8	$^{c}7.8^{C}$
10.67	22.00	7.4	7.1	8.4	8.1	$^{c}7.5^{B,C}$
21.29	22.00	7.9	7.3	8.4	8.0	$^{c}8.1^{C}$

*Each value represents the average of duplicate pens of six males and six females.

**Means bearing the same capital letter superscripts do not differ significantly ($P < .01$). Means bearing the same small letter superscripts (preceeding the mean) do not differ significantly ($P < .05$).

receiving the chicks diet containing 22.00 milligrams of xanthophyll per pound, the group representing the 10.67 milligrams per pound breeder diet did not differ significantly in pigmentation from the chicks receiving the diet containing 11.46 milligrams per pound. Likewise, within the groups of chicks receiving the chick diet containing 11.46 milligrams of xanthophyll per pound, those chicks representing breeder diets containing 0 and 10.67 milligrams of xanthophyll per pound did not differ significantly in pigmentation from chicks receiving the diet containing 22.00 milligrams per pound.

Trial 2. Table X gives a summary of average pigmentation scores of chicks used in Trial 2 of Experiment 2. As in Trial 1 when the pigmentation scores of all day-old chicks hatched from eggs of hens receiving the three breeder diets were subjected to statistical analysis, results showed that the addition of each level of xanthophyll to the breeder diets resulted in a significant ($P < .01$) improvement in pigmentation. Average scores were 0, 6.8 and 8.2 for chicks representing, respectively, breeder diets containing 0, 10.67 and 21.29 milligrams of xanthophyll per pound of diet.

Trial 2 differed from Trial 1 in design only in that chicks from breeder groups receiving 10.67 milligrams of xanthophyll per pound were omitted from the growth study. All chick diets were employed. Results of feeding the chick diets were essentially the same as in Trial 1. Again the scores of chicks at four weeks of age fell into three groups depending on

TABLE X

AVERAGE FOOT PIGMENTATION SCORES*
EXPERIMENT 2--TRIAL 2

Treatment		Age				
Xant. level breeder diet	Xant. level chick diet	1 day	1 wk.	2 wks.	3 wks.	4 wks.**
0	0	0.0	0.0	0.0	0.0	0.0[a]
21.29	0	8.9	6.4	3.4	0.8	0.6[a]
0	11.46	0.0	2.4	5.3	6.0	5.6[b]
21.29	11.46	9.2	8.0	7.4	6.2	6.0[b]
0	22.00	0.1	3.4	6.8	7.3	7.6[c]
21.29	22.00	7.9	7.5	8.5	8.7	8.1[c]

*Each value represents the average of duplicate pens of six males and six females.

**Means bearing the same small letter suprescript do not differ significantly ($P < .05$).

the xanthophyll content of the chick diet. The three groups differed significantly ($P < .05$) from each other but within the groups, chicks receiving the same chick diet did not differ significantly in average pigmentation score regardless of the diet of the breeder hens from which they came.

In Trial 2 the average pigmentation score of four week old male chicks, which was 7.08, differed signficantly ($P < .01$) from the average score of the same age females, which was 6.54.

A summary of blood xanthophyll determinations is given in Table XI. Since each value represents one individual bird, the small number of birds tested per treatment created doubt regarding the value of statistical analysis of the data. The xanthophyll content of a pooled sample of blood obtained at one day of age from four chicks hatched from the breeder group fed the xanthophyll-free diet was 3.04 micrograms per milliliter of serum. A value of 40.96 micrograms per milliliter of serum was determined for a pooled sample of blood from chicks whose dams had received the breeder diet containing 10.67 milligrams of xanthophyll per pound. This approximate value was determined by extension of the standard curve beyond the limits of the greatest standard concentration.

Values from week to week are of blood obtained from different birds, since the birds often died as a result of the bleeding process. The blood xanthophyll level of those chicks fed the xanthophyll free diet decreased by the end of the first week to a lower level than at one day, while that of chicks fed

TABLE XI

BLOOD XANTHOPHYLL VALUES*--EXPERIMENT 2--TRIAL 2

Treatment						
Xanthophyll level of breeder diet	Xanthophyll level of breeder diet	Sex	Weeks of age** 1	2	3	4
0	0	M			0.64	0.16
		F			0.16	0.16
		Av.	0.32	0.16	0.40	0.16
0	11.46	M			11.20	6.08
		F			5.92	4.32
		Av.	12.20	10.48	8.56	5.20
0	22.00	M			15.84	14.88
		F			12.24	12.88
		Av.	22.24	20.92	14.04	13.88
10.67	11.46	M			16.28	8.80
		F			8.56	5.92
		Av.	16.59	14.40	12.42	7.36

*Micrograms of xanthophyll per milliliter of serum. Each value represents one bird.

**Blood samples at one and two weeks were pooled for analysis due to the small amount of blood obtained from each bird.

the diet containing 11.46 and 22.00 milligrams per pound had risen, respectively, to 12.20 and 22.24 micrograms per milliliter of serum.

The decline in blood xanthophyll levels from the first week to the fourth week as shown in Table XI may be somewhat exaggerated due to the small numbers of birds and due to the use of different birds each week. However, this same tendency for decline with age was noted in Experiment 1 as shown in Table VIII. In Experiment 2, the decline seen in blood xanthophyll levels is not reflected in pigmentation of other birds from the same breeder group which received the same chick diet. When all treatments are considered, there appeared to be a tendency for pigmentation to drop from the second to the fourth week of age.

When blood xanthophyll levels of males and females were determined separately, in seven out of eight determinations higher levels were observed in the blood of males than females, the eighth determination being equal. This is reflected in pigmentation scores of males compared to females.

Analysis of chick body weights at four weeks showed no significant differences between treatments, indicating no nutritive value from xanthophyll. These results do not agree with a report by Temperton and Dudley (1947) in which the authors conclude that the xanthophyll extract of dried grass posesses biological activity for growing chicks. This activity was

reflected by decreased mortality and increase in growth rate when added to a basal starter ration deficient in carotenes and vitamin A. The suggestion was made that phsiological effects are independent in action from vitamin A activity. All chick diets used in Experiment 2 were calculated to contain 1891 I.U. of added vitamin A per pound.

The work of Mitchell (1961) showed that satisfactory pigmentation of xanthophyll-depleted chicks was obtained after a four-week feeding period. Depletion was accomplished from one day to four weeks of age, and the chicks were then fed experimental diets from four to eight weeks of age. Increasing the level of dietary xanthophyll above 10 milligrams per pound did not hasten pigmentation.

Results of Experiment 2, showed that chicks normal in all respects except depleted of blood xanthophyll and skin pigmentation can be hatched from dams fed diets containing no xanthophyll. This is in agreement with the report of Palmer and Kempster (1919a). The blood xanthophyll levels of depleted chicks began to rise and xanthophyll was deposited in the shanks of these chicks within a few days after the chicks were fed diets high in xanthophyll. These results are not in agreement with the conclusions of Mann (1946) that chicks are not able to utilize xanthophyll until they are about four weeks of age.

The four-week pigmentation scores were found to be dependent mainly upon the level of xanthophyll in the chick diet

and not upon the xanthophyll content of the diet fed to their dams.

When results of Experiment 2 are viewed with results of Experiment 1 and with reports of other workers (Camp et al., 1963; Davis and Kratzer, 1958), it would appear that the effect of dietary treatment upon pigmentation of chicks from hatching to four weeks of age can best be measured by the use of four-week pigmentation scores instead of change in scores from one day to four weeks. This may not be the case when experimental treatments start with birds which are several weeks old.

Experiment 3

A summary of foot pigmentation scores of eight week old birds is given in Table XII. Statistical analysis of pigmentation scores revealed considerable overlaping as to significant ($P < .01$) differences between groups receiving different dietary treatments. In general, treatments involving higher energy-lower xanthophyll containing finisher diets from six to eight weeks produced chicks with lower pigmentation scores than scores produced by feeding the starter diets during the entire eight-week period.

The reason for this is not readily apparent. However, it should be pointed out that in other studies at this Station involving similar finisher diets, chicks consumed much less of the finisher diet than they did of the starter diet. This would

TABLE XII

AVERAGE FOOT PIGMENTATION SCORES--EXPERIMENT 3

Treatment	Protein source*	Fat source*	Type of diet 6-8 wks.	Scores Male**	Scores Female**	Scores Av.***
13-13	PM	F	Starter	8.1	8.1	$^{a}7.8^{A}$
14-14	PM	PO	Starter	7.5	7.5	$^{a}7.5^{A,B}$
13-13A	PM	F	Finisher	8.0	6.9	$^{a}7.4^{A,B,C}$
12-12	SBOM	F	Starter	6.9	6.9	$^{b}6.9^{B,C,D}$
12-12A	SBOM	F	Finisher	7.3	6.3	$^{b}6.8^{C,D}$
14-14A	PM	PO	Finisher	6.7	6.9	$^{b}6.6^{D}$
			Average	7.4	6.9	

*SBOM = soybean oil meal, PM = peanut meal, F = stabilized, fancy bleachable fat, PO = peanut oil.

**Each score represents an average of 15 birds. Average score of males was significantly ($P < .01$) different from that of females.

***Means bearing the same capital letter superscript do not differ signficantly ($P < .01$). Means bearing the same small letter superscript (preceeding the mean) do not differ significantly ($P < .05$).

result in a lower xanthophyll intake and might be the reason for the lower pigmantation previously noted. In this study accurate feed consumption data could not be obtained due to an outbreak of Avian nephritis-nephrosis (gumboro disease) which caused high mortality during a three-day period, resulting in unreliable feed intake data per bird, since feed intake of the dead birds would have had to have been approximated. It was felt that this disease outbreak caused no effect on pigmantation since only apparently healthy birds were selected for determination of pigmentation scores. Also growth rate data appeared to be within expected limits. No significant differences were found in average body weights of eight week old chicks subjected to the various dietary treatments.

Another observation was that the pigmentation scores of birds fed diets containing soybean oil meal were generally lower than those fed diets containing peanut meal, although the treatment producing the lowest scores was one in which peanut meal was fed. It is doubtful if conclusions drawn from these differences based upon the 1 percent level of probability would be very meaningful.

When the 5 percent level of probability was used, average scores fell into two distinct groups. Average scores of chicks receiving the peanut meal-fat-starter, the peanut meal-peanut oil-starter and the peanut meal-fat-finisher all were significantly higher than scores of chicks receiving the other three

diatery treatments. Within these two groupings, scores did not differ significantly. Conclusions or proper interpretations based upon these differences at the 5 percent level of probability would be questionable. There is, however, the possibility that soybean oil meal could have had a depigmenting effect as was reported by Culton and Bird (1941).

A significant correlation ($r = 0.40$) was found between foot pigmentation scores of 173 live birds and skin pigmentation scores of the carcasses after dressing and chilling. The average score of the dressed carcasses was only 3.5 while the average foot score of the live bird was 7.2. These data show that foot pigmentation scores are a good indication of the skin pigmentation of dressed birds, when picking is aided by semi-scalding.

The average pigmentation score of males was significantly ($P < .01$) higher than that of females.

Experiment 4

Results of Experiment 4 are given in Table XIII. Change in foot pigmentation scores of roasters from twelve to sixteen weeks of age were used for analysis. The average scores of birds on all treatments increased from twelve to sixteen weeks of age. The average change in score of birds fed the diet containing 14 percent protein for the two periods (twelve to fourteen weeks of age and fourteen to sixteen weeks of age) was significantly ($P < .01$) greater than those of any other treatment except the treatment in which 16 percent protein was fed for the first period and 12

TABLE XIII

AVERAGE CHANGE IN FOOT PIGMENTATION SCORES* 12-16 WEEKS
EXPERIMENT 4

Percent dietary protein		
12-14 weeks	14-16 weeks	Average score change**
14	14	$^{a}3.2^{A}$
16	12	$^{a,b}2.8^{A,B}$
16	16	$^{b,c}2.0^{B,C}$
14	12	$^{c}1.9^{B,C}$
16	14	$^{c}1.3^{C}$

*Each figure represents an average of two replicate pens of ten birds each.

**Means bearing the same capital letter superscript do not differ significantly ($P < .01$). Means bearing the same small letter superscript (preceeding the mean) do not differ significantly ($P < .05$).

percent for the last.

Average change in pigmentation scores of roasters receiving treatment 16-12 (16 percent dietary protein from twelve to fourteen weeks of age, 12 percent dietary protein from fourteen to sixteen weeks of age) did not differ significantly from those of roasters receiving treatment 16-16 but differed significantly ($P < .01$) from those of roasters receiving treatment 14-12 and differed significantly ($P < .05$) from those of roasters receiving treatment 16-14. Average change in pigmentation scores of roasters receiving treatments 16-16, 14-12 and 16-14 did not differ significantly ($P < .05$) from each other.

No reason can be given for the differences in pigmentation score changes due to various dietary treatments. The energy and xanthophyll content of the various diets used did not differ greatly. While the differences in dietary composition were mainly in soybean oil meal resulting in the various protein levels, changes in pigmentation from 12 to 16 weeks of age did not correspond consistently to any change in protein content of the diets.

When foot pigmentation scores of live roasters were correlated at 16 weeks of age with the pigmentation score of the skin of the dressed carcasses, the correlation was ($r = 0.16$). However, when foot pigmentation scores were determined after the birds were dressed, there was a significant ($P < .05$) correlation ($r = 0.41$) between the pigmentation score of the skin of the

dressed carcass and foot scores. Foot pigmentation scores of live birds averaged 8.2 with several birds showing a redness in the pigment making it somewhat difficult to match with a standard color. Following processing, average foot pigmentation score was 6.8. The redness had disappeared indicating that the cause was probably an increased blood supply in the foot which disappeared when the birds were bled. The redness may have been a factor in the confusing results discussed earlier concerning change in pigmentation.

These results indicate that foot pigmentation of a live roaster at 16 weeks of age may not be a true indication of the skin pigmentation of the dressed carcass due to the redness of color. This was not the case with eight week old birds in Experiment 3.

Experiment 5

A summary of pigmentation scores and xanthophyll intake per bird is shown in Table XIV. Since chicks were randomly assigned to dietary treatment at one day of age, four-week pigmentation scores were used for statistical analysis. No significant differences were found in pigmentation scores of four week old chicks fed diets containing different levels of dried whey. There was a significant difference ($P < .01$) between average scores of chicks housed in the normal temperature room (80° reduced to 70° F.) and scores of chicks housed in the hot

TABLE XIV

AVERAGE FOOT PIGMENTATION SCORES AND XANTHOPHYLL INTAKE PER BIRD--EXPERIMENT 5

Diet No.	Percent whey	4 week scores*			Mg. xanthophyll intake per bird**		
		Normal temp.	High temp.	Av.	Normal temp.	High temp.	Av.
18	0	4.8	4.2	4.5	22.48	18.21	20.34
19	2	4.9	4.2	4.6	23.98	15.52	19.76
20	4	5.0	4.2	4.6	22.42	18.73	20.58
21	6	4.7	4.2	4.4	23.49	18.32	20.90
	Av.***	4.8[A]	4.2[B]	4.5	23.10[A]	17.70[B]	20.40

*Scores reported for each temperature group represent averages of five males and five females each in two replicate groups.

**Values reported for each temperature group represent averages of two replicate pens each containing ten birds.

***Means bearing the same capital letter superscript do not differ significantly. Mean scores should not be compared to mean xanthophyll intake.

temperature room (90° F. constant) with the highest scores being produced in the normal temperature room.

Because of a reduction in feed intake, average xanthophyll intake per bird from one day to four weeks of age was significantly ($P < .01$) reduced in the hot room compared to the normal room. Chicks fed diets containing various levels of dried whey did not differ significantly in average xanthophyll intake per bird.

When the average pigmentation score of birds in each pen was compared to the average xanthophyll intake of birds in that pen, a significant positive correlation ($r = 0.78$) was found. From this, it appears that the effect of the heat was to cause a reduction in feed intake, consequently a reduction in xanthophyll intake which, in turn, led to a reduction in pigmentation. However, since this experiment was not designed to study the effect of feed intake upon pigmentation, birds in the room maintained at "normal" temperatures were not restricted in feed intake to a level equal to that of birds in the hot room.

Results of Experiment 1 showed that pigmentation of birds which consumed 8.30 milligrams of xanthophyll did not differ significantly from pigmentation of birds which consumed 19.10 milligrams per bird. It would appear therefore that the change in xanthophyll consumption of 23.10 to 17.70 milligrams per bird noted in Experiment 5 should not have caused a significant change in pigmentation. It should be mentioned, however, that in Experiment 1, birds which consumed 19.10 milligrams of xanthophyll

were fed different sources of xanthophyll than those which consumed 8.30 milligrams of xanthophyll, while the source of xanthophyll was the same for all birds in Experiment 5.

Milligan and Winn (1964) reported that pigmentation of chicks was adversely affected by temperatures of 80° and 100° F. when compared to pigmentation of chicks housed in rooms with temperatures of 70°, 60°, 53°, and 46° F. Results of Experiment 5 generally agree; however, the effect of feed intake must be a factor for consideration.

The average weight of four week old chicks housed in the hot room was significantly ($P < .01$) lower than the average weight of those housed in the normal temperature room while no significant differences were detected in average weights of chicks fed diets containing various levels of dried whey. When xanthophyll intake per gram of chick body weight was calculated, it was found that chicks housed in the cool room (average weight of 619 grams) consumed 0.037 milligrams of xanthophyll per gram of body weight while chicks housed in the room with normal temperature (average weight of 515 grams) consumed 0.034 milligrams of xanthophyll per gram of body weight. Since the chicks consumed about an equal amount of xanthophyll per unit of body weight and pigmentation differed, it would appear that some unidentified factor caused the differences in pigmentation. In this experiment, it appeared that the increased amount of heat could have resulted in destruction of some xanthophyll in the feed since the feed was maintained in

the room with the chicks or could have affected xanthophyll utilization or metabolism in the tissues.

Experiment 6

Results of Experiment 6 are given in Table XV. The total number of birds fed the various diets and showing each degree of pigmentation is given without benefit of statistical analysis. None of the birds on any of the levels of beta-apo-8'-carotenal showed deposition of pigment in the beak although the eye ring of a few birds on the higher two levels showed faint color. All birds which received diets containing alfalfa meal showed pigmentation at the base of the beak varying in degree from faint to moderate depending on the level of alfalfa meal. Pigment was also evident in the eye ring.

Pigmentation of the birds fed diets containing marigold petal meal was less intense than pigmentation of those birds receiving diets containing the highest level of alfalfa meal. Pigmentation of birds fed diets containing 30.00 milligrams of xanthophyll per pound from marigold petal meal was about the same as that of those birds receiving the diet containing the 22.50 milligrams of xanthophyll per pound from alfalfa meal.

Corn gulten meal was equal to marigold petal meal in producing pigment in the beak. The beak, eye ring and vent of birds fed the basal xanthophyll-free diets were completely bleached. Those birds receiving diet 34, which contained

TABLE XV

PIGMENT DEPOSITION IN THE BEAKS OF LAYING PULLETS
EXPERIMENT 6

Diet No.	Xanthophyll mg./lb. diet	Xanthophyll source**	Degree of pigmentation***			
			0	1	2	3
			no. of birds			
25	0	---	6			
25A	7.50	B	6			
25B	15.00	B	6			
25C	22.50	B	6			
25D	30.00	B	6			
26	7.50	A		6		
27	15.00	A		5	1	
28	22.50	A		1	5	
29	30.00	A				6
30	0	---	6			
30A	7.50	M	5	1		
30B	15.00	M	1	5		
30C	22.50	M		1	4	1****
30D	30.00	M		1	5	
31	7.50	CGM	4	2		
32	15.00	CGM		4		1****
33	22.50	CGM		2	4	
34	10.43	A,C			6*****	

*Pullets fed all diets except diet 34 were fed a pigment-depletion diet for 28 days prior to feeding other diets. Pullets fed diet 34 were fed this diet for the entire depletion and experimental period.

**B = beta-apo-8',carotenal, A = alfalfa meal, M = marigold petal meal, CGM = corn gluten meal, C = yellow corn.

***Pigmentation at the base of beak given scores from 0 to 3 with 3 showing the highest pigmentation. Total of six birds per diet.

****Out of production.

*****Five birds showed pigment at the tip of the beak and one at the base.

10.43 milligrams of xanthophyll per pound supplied by yellow corn and alfalfa meal, for the entire depletion and supplemental periods, showed what might be described by the poultryman as "normal" bleaching for birds in the early stages of production. Depletion of pigment from the eye ring and vent was complete with the base of the beak showing bleaching and the tip of the beak still pigmented. When these birds were compared with birds receiving the basal xanthophyll-free diets, it was evident that even the base of the beak of those birds experiencing normal bleaching were somewhat colored.

It is significant to note that all birds, except those indicated in Table XV as out of production, were in a high rate of production. These results are in disagreement with reports of Blakeslee and Warner (1915) and Palmer and Kempster (1919b). Results of this experiment showed that pigment was being deposited in the beaks, eye ring and vent while the hens were laying at a high rate, as pigment was found at the base of the beak while the tip was bleached. These hens had been bleached of pigment in these areas by receiving a xanthophyll-free diet for four weeks prior to being fed the experimental diets for the next four weeks.

Average blood xanthophyll levels determined after the four week depletion period of six pullets which had received the ANRC basal diet and of six pullets which had received diet 34, containing 10.43 milligrams of xanthophyll per pound, were

1.07 and 6,09 micrograms of xanthophyll per milliliter of serum, respectively. The rate of production of both groups of pullets was approximately 85 percent at that time.

Experiment 7

A summary of blood xanthophyll measurements is shown in Table XVI. Since levels of blood xanthophyll were quite variable when Experiment 7 began, the use of changes in blood xanthophyll values from beginning to the end of the experimental period seemed to be the best measure of the effect of treatment. The average blood xanthophyll level of birds which were full fed on the diet containing 7,22 milligrams of xanthophyll per pound showed a drop each week when compared with the level at the start of treatment. No explantaion as to the reason for this drop is proposed except that blood was drawn from the same birds each week, and this drop may represent a dilution effect. This same trend in blood xanthophyll levels was noted by Mitchell (1961).

A measure of change in blood xanthophyll levels after one and two weeks of treatment showed no significant differences due to a reduction in the total feed intake to 75 percent and 50 percent of full-feed or by feeding the diet containing 5.41 milligrams of xanthophyll per pound. There was a significant ($P < .01$) difference in average change of blood xanthophyll of chicks fed the diet containing 3.61 milligrams of xanthophyll

TABLE XVI

AVERAGE CHANGE IN BLOOD XANTHOPHYLL LEVELS--EXPERIMENT 7*

No.	Dietary treatments: Mg. xanthophyll per pound	Dietary treatments: Amount fed	Period of change: 0-1 wk.**	Period of change: 0-2 wks.**	Period of change: 0-3 wks.***
1	7.22	ad libitum	-1.32^{A}	-0.77^{A}	-1.95^{a}
2	7.22	75% of 1	-1.19^{A}	-1.59^{A}	-2.50^{a,b}
3	7.22	50% of 1	-0.86^{A}	-0.88^{A}	-1.54^{a}
4	5.41	same as 1	-1.34^{A}	-1.72^{A}	-2.21^{a}
5	3.61	same as 1	-3.48^{B}	-3.81^{B}	-3.85^{b}

*Each value represents the average change in micrograms of xanthophyll per milliliter of blood serum for three replicate groups of three males and four females.

**Means, within each period, bearing the same capital letter superscript do not differ singificantly ($P < .01$). Means not differing at the 1 percent level of probability do not differ at the 5 percent level of probability.

***Means bearing the same small letter superscript do not differ significantly ($P < .05$).

per pound when compared to chicks full fed the diet containing 7.22 milligram of xanthophyll per pound. The drop in blood xanthophyll was significantly ($P < .01$) greater when xanthophyll intake was reduced by lowering the xanthophyll level in the diet than by restricting the amount of diet eaten by the chicks.

Changes in blood xanthophyll levels after three weeks of treatment were essentially the same as that after one and two weeks except that there was no significant difference in reduction in blood xanthophyll between the chicks fed the diet containing 7.22 milligrams per pound but reduced to 75 percent of the full fed group and chicks fed the diet containing 3.61 milligrams per pound. There had been a significant ($P < .01$) difference in these average values after one week and two weeks of treatment.

Average foot pigmentation scores (Table XVII) likewise measured as a change from beginning scores did not differ significantly after two weeks of experimental treatment, although birds fed the diet containing 3.61 milligrams of xanthophyll per pound did show the greatest reduction in pigmentation. The average score of all birds was less after both two and three weeks of experimental treatment than at the beginning.

At the end of three weeks of experimental treatment, chicks fed the diet containing 3.61 milligrams of xanthophyll per pound had lost a significantly ($P < .01$) greater amount of pigment than had chicks on any other dietary treatment. The

TABLE XVII

AVERAGE CHANGE IN FOOT PIGMENTATION AND AVERAGE FINAL BODY WEIGHTS*--EXPERIMENT 7

Dietary treatments			Pigmentation score changes** period		Average final body weights** (grams)
No.	Mg. xanthophyll per pound	Amount fed	0-2 wks.	0-3 wks.	
1	7.22	ad libitum	-0.5^{A}	-0.6^{A}	1371^{A}
2	7.22	75% of 1	-0.4^{A}	-0.6^{A}	1258^{B}
3	7.22	50% of 1	-0.1^{A}	-0.2^{A}	1016^{C}
4	5.41	same as 1	-0.3^{A}	-0.5^{A}	1354^{A}
5	3.61	same as 1	-0.7^{A}	-1.2^{B}	1348^{A}

*Each value represents the average of three replicate groups of three males and four females,

**Means, within each column, bearing the same capital letter superscript do not differ significantly ($P < .01$). Means which do not differ at the 1 percent level of probability do not differ at the 5 percent level of probability.

average score of other lots of birds was not significantly different one from the other.

Average body weights of birds after three weeks of experimental treatment are given in Table XVII. Average weights are a direct reflection of feed intake with a significant ($P < .01$) reduction in weight with each reduction in feed intake. The average weight of birds full fed the diet containing 7.22 milligrams of xanthophyll was significantly ($P < .01$) heavier than for those receiving only 75 percent as much feed which was significantly ($P < .01$) heavier than those receiving only 50 percent as much feed. Body weights were not reduced in the same proportions as feed restriction as shown by the fact that those birds which were fed only 75 percent as much feed weighed 90 percent as much as those on full feed. Birds receiving only 50 percent as much feed weighed 75 percent as much as those on full feed. There was a greater percent utilization of nutrients as well as xanthophyll when birds were restricted in feed intake as evidenced by the fact that pigmentation scores did not differ significantly. Average xanthophyll intake per gram of body weight was higher in those birds receiving full feed than in those receiving a restricted amount. Average body weights of those birds receiving diets containing 5.41 and 3.61 milligrams of xanthophyll per pound did not differ significantly from each other, nor did either differ significantly from those fed the diet containing 7.22 milligrams of xanthophyll per pound.

Experiment 8

A summary of changes in blood xanthophyll levels of birds is given in Table XVIII. Again, changes in blood xanthophyll levels from the beginning of the experiment were used for statistical analysis since there was considerable variation among individual birds at the beginning. After two weeks of experimental treatment, those birds which had been parasitized with E. maxima showed a drop in average blood xanthophyll level which was found to be significantly different ($P < .05$) from blood xanthophyll changes of birds on any other tratment. Those birds which had received the same amount of feed as the parasitized chicks showed a gain in average blood xanthophyll level. These results indicate that feed intake per se was not a major factor in the decrease in blood xanthophyll level of parasitized chicks.

Feed intake of non-parasitized chicks receiving feed ad libitum and parasitized chicks also receiving feed ad libitum did not differ greatly as may be seen in Table XIX. These data further substantiate the fact that the drop in blood xanthophyll level and loss in pigment of chicks parasitized with coccidia is not a result of reduced feed intake.

After two weeks, chicks which had been restricted to 75 percent of full feed showed a significantly greater increase in blood xanthophyll than did those chicks receiving full feed. This difference was significant at the 5 percent but not at th 1 percent level of probability. Again this points out the

TABLE XVIII

AVERAGE CHANGES IN BLOOD XANTHOPHYLL LEVELS* AND FOOT PIGMENTATION SCORES**--EXPERIMENT 8

Treatment			Blood xanthophyll changes***		Pigmentation score changes***	
	Amount of	E. maxima oocysts	Period		Period	
No.	feed	per bird	0-2 wks.	0-3 wks.	0-2 wks.	0-3 wks.
1	ad lib.	None	0.14^{b}	0.64^{b}	0.2^{b}	0.1^{B}
2	75% of 1	None	2.12^{a}	2.16^{a}	0.9^{a}	1.1^{A}
3	ad lib.	20,000	-1.81^{c}	0.48^{b}	-0.2^{c}	-0.5^{C}
4	same as 3	None	1.07^{a,b}	0.12^{b}	0.4^{b}	0.1^{B}

*Each value represents the average change in micrograms of xanthophyll per milliliter of blood serum for three replicate groups of two males and two females.

**Each value represents the average change in foot pigmentation score of three replicate groups of five males and five females.

***Means, within each column, bearing the same small letter superscript do not differ significantly ($P < .05$) while those bearing the same capital letter superscript do not differ significantly ($P < .01$). Means which do not differ at the 1 percent level of probability do not differ at the 5 percent level of probability.

TABLE XIX

AVERAGE DAILY FEED INTAKE OF PARASITIZED AND NON-PARASITIZED CHICKS FED AD LIBITUM--EXPERIMENT 8

Day after inoculation	Non-parasitized	Parasitized
	grams	grams
0	72	72
1	81	84
2	64	71
3	76	79
4	76	70
5	61	50
6	71	55
7	89	86
8	77	82
9	72	85
10	82	86
11	70	74
12	85	83
13	93	93
14	83	83
15	98	101
16	83	89
17	94	97
18	94	100
Total	1521	1540

increased utilization of xanthophyll by chicks on a restricted feed intake. It should be mentioned that unlike Experiment 7 where a dietary level of 7.22 milligrams of xanthophyll per pound was used, in Experiment 8, a diet containing 11.46 milligrams of xanthophyll per pound was used. Restricting feed to 75 percent of full feed would reduce xanthophyll intake equivalent to a level of 8.6 milligrams per pound.

After three weeks, changes in blood xanthophyll levels were significantly different ($P < .05$) between birds subjected to 75 percent restriction and birds receiving all other treatments. Blood xanthophyll levels of the restricted-fed birds showed a significantly ($P < .05$) greater increase than those of birds of any ogher treatment. No significant differences were noted within the other groups in blood xanthophyll level changes.

Pigmentation scores, reported as change from beginning to two weeks, reflected blood xanthophyll levels. Birds restricted to 75 percent of full feed gained a significantly greater ($P < .05$) amount of pigment than birds on any other treatment while the parasitized group showed a loss in average pigmentation score which was significantly different from changes in score of any other group. These same differences were true after three weeks of experimental treatment except that they were significant at the 1 percent level of probability.

Average body weights as given in Table XX show the effect of feed restriction. After one week of experimental treatment those birds on 75 percent of full feed had significantly ($P < .05$)

TABLE XX

AVERAGE CHICK BODY WEIGHTS*--EXPERIMENT 8

Treatment No.	Amount of feed	*E. maxima* oocysts per bird	Age of chick - weeks** 5	6	7	8
1	ad libitum	None	519	711^{a}	937^{A}	1179^{A}
2	75% of 1	None	519	665^{b}	820^{C}	967^{B}
3	ad libitum	20,000	519	709^{a}	907^{B}	1160^{A}
4	same as 3	None	518	709^{a}	926^{A,B}	1150^{A}

*Each value represents the average body weight of three replicate groups of five males and five females.

**Means bearing the same small letter superscript do not differ significantly ($P<.05$). Means bearing the same capital letter superscript do not differ significantly ($P<.01$). Means which did not differ at the 1 percent level of probability, did not differ at the 5 percent level of probability.

lower average body weights than birds on any other treatment. After two weeks of experimental treatments the restricted birds were significantly ($P < .01$) lighter in weight than birds on any other treatment. Furthermore, the parasitized birds had significantly ($P < .01$) lower average body weights than those birds on full feed but non-parasitized, while the average weight of the non-parasitized birds fed the same amount of feed as the parasitized ones was not significantly different from either the non-parasitized full fed or the parasitized groups.

The only significant differences in body weights after three weeks of treatment were those birds on restricted feed which were significantly lower ($P < .01$) than those of chicks on any other treatment.

Experiment 9

A summary of changes in foot pigmentation scores of chicks representing the dietary xanthophyll phase of Experiment 9 are given in Table XXI. Although chicks were assigned to pens so that their average pigmentation scores were approximately equal among pens, they were not given the oocysts of *E. maxima* until after they had been fed the various experimental diets for one week. It was believed, therefore, that the effect of coccidiosis would be more correctly measured if changes in pigmentation scores from the time of inoculation were used. The time of zero in the tables represent time of inoculation.

TABLE XXI

AVERAGE CHANGES IN FOOT PITMENTATION SCORES*--DIETARY XANTHOPHYLL LEVEL PHASE--EXPERIMENT 9

Diet No.	Mg. xanthophyll per lb. of diet	Period of change**					
		0-2 weeks			0-3 weeks		
		E. maxima, oocysts per bird			E. maxima, oocysts per bird		
		0	10,000	Av.	0	10,000	Av.
1	0	-3.5	-2.7	$^{a}-3.1^{A}$	-3.5	-2.8	$^{a}-3.2^{A}$
2	3.97	-2.4	-2.6	$^{b}-2.5^{A}$	-2.8	-3.0	$^{a}-2.9^{A}$
2B	5.97	-1.0	-1.4	$^{c}-1.2^{B}$	-0.9	-1.5	$^{b}-1.2^{B}$
4	11.46	0.1	-1.2	$^{d}-0.5^{C}$	0.0	-1.3	$^{c}-0.6^{B}$
	Av.	-1.7	-2.0		-1.8	-2.2	

*Each value represents the average change in foot pigmentation score of two replicate groups of six males and four females.

**Means bearing the same capital letter superscript do not differ significantly ($P < .01$). Means bearing the same small letter superscript (preceeding the mean) do not differ significantly ($P < .05$).

After a period of two weeks of experimental treatment, changes in pigmentation scores were found to be significantly different ($P < .05$) between birds fed each dietary level of xanthophyll, however, differences between lots of birds fed the diet containing 3.97 milligrams of xanthophyll per pound were not significantly different at the 1 percent level of probability from lots of birds fed the xanthophyll-free diet. Although the overall effect of coccidiosis upon pigmentation was not significant, there was a significant ($P < .01$) diet times coccisiosis interaction. All parasitized chicks showed a greater loss in pigmentation than non-parasitized chicks given the same diet, except that those chicks fed the xanthophyll-free diet, where the reverse was true. The reason for this is that the average pigmentation score of the chicks fed the xanthophyll-free diet was lower at the time when the birds were parasitized than chicks fed the same diet but receiving no coccidia. Since scores of all chicks receiving the xanthophyll-free diet fell to the lowest score on the standard after two weeks, the parasitized chicks on this diet did not lose as much pigment as the non-parasitized chicks as their initial score did not have as far to fall to reach the lowest score. It is postulated that parasitized birds would have shown a significant loss in pigmentation if this had not been a factor.

After three weeks of experimental treatment, changes in pigmentation scores from the beginning of the experimental

period were a direct reflection to the dietary xanthophyll level. The chicks fed diets containing the lower two levels of xanthophyll were singificantly different ($P < .05$) in pigmentation changes than chicks fed the diet containing 5.97 milligrams per pound, which were, in turn, different from the chicks fed the diet containing the highest level. Although there was again a significant ($P < .01$) interaction between diets and coccidiosis, the overall effect of coccidiosis caused a significantly ($P < .05$) greater loss in pigmentation scores.

Higher dietary levels of xanthophyll did not affect loss of pigment of parasitized chicks as shown by the fact that the difference in values (change in pigment scores) between parasitized and non-parasitized chicks fed the higher level of xanthophyll was greater than between similar comparison of chicks fed lower levels of dietary xanthophyll in all cases except one.

Another significant interaction after three weeks was between sex and coccidiosis. This is reflected in that parasitized males lost more pigment compared to non-parasitized males while parasitized females lost the same amount of pigment as non-parasitized females.

A summary of cahnges in foot pigmentation scores of chicks subjected to the coccidiostatic phase of Experiment 9 is shown in Table XXII. As was true in the other phase of the experiment, a significant interaction between diet and coccidiosis was evident after two weeks and after three weeks

TABLE XXII

AVERAGE CHANGES IN FOOT PIGMENTATION SCORES*--COCCIDIOSTAT PHASE--EXPERIMENT 9

Coccidiostat** added to diet No. 4	Period of change					
	0-2 weeks			0-3 weeks***		
	E. maxima, oocysts per bird			E. maxima, oocysts per bird		
	0	10,000	Av.	0	10,000	Av.
---	0.1	-1.2	-0.5	0.0	-1.3	-0.6
Amprol	0.0	-0.1	0.0	-0.2	-0.6	-0.4
Amprol plus	-0.6	0.0	-0.3	-0.8	-0.3	-0.6
Zoamix	0.3	0.0	0.1	0.4	-0.5	0.0
Unistat	0.0	-0.4	-0.2	0.0	-0.8	-0.4
Av.	0.0	-0.3		-0.1[A]	-0.7[B]	

*Each value represents the average change in foot pigmentation score of two replicate groups of six males and four females.

**Composition of coccidiostats given in Experimental Procedure on page 44.

***Means of non-parasitized and parasitized birds were significantly different ($P < .01$).

of experimental treatment. This interaction can again be explained by the fact that parasitized chicks fed Amprol Plus did not drop as much in pigmentation scores as non-parasitized chicks fed the same coccidostat. As shown in this experiment Amprol Plus was effective in preventing loss of pigmentation from infection with E. maxima.

After three weeks of experimental treatment, parasitized chicks lost significantly more ($P < .01$) pigment than non-parasitized chicks. Also the interaction between sex and coccidiosis was again found to be significant ($P < .05$).

Because of the small number of blood samples available, and the variation in individual samples, proper evalutaion of the effects of different treatments upon xanthophyll level in the blood was considered doubtful.

Experiment 10

A summary of average changes in foot pigmentation scores is given in Table XXIII. Changes did not differ significantly between groups after one week following parasitization. After two weeks those chicks parasitized with E. necatrix had lost significantly ($P < .01$) more pigment than the non-parasitized control chicks. Chicks parasitized with E. maxima had lost significantly ($P < .05$) more pigment than the control chicks. Average changes in pigmentation scores between groups parasitized with different species of coccidia did not differ significantly. Differences

TABLE XXIII

AVERAGE CHANGES IN FOOT PIGMENTATION SCORES*
EXPERIMENT 10

Coccidia species	Period of change		
	0-1 week	0-2 weeks**	0-3 weeks***
None	0.0	-0.2^{B}	0.0^{b}
E. maxima	-0.1	$-0.6^{A,B}$	0.4^{a}
E. necatrix	0.5	-0.7^{A}	-0.5^{a}
E. tenella	0.2	$-0.4^{A,B}$	$-0.3^{a,b}$

*Each value represents the average change in foot pigmentation score of four replicate groups of 12 females each.

**Means bearing the same capital letter superscript do not differ significantly ($P < .01$). However, means of chicks parasitized with E. maxima differed significantly ($P < .05$) from means of the non-parasitized controls.

***Means bearing the same small letter superscript do not differ significantly ($P < .05$).

between non-parasitized chicks and thos parasitized with E. tenella were not significant.

The same relative changes in pigmentation scores were found three weeks after inoculation except that differences were significant at only the 5 percent level of probability. The changes show a gain in pigmentation scores during the third week. This would be expected since the lapse in time gave the chicks time to recover from the effects of coccidiosis.

Results of this experiment indicate that the effect of coccidia upon pigmentation is caused by damage to the small intestine. This is the point where E. necatrix and E. maxima cause the most damage to intestinal tissue. E. tenella is most damaging not in the small intestine but in the cecae which is not of major importance in nutrient absorption. Loss of pigment of chicks parasitized with E. tenella was not significantly different from loss of pigment in the non-parasitized control chicks. These data indicate that the primary effect which coccidia have upon pigmentation of chicks is by blocking absorption of xanthophyll from the intestine or a destruction of xanthophyll in the intestine.

Experiment 11

Preliminary to Experiment 11, studies were conducted to test various xanthophyll concentrates and various methods of injection of these materials into chicks in an attempt to by-

pass the intestine as an avenue of xanthophyll intake in chicks parasitized with E. maxima. Results of the preliminary studies showed that a lutein concentrate mixed with Wesson oil and Tween-80, an emulsifier, could be injected into the breast muscle of the chick and absorbed into the blood stream.

The average blood xanthophyll levels of chicks used in Experiment 11 are given in Table XXIV. Average xanthophyll levels of chicks fed the diets containing 11.46 milligrams of xanthophyll per pound of feed were both significantly ($P < .01$) higher than that of chicks fed the diet containing no xanthophyll. Diet 4 containing xanthophyll from yellow corn, alfalfa meal and corn gluten meal produced significantly ($P < .01$) higher blood xanthophyll levels than the diet containing the same xanthophyll level but supplied by the lutein concentrate.

Chicks which had been parasitized with E. maxima had significantly ($P < .01$) lower blood xanthophyll levels than did non-parasitized chicks. The injection of lutein into the breast muscle of chicks resulted in a significant ($P < .01$) improvement in blood xanthophyll levels compared to that of non-injected chicks.

A significant interaction between coccidiosis and injection was found in the statistical analysis showing that parasitized chicks responded differently to injection of xanthophyll than did non-parasitized chicks. Although blood xanthophyll levels of parasitized chicks increased when chicks

TABLE XXIV

AVERAGE BLOOD XANTHOPHYLL LEVELS*--EXPERIMENT 11

E. maxima oocysts per bird	Diet No.**	Dietary xanthophyll content	Lutein injection: None	Lutein injection: 7.5 mg. per bird***	Lutein injection: Av.****
None	1	0	0.51	3.13	1.82
None	1A	11.46	4.53	5.99	5.25
None	4	11.46	8.23	11.09	9.66
	Av.		4.42	6.74	5.58^{A}
20,000	1	0	0.25	1.48	0.86
20,000	1A	11.46	0.80	1.84	1.32
20,000	4	11.46	1.28	2.65	1.97
	Av.		0.78	1.99	1.38^{B}
Average	1	0	0.38	2.31	1.34^{AA}
	1A	11.46	2.66	3.91	3.29^{BB}
	4	11.46	4.75	6.87	5.81^{CC}
	Av.		2.60^{AAA}	4.36^{BBB}	3.48

*Each value represents the average of duplicate pens of six males, reported as microgram per milliliter of serum.

**Diet 1A was formulated by adding 130 grams of lutein concentrate (4 grams per pound) to 100 pounds of Diet 1.

***Injections of 2.5 milligrams of lutein per bird were given into the breast muscle on each of three consecutive days.

****Means bearing the same capital letter superscript do not differ significantly ($P < .01$). Only menas bearing the same number of letters in the superscript should be compared with each other.

were injected with xanthophyll, the response was not as great as when non-parasitized chicks were injected.

These data indicate that, while the intestine was by-passed as a means of getting xanthophyll into the blood stream, there appear to be some other factor or factors involved in the metabolism of xanthophyll from the intestine than the destruction of intestinal tissue by coccidia.

General Discussion

The chicken is generally considered to have no known nutritional of physiological requirement for the xanthophyll pigments. The elucidation of processes involved in xanthophyll metabolism has been of interest. A more complete understanding of factors affecting absorption, transport, deposition and destruction of xanthophyll is important if xanthophyll is to fulfill its intended role in poultry nutrition. This role involves the consistant production of poultry products with uniform pigmentation which will present a product with more consumer appeal.

Studies reported have confirmed the non-essential nutritive nature of xanthophyll for the chick. When diets containing a high level of xanthophyll were fed to chicks hatched from eggs laid by hens depleted of xanthophyll, a high degree of pigmentation was observed in the chicks within three to four weeks. Contrary to the report of Mann (1946), these depleted chicks began to utilize and deposit pigment within a few days after receiving feed containing a high xanthophyll content. This is an important

consideration for broiler producers who need not supply feed containing high levels of xanthophyll to breeder hens producing hatching eggs for the purpose of influencing hatchability, the health of the chick or final pigmentation of the broiler.

Production of poultry products with uniform pigmentation of the desired degree will continue to be a problem due to many factors. Variation in availability of xanthophyll from various feed products, variation in capacity of birds to deposit pigment, the physiological stress of disease and excessive environmental temperatures are all factors which may be involved. Therefore, the inclusion of high levels of xanthophyll in the diet does not always insure the production of a uniformly pigmented product.

Feed intake *per se* does not seem to be a major factor in pigmentation of chicks. When a diet containing approximately 11 milligrams of xanthophyll per pound was fed to normal, healthy chicks, a reduction in feed intake by 25 or 50 percent did not significantly reduce pigmentation in the chicks. The major effect of coccidiosis produced by *E. maxima* appeared to be in the prevention of absorption of xanthophyll from the intestine. The intramuscular injection of lutein was effective in increasing xanthophyll levels in the blood of birds infected with *E. maxima*. Other factors may be involved, since the effect of coccidiosis was not completely overcome by injections.

Many coccidiostats used in poultry feeds, which control the more pathogenic species of coccidia, do not effectively

control E. maxima, a primary cause of loss of pigmentation. Of the four coccidiostats studied, only Amprol Plus was effective in preventing loss of pigmentation. A more complete control of E. maxima could help in the production of well pigmented broilers.

The depigmenting effect of high environmental temperature appears to include many possible factors. While there was a reduction in feed intake, it is possible that there was destruction of xanthophyll in the feed, in the tissue or intestine of the birds or an ineffective absorption of xanthophyll from the intestine. The use of antioxidants in the feed may have an important role in the prevention of destruction of xanthophyll.

Laying hens, which had been depleted of pigmentation, were found to deposit pigment in their beak, eye ring and other exposed pigmented areas. This is contrary to the report of Palmer and Kempster (1919a) which stated that it was impossible to restore xanthophyll to pigmented areas of laying hens, no matter how large an excess of pigment was fed. The use of pigmentation of the hen as an index of past laying performance must include a consideration of the xanthophyll content of the layer diets fed during the production period.

SUMMARY

A total of 11 experiments were conducted to study the effect of certain factors upon xanthophyll metabolism in the chicken. Studies were conducted with growing chicks, broilers, roasters and laying pullets. Blood xanthophyll levels and visual pigmentation scores of chickens were used to evaluate xanthophyll metabolism.

Increasing the dietary xanthophyll level from 5.97 to 11.46 milligrams per pound did not significantly affect pigmentation in chicks which were fed these diets from one day to four weeks of age. Average pigmentation scores of chicks fed a diet containing 3.61 milligrams of xanthophyll per pound from four to seven weeks of age were significantly ($P < .01$) lower than those of chicks fed a diet containing 7.22 milligrams of xanthophyll per pound for the same period.

In two out of three comparisons, average pigmentation scores of chicks were higher when diets contained added fat than when the diets contained the same level of xanthophyll but without added fat. This points out the variable results of added dietary fat upon pigmentation.

Chicks hatched from eggs laid by hens fed a xanthophyll-free diet showed almost no visible pigment and a very low blood xanthophyll level. The blood xanthophyll level began to rise and xanthophyll was deposited in the shanks of these

chicks within a few days after the chicks were fed diets high in xanthophyll, indicating that chicks are able to utilize xanthophyll at an early age. Four-week pigmentation scores were dependent mainly upon the level of xanthophyll in the chick diet and not upon the xanthophyll content of the diet fed to their dams.

Pigmentation scores of males were generally higher than for those of females. A significant ($P < .05$) positive correlation was found between blood xanthophyll level and foot pigmentation scores of chicks. Foot pigmentation scores of eight week old broiler type chicks were significantly ($P < .05$) positively correlated with the pigmentation scores of the skin of the dressed carcass.

Average foot pigmentation scores were not affected by reducing feed intake to 75 or 50 percent of the amount consumed by birds receiving feed free choice, indicating a greater utilization of xanthophyll when feed intake was restricted. Scores of four week old chicks housed in high temperature rooms (90° F. constant) were lower than those of chicks housed at normal temperatures (80° reduced to 70° F.).

Chicks parasitized with E. maxima or E. necatrix lost pigment after two and three weeks when compared to non-parasitized chicks. Feed intake per se was not a factor in the loss of pigment due to infections of E. maxima. Increasing the level of xanthophyll in the diet did not overcome the effect of coccidia. Injection of xanthophyll into the breast muscle resulted in a

significant improvement of pigmentation of chicks parasitized with E. maxima when compared to non-injected parasitized chicks. This suggests that a primary effect of coccidia is in the prevention of xanthophyll absorption from the intestine of the chicken.

Observation of pullets in production disclosed that laying hens were capable of depositing xanthophyll in their beak, eye ring and vent when fed diets high in this pigment.

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