T H E S I S

on

THE COMPARATIVE VALUES OF LIGHT AND DARK EGG YOLKS WITH RESPECT TO VITAMIN A CONTENT

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PURPOSE

The purpose of this study is to determine the relative values of light and dark egg yolks with respect to their vitamin A content. This problem is of economic importance in the commercial egg industry. It is generally agreed that New York City, the largest egg market, became partial to pale egg yolks because of the doubt aroused concerning the quality of the eggs having highly colored yolks. Eggs shipped from the Pacific coast to New York are sold at a premium over eggs produced in the Middle West and East. The pale yolks, however, are more difficult to produce than dark yolks because of certain feed requirements. In view of the fact that vitamin A is generally associated with yellow pigments, the question arises as to whether or not dark yolks are more valuable than pale yolks from a nutritional standpoint.

STATEMENT OF THE PROBLEM

The problem in this study was that of determing the comparative values of light and dark egg yolks with respect to their vitamin A content. The materials and method of procedure consisted of a literature review and experimental work. The general outline of the study embodies the egg yolk as a source of vitamin A, the factors affecting the color of egg yolks, the biological relation between yellow pigments and vitamin A, and a report on the experimental work.

THE EGG YOLK AS A SOURCE OF VITAMIN A

The history of evidence involving the hen's egg as a source of vitamin A dates back to experiments performed long before the importance of this food factor in nutrition was recognized. In 1891, Socin, reported having successfully fed mice on a mixture of starch, cellulose, and egg yolk for ninty-nine days and that he believed that they would have continued to thrive on this diet. The nutritional properties of vitamin A in foods was discovered as the result of a characteristic eye disease appearing among experimental animals. Osborne and Mendel found this to be true with their experimental animals in 1913. This disease is known as xerophthalmia. Thus the vitamin A content of all materials has been determined largely by the preventive or curative effects possessed with respect to this characteristic eye disease. Various animals have been used in determining the vitamin A content of foods, but young rats have been used by the largest number of investigators.

Among the early investigators to associate vitamin A with egg yolks were E. V. McCollum and M. Davis (1913) who state:

"After numerous attempts to prevent the occurrence of growth suspension by nice adjustments of our diets, we found that the failure of rats to make further growth after being brought to this "critical" point on mixtures of isolated food substances, is due to a lack of certain ether soluble substances in the diet. These can be supplied by ether extract of egg or butter. In 1914 McCollum and Davis found also that when rats had been fed on a diet deficient in vitamin A until growth and development ceased, they would make a new growth if small amounts of ether extract of butter or egg were added."

Murphy and Jones (1924), in their attempt to find the smallest quantity of fresh whole egg that would cure rats of xerophthalmia and enable them to grow at a normal rate, fed different amounts. They report that their first trials were made with 0.5 gm. of fresh whole egg given daily after the onset of xerophthalmia. The next trials were made with 0.75 gm. of egg, and finally with 0.25 gm. The results of their experiments are given as follows:

"Feeding experiments have shown that from 0.50 gm. to 0.75 gm. of fresh, whole egg fed supplies young rats with sufficient vitamin A for growth at a normal rate. A smaller quantity, 0.25 gm., was found to be adequate to cure well advanced cases of xerophthalmia."

Quoting Sherman and Smith (1930) from their text, "The Vitamins", we find the following paragraph concerning the egg yolk as a source of vitamin A:

"Eggs are an important source of vitamin A. The vitamin is contained essentially in the yolk of the egg and the concentration depends to an extent not fully determined, upon the food of the fowl. Undoubtedly the fowl depends upon its food for the vitamin A which the egg contains; but to what extent a shortage of this vitamin in the food results in the production of vitamin A-poor eggs, as against a simple diminution of egg production, remains to be investigated in detail. There is little doubt that the egg is a more constant source of vitamin A than is liver, which alone among animal body tissues approaches it in richness in this vitamin. The egg as a whole may be expected to contain about 15 to 20 units of vitamin A per gram; and the yolk about three times this concentration."

It is generally known that the egg is an important source of vitamin A, however, investigators are not sure of the factors affecting the amount contained in the egg yolk. From some of the foregoing statements it is partly evident that the amount of vitamin A present in the egg yolk depends to some extent upon the amount contained in the food which the hen eats. However, from the latest experimental data available we are unable to determine definitely to what extent food affects the amount of vitamin A stored in the egg. Many experiments have been performed which show very clearly that food does have a noticeable effect upon the vitamin A content of eggs. Experiments have also been carried on in an attempt to determine the effect of long continued storage on the vitamin A content of eggs. McCollum and Davis (1924) reported that the vitamin A physiological property for inducing growth was not impaired even when the egg was boiled. However, scientists agree that eggs heated in contact with air have a tendency to lose their vitamin A potency. In this paper the author is primarily interested in the factors that affect the vitamin A content as found under normal condition in the fresh egg.

Having learned that food does have an appreciable effect upon the vitamin A content of egg yolks, it might be of interest to the reader to have a list of some of the materials commonly fed to poultry and the relative distribution of vitamin A therein. S. L. Smith (1929), in U. S. D. A. Circular 84, gives a list of foods, and he indicates the relative distribution of vitamin A in these foods by plus signs. For example, one plus sign following a particular food indicates that the food contains vitamin A. Two plus signs indicate that the food is a good source of vitamin A. Three plus signs indicate that the food is an excellent source of vitamin A, and a minus sign indicates that the food contains no appreciable amount of vitamin A. The list of foods which follow is taken from the list given in the circular, but the sign(x) is used instead of plus for convenience. Zero is used to indicate lack of vitamin A.

White corn .... (0)

Yellow corn .... (xx)

Yellow corn gluten .... (xx)

Oat meal .... (0 to x)

Oats, whole	(0 to x)
Millet seed	(x)
Rye, germ	(xx)
Rye, whole	(x)
Wheat, bran	(x)
Wheat middlings, commercial	(0)
Wheat, germ	(xx)
Wheat, whole	(x)
Milk, cow's skimmed, dried	(x)
Milk, cow's skimmed raw	(x)
Milk, cow's whole, pasteur.	(xxx)
Milk, cow's whole, dried	(xxx)
Milk, cow's whole, raw	(xxx)
Buttermilk	(x)
Cod-liver oil	(xxx)

<u>Greens</u>

Alfalfa	(xx)
Beets, leaves	(xx)
Beets, roots	(0 to x)
Cabbage, leaves, green, dried	(x to xx)
Cabbage, leaves, green, fresh	(xx)
Carrots, raw	(xxx)
Collards, raw	(xx)
Kale	(xx)
Lettuce, head	(x to xx)
Lettuce, leaves, green	(xxx)

Rutabagas .... (x)

Spinach .... (xxx)

Turnip, greens, raw .... (xxx)

The above list is far from being complete in the case of poultry feed, but many of those commonly used are to be found there.

FACTORS AFFECTING COLOR OF EGG YOLKS

Poultrymen throughout the country agree that the hen's egg represents a finished product of the food intake in excess of the amount required for body maintenance. It is reasonable then, to expect the raw materials to have some effect on the color and quality of the finished product. Thus, the idea has developed that feed and management exert considerable influence on the color variation in egg yolks.

In studying the factors which affect the color of egg yolks it is important to consider the source of coloring material. In plants the yellow color is due to certain pigments known as carotinoids. However, in many plants the yellow color is masked by chlorophyll. Carotinoids are subdivided into carotin and xanthophyll. Carotin is found in butter, carrots, and the leaves of many plants. Xanthophyll is found in leaves of certain plants, in yellow corn, and is the chief coloring matter in egg yolks. In the foregoing pages of this study a list of feeds is given together with the comparative vitamin A distribution. It is of interest to note that the foods given in the list are among those containing carotin and xanthophyll. The question arises as to what part these color pigments of food play in the coloring of egg yolks. Numerous experiments have been performed by investigators who have attempted to determine the role of these yellow color pigments in the color of egg yolks.

According to L. S. Palmer and H. L. Kempster (1919) xanthophyll is the chief plant carotinoid responsible for the yellow pigmentation in egg yolks.

H. L. Kempster (1930) gives the following report:

"In 1915 Palmer, of the Missouri Agricultural Station, in cooperation with the writer, conducted tests with laying hens in a study of this interesting problem. By this time the identity of the principal pigment in the egg yolk had been determined as xanthophyll, and its relation to plant xanthophyll had also been established. Studies by Palmer and Eckles had also shown that the natural yellow pigment that characterizes the milk fat of the cow is physiological as well as identical with carotin of the chloroplast and depends upon the presence of this pigment in food for its presence in body tissue, and secretions of the animal body. A similar relation between the xanthophyll of the chloroplast naturally suggested itself. Should such a relationship be confirmed, it presented the interesting phenomena of the inability of the cow to take up xanthophyll pigments to any extent and the similar inability of the fowl to make use of the carotin.

Accordingly feeding tests were outlined in which, in one case, the ration was rich in xanthophyll; in another case rich in carotin; and, in a third case, devoid of either. The results of the feeding tests showed that the hens fed ration rich in xanthophyll laid eggs in which the yolks were highly pigmented. While with the two other rations the yolks became very pale. This confirmed the theory that the hen utilized only the xanthophyll pigment and not the carotin.

It was not, however, until 1919 that sufficient evidence had accumulated to definitely come to this conclusion. By that time it had become possible to raise White Leghorn pullets successfully on ration devoid of either carotin or xanthophyll. These birds were normal aside from the absence of yellow pigment in the sections which normally carry yellow pigment. Feeding tests then showed that where xanthophyll was added to the ration yellow color began to appear in the skin surface within seventy-two hours and that after four weeks of feeding ration rich in carotin there was no evidence of the utilization of carotin. Again feeding tests in which the hens used were, prior to the tests, laying eggs in which the yolks were devoid of any yellow pigment, the hard boiled eggs showing no difference in color of yolk and white, showed that of the common feedstuffs the principal sources of xanthophyll are yellow corn and the green leafy parts of plants. This explains why previous investigators had observed that ration rich in yellow corn and green feed had produced highly colored yolks. The degree to which the yolk is colored is directly dependent upon the amount of xanthophyll consumed. It is thus seen that if hens vary in the amount of yellow corn or green feed consumed then the different hens will produce eggs with yolks of different color. Also the same hens will vary in the color of the yolk produced, depending upon the amount of xanthophyll consumed. This variation may occur in a very short period of time since a major portion of the yolk is produced just prior to its release from the egg follicle. Therefore, yolk color is controlled by limiting the amount of yellow corn and green leafy parts of plants that are consumed. If the yolks are too pale, more green feed and yellow corn should be fed, and if the yolks are too rich in color, the amount of yellow corn or green feed should be reduced."

Stewart and Atwood (1903) in a number of experiments conducted to study the effect of feeding different foods upon the flavor of eggs include the following statements in their report:

"The different rations, however, very clearly affected the color of the yolks. When the grain ration consisted of wheat, oats, or white corn fed either alone or in combination with each other the yolks were so light colored that the eggs would be quite undesirable for fancy trade."

The Oregon Experiment Station (1928) performed an experiment in an effort to determine the effect of yellow corn and green feed upon the color of the yolk and density of the white. In this study we are interested in the experiment only in so far as it has bearing upon the coloring of egg yolks. It is evident that the experiment was carried out with the requirements of commercial production and market demands as the underlying stimulus, but the general summary which follows shows that varied feed practices tend to produce variations in yolk colors:

"There is considerable variation in commercial grading of so called dark yolked eggs. There is also quite a variation in the production of different colored yolks by hens upon the same feed. Alfalfa leaves and blossoms at the rate of 5% of the mash produced slightly lighter yolks than 5 pounds of kale per 100 birds daily, but was satisfactory as a class for pale yolk market demands. Two and one-half per cent of alfalfa leaves and blossoms produced yolks lighter than three pounds of kale per 100 birds daily and in both cases too light in color for general use because some eggs were pale or sickly looking in appearance.

Kale at the rate of 5 pounds per 100 birds daily or alfalfa leaves and blossoms to the extent of 5% of the mash or the amount generally recommended does not appear to produce yolks too dark to meet the demand of the New York market as understood by Oregon graders."

W. A. Morgan and J. G. Woodroof (1927) demonstrated the pronounced influence of Pimiento pepper on the coloring of egg yolks. They fed twenty Rhode Island Red hens on the following ration:

Mash		Scratch Feed	
Wheat bran	5 kg.	White corn	10 kg.
Wheat shorts ...	5 kg.	Wheat	10 kg.
Beef scraps	3 kg.	Oats	5 kg.

The following is included in their report:

"After several days on this feed, ground, dried Pimiento pepper was fed to half the hens at the rate of 0.5 gram (1/60 ounce) each day per hen, the other half receiving the same feed with no pepper.

The appearance of coloration in the yolks after the hens were put on pepper feed was on the fifth day and full intnesity was reached on the tenth day.

Regardless of the quantity of pepper fed the white of the egg remained unchanged, while the yolk showed definite changes in color. The final intensity of the yolk was in direct proportion to the amount of pepper included in the feed."

L. F. Payne (1925) discovered that birds would produce olive colored yolks if allowed access to ranges in which certain weeds were prevalent. Upon investigation Payne found that where birds ate freely of two weeds later identified as Shepherd's Purse and Penny Cress the yolks were dark or olive colored in appearance.

Benjamin (1918) states: "Excessive consumption of green food, such as the ravenous eating of rape, which often occurrs when the hens are first allowed access to it in the spring, sometimes makes the yolks very dark. In such cases, when the egg is candled, the yolk will often appear dark, and will have a greenish rather than a red tint.

Yellow corn, green food, and general ranging causes the yolks to be dark yellow, while white corn, wheat, buckwheat, and lack of exercise cause light yolks. These slight variations in color of yolks do not indicate any difference in the food value of the eggs."

Thompson (1918) reported cottonseed meal spots on the yolks of eggs from hens but none on the yolks of the eggs from pullets.

Later the New Mexico Station (1927) reported as follows: "The pen which received 38 per cent of cottonseed meal in the mash, produced eggs which were so badly affected by the cottonseed meal spots as to be unmarketable. The yolks of these eggs turned black in color, as the eggs were kept for a few days, so that when a week old the yolks were almost entirely black."

Aside from the factors previously mentioned in connection with egg yolk color, there has been some controversy as to whether or not there are seasonal variations in egg yolk colors. According to S. L. Parker (1927) a study was made to determine whether or not there are seasonal variations in egg yolk color. Eggs were shipped to New York City from two Pacific coast states and reshipped to the experiment station in California. The test was made on two different series of eggs: (1) biweekly samples shipped to New York City and reshipped to California and (2) weekly samples of eggs shipped to the laboratory directly from the packing plant in Petaluma. The following summary is given:

"There was found to be no definite seasonal trend in egg yolk color.

This was the case when tested on two different series of eggs: (1) biweekly samples of eggs which had been shipped from the Pacific coast to New York City and reshipped to the Experiment Station in California, (2) weekly samples of eggs shipped to the laboratory directly from the packing plant in Petaluma."

S. L. Parker's conclusions would seem to differ in some measure from those of H. L. Kempster (1930) who makes the following statements:

"The yolk color in eggs is a variable factor. That fact has been known for a number of years, but its economic importance has only recently been recognized. These variations in yolk occur at different periods of the year and even the same hen will produce eggs with different degrees of yolk color. It has been observed that feed has considerable influence on the color of yolks laid by hens."

From the foregoing conclusions of the various investigators quoted herein the reader can see that feed is the chief controlling factor in the coloring of egg yolks.

BIOLOGICAL RELATION BETWEEN YELLOW PIGMENTATION AND VITAMIN A

It is evident from the foregoing pages that xanthophyll has been generally accepted as the chief color pigment in egg yolks. It is also clear that feed has considerable influence upon the color of egg yolks. Scientists and investigators in many cases have associated vitamin A with the yellow pigments of plants and foods. If this were found to be true it would be reasonable to expect that egg yolks containing excessive amounts of yellow pigment would also contain large amounts of vitamin A. Investigators have made various attempts to identify vitamin A with yellow pigments.

Palmer and Kempster (1919) performed an unusual piece of experimental work in an attempt to show the relation of carotinoids to growth, fecundity, and reproduction.

These men state that their problem was primarily one of selecting ration entirely devoid of carotinoids, particularly xanthophyll, but otherwise presumably adequate for normal growth.

Palmer and Kempster performed three experiments on this particular piece of work, and as the third experiment is of interest in this study the procedure is quoted as follows:

"Sixty newly hatched White Leghorn chicks were immediately placed on the carotinoid-free ration about April 15,

1918. This ration consisted of white corn for scratch feed, a mash of white corn meal, and white corn, bran, with skim milk and bone meal and paper pulp ad libitum. Rice flour was used in the mash for a brief period but was discontinued following the discovery that it contained traces of carotinoids. The chicks were placed in a pen on a board floor covered with dirt, and had the run of a small dirt yard, absolutely free from vegetation. The chicks soon learned to eat paper which was frequently supplied merely by throwing newspaper into the pen.

Summary

1. White Leghorn chicks have been raised from hatching to maturity on ration containing the merest traces, if not entirely devoid, of carotinoids. The full grown hens have shown normal fecundity, and no abnormalties with respect to fertility of the carotinoid-free eggs have developed. A second generation of chicks, free from carotinoids at hatching, has been started with every evidence of being normal except the absence of the natural yellow pigmentation of the skin.

2. It is concluded that the natural yellow pigment of fowls which is derived from the xanthophyll of the food bears no important relation to growth or to function of fecundity and reproduction, at least for one generation.

3. When yolks of hen eggs are devoid of their natural xanthophyll pigmentation, as a result of the absence of

carotinoids from food, a small residual pigmentation of the raw egg yolks is observed. This pigmentation is readily extracted by acetone but attempts to identify it with bilirubin or other known yellow animal coloring matters have so far been unsuccessful."

Again we have L. S. Palmer in an article in "Science" (1919) stating:

"My attention has been called to Steenbock's interesting observation, in "Science" Oct. 10, 1919, that yellow corn and colored roots, such as carrots and sweet potatoes, are richer in fat-soluble vitamin than white corn and pigmentless roots and tubers. A number of other instances are noted in which fat-soluble vitamin and carotinoid pigments occurr simultaneously. The fact that these relations have led Steenbock to the provisional assumption that the fat-soluble vitamin is one of the carotinoid pigments has prompted me to call attention to a number of cases where this relation apparently breaks down.

Drummond has recently tested the possibility of carotin being the fat-soluble vitamin by feeding both crude and crystalline preparation of the pigment to rats, although the question may be raised as to the logic of testing the relation to fat-soluble vitamin of a substance of which is not natural to the body of the animal upon which the test is performed. Carotin is not found in the body of the rat.

The writer has recently reported that it is possible to raise a flock of chickens from hatching to maturity on a diet free, or at most containing the merest traces, of carotinoids. Not only did the mature hens lay eggs whose yolks were free from carotinoids, but a second generation of carotinoid-free chicks were hatched from them. Only one of two possible conclusions can be drawn from this experiment. Either the fat-soluble vitamin and the yellow plant pigments are not related physiologically or the fat-soluble vitamin requirements of fowls differ from that of mammals. The diets which were used for the successful growth of chicknes contained abundance of fat-soluble vitamin, however, in the form of carotinoid-free pork liver.

Another interesting case of negative relation between carotinoids and fat-soluble vitamin is seen in the fact that a number of species of animals, such as sheep, swine, dogs, cats, rats, rabbits, and guinea pigs, are free from carotinoids in blood and adipose tissues, and nerve cells. The milk fat of the mammals of these species is also colorless. How is one to make the successful raising of young on carotinoid-free milk coincide with the assumption that fat-soluble vitamin is one of the yellow plant pigments?

Still another instance of negative relation between carotinoids and fat-soluble vitamin is seen in the case of certain vegetable oils, like cottonseed oil. Fresh cottonseed oil after being purified from resinous material, has a beautiful golden yellow color and is rich in carotinoids.

It should also contain abundance of fat-soluble vitamin to be in keeping with Steenbock's assumption. Apparently this is not the case since both bleached and unbleached cottonseed oil has been found to be free from vitamin. The oil from yellow corn similarly, should contain the vitamin, but the same investigation has reported failure to obtain growth with diets containing the commercial unbleached corn oil.

It is thus possible to cite a number of instances where the probable relation between carotinoids and fat-soluble vitamin breaks down. No doubt others could be found. The writer regards the instances of a simultaneous occurrence of fat-soluble vitamin and plant carotinoids as fortuitous. The similarity of certain of the properties of the two kinds of material admittedly offers a working basis for the ultimate isolation of the fat-soluble vitamin and research in this direction offers many facinating possibilities. The relation between the vitamin and color in the case of corn may be a genetic one, in which case it should be possible to transfer the vitamin to white corn. Further attempts, however, to establish an identity of the vitamin with one of the carotinoid pigments is not likely to lead to profitable results."

Willimott and Moore (1926) in their work with nettle leaves state that: "Pure crystalline xanthophyll prepared from nettle leaves cannot be identical with vitamin A."

Stephenson (1920) removed the carotene of butter fat by absorption with charcoal without affecting its content of vitamin A.

In an article published by H. L. Kempster (1930), in the U. S. Egg and Poultry Magazine, Dec. 1930, he states:

"There may be a close relationship between the degree of yellow color in the yolk and the vitamin A content of the egg. The feeds rich in xanthophyll are also rich in vitamin A and one can rest assured that eggs with highly pigmented yolks possess an abundance of vitamin A. Under normal conditions, the degree of yellow color in the egg yolk is a fair index of the vitamin A content of the egg, and feeding tests have demonstrated that pale yolked eggs when fed to chicks will not produce as satisfactory growth as will eggs with highly colored yolks. However, it is possible to produce pale yolked eggs rich in vitamin A provided a source of vitamin A not associated with xanthophyll is employed. It is thus seen that the consumer demanding a pale yolked egg, is running the risk of securing eggs of lower nutritional value, due to the possibility that they may have a low vitamin A content."

Sherman and Smith (1930) in their text, "The Vitamins", give the following explanation concerning the relation of plant pigments to vitamin A:

"Moore (1929) reported preliminary experiments suggesting the possibility that carotin, while not identical with vitamin A, may be the precursor from which the vitamin is formed in vivo. These experiments consisted briefly in feeding rats depleted of their vitamin A reserves graded doses of carotene, from 0.0001 to 0.75 mg. daily, and after 36 days killing the rats and examing their liver fats for carotin and for vitamin A by colorimetric and spectrographic methods.

It was noted first that although the largest amount of carotin was sufficient to color the whole rat intensely yellow if absorbed unchanged, the body fat was found to be as colorless as that of the controls and the liver to show only feeble yellow pigmentation. On the other hand, an intense blue color was given with antimony trichloride and on spectrographic examination the absorption band was found to be at 610 to 630 uu, characteristic of vitamin A, rather than 590 uu characteristic of carotin. A comparison of the blue and yellow units showed that in cases where the amount of carotin fed was below the minimal physiological dosage for vitamin A the blue units did not rise above those of negative controls, while in the case of all three rats receiving carotin in excess of the minimal dose, higher values in the blue units were noted, with no corresponding changes in the yellow such as would have been the case if carotin had been stored unchanged.

In the detailed report of this important development concerning the relationship of carotin to vitamin A Moore

(1930) has cleared up many doubtful points raised by various investigators and has presented apparently convincing evidence that the carotin in plant materials is more or less completely converted into vitamin A in the animal body. Some features of the conversion are summarized by Moore as follows:

Carotin	Vitamin A
Synthesized in plant	Stored in animal
Intnesely yellow	Almost colorless
328 uu absorp. band absent	328 uu absorp. band developed
Greenish blue $SbCl_3$ reaction at 590 uu	Vivid blue $SbCl_3$ reaction at 610 to 630 uu

Evidence along similar lines has also been reported by Wolff, Overhoff, and Van Eckelen (1930) who state that they have demonstrated the synthesis of vitamin A in the livers of rabbits following the administration of carotin. They have attempted to distinguish between carotin, xanthophyll, and vitamin A in food material by means of (1) spectrographic analysis of color produced by the antimony trichloride reaction, (2) the intensity of color of the materials, and (3) separation of carotin from vitamin A and xanthophyll by shaking a petroleum ether solution of the material with 90 per cent alcohol, the carotin remaining in the petroleum ether and the xanthophyll and vitamin A being taken up by the alcohol. Of the materials tested, all gave positive results by the methods followed for carotin, xanthophyll, and vitamin A; butter for carotin and vitamin A; and extract of carrots, green cabbage and spinach for carotin only.

Meanwhile continued confirmation of the ability of purified carotin to function as vitamin A was reported from various laboratories. Kawakami and Kimm (1930) reported that carotin purified by repeated crystallization from carbon disulfide and ethyl alcohol cured xerophthalmia in rats in doses of between 0.03 and 0.05 milligram daily. Javillier and Emerque (1930) found that samples of spinach and carotin which had been preserved for 40 years in an atmosphere of hydrogen in a sealed tube in diffuse light was effective as a source of vitamin A in a dosage of 0.01 milligram per 100 grams weight of rat.

Green and Mellanby (1930) reported complete protection of rats against infection with purified carotin, m. p. 170 degrees C., in doses of 0.02 milligram daily and stated their belief that carotin itself is the specific substance responsible for the vitamin A activity of green vegetables, carrots, and butter, and possibly egg yolk."

N. S. Capper (1930) further corroborates the foregoing report on vitamin A activity in carotin in his note on carotin as a precursor of vitamin A in the fowl. The note is stated as follows:

"Recent work by Moore and others (see for example, Moore Biochem. Jour., V-24, p. 692; 1930) has left little doubt that in the rat, carotene can function as a precursor of vitamin A. Experiments which I have just carried out have indicated that the same holds true in the fowl also. White Leghorn chickens, six weeks old, were given a synthetic diet free from vitamin A to which irradiated ergosterol was added to supply the vitamin D. Control birds receiving this diet succumed in about six weeks, their livers giving negative tests for vitamin A either by the antimony chloride test or by the adsorption spectrum. To other birds, after a preliminary period of vitamin A depletion, daily doses of carotene (1 mgm.) or cod-liver oil (10 mgm.) were given, with the result that complete cures were affected and satisfactory growth restored. The livers of all these birds, receiving either carotene of concentrates, gave positive tests for vitamin A, the oils yielding an intense blue color with antimony chloride and showing strong adsorption band in the region of 328 uu.

As well as indicating that the ability to transmute carotene into vitamin may hold fairly general throughout the animal kingdom, the experiment would seem to afford an explanation of the results of Palmer and Kempster (Jour. Biol. Chem., V-39, p. 331, 1919) who found that while xanthophyll fed to fowls reared on a diet free from carotinoids quickly increased their pigmentation, carotene had no such effect. The transmutation of carotene into the colorless vitamin A would account for this very simply."

The object of the foregoing literature review has been to obtain a historical background of the factors having a bearing upon the question of the comparative values of the vitamin A content of light and dark egg yolks. From this review it is evident; first, that the egg has long been considered as an important source of vitamin A; second, that food is the chief factor of control in egg yolk color; third, that the amount of vitamin A present in the food has considerable influence upon the amount stored in the egg; and fourth, that while the yellow plant pigments and vitamin A do not appear to be identical, it is generally agreed that carotin may act as a precursor of vitamin A in the animal body, and that there is a definite relationship between the vitamin A content of foods and yellow pigmentation. Just what this relationship is between xanthophyll, the yellow pigment of egg yolks, and vitamin A has not been determined.

EXPERIMENTAL WORK

On November 16, 1931 an experiment was started for the purpose of testing light and dark egg yolks for their comparative vitamin A content. The test was made by feeding young albino rats on a vitamin A-free ration to which different levels of fresh egg yolk had been added as the sole source of vitamin A. The eggs used in this experiment were obtained from an experiment at the college poultry plant, designed to show the effect of greens on yolk color and were not considered ideal for use in determining the relation between vitamin A content and yellow pigmentation of egg yolks. These eggs were produced by White Leghorn pullets fed on the following rations:

Mash

320	pounds	mill-run
400	"	ground wheat
400	"	ground corn (yellow)
200	"	ground oats
100	"	ground barley
200	"	meat meal
100	"	fish meal
100	"	dried milk
80	"	alfalfa meal
50	"	bone meal
20	"	O. P. oil meal

20 pounds oyster-shell flour

10 " dairy salt

One per cent cod-liver oil

Scratch Feed

800 pounds wheat

800 " cracked corn (yellow)

200 " oats (gray)

The hens producing the eggs with light yolks received the above rations with the exception of the alfalfa meal. They were confined and had access to wire runs. The hens producing the eggs with dark yolks received the above rations with liberal amounts of fresh green kale and also had access to lots planted to alfalfa.

Procedure

As far as conditions would permit the methods followed in this experiment were those suggested by Sherman. Young albino rats at the age of 3 to 4 weeks were placed on a basal ration found experimentally to be free from vitamin A and kept until they became constant in weight. The basal ration consisted of the following materials and percentages:

Casein (alcohol extracted) ..	18 per cent
Salt mixture	4 " "
Yeast	10 " "
Starch	67 " "

At the close of the depletion period the rats were placed in separate cages and fed the basal ration containing different levels of fresh egg yolk.

Results

Three groups of rats were fed on the experimental diet. The first group received the basal ration plus 0.3% fresh egg yolk. The two remaining groups received the basal ration plus 0.5% fresh egg yolk. In each group the animals were divided so that one half received ration containing light yolks, and the other half received ration containing dark yolks. The results are given in tables 1 and 2. As can be noted from the tables the ration containing the light yolks produced weekly gains which were slightly higher than that containing the dark yolks, although the difference is not significant.

Discussion

In this experiment a colony of animals was used which had not previously been standardized for vitamin A work. It was soon found that the results with this colony were not strictly comparable with those obtained in some other laboratories. This was probably due to the fact that the animals used were of a larger strain than those used by Sherman and other investigators in vitamin A work. On the other hand, it was found that the rats used in the experiment were depleted in periods comparable to those reported by Sherman. The cessation of growth usually occurred in 30 to 35 days, indicating that the basal ration was free from any significant amounts of vitamin A.

In general, it was the aim to feed the rats on the experimental diet for a period of five weeks, but in many cases this was not possible due to the irregular temperature conditions in the laboratory which were conducive to lung troubles that interfered with normal food intakes. However, animals were not discarded in any case unless there was an immediate and almost complete failure of appetite.

Because of the conditions just mentioned it was difficult, if not impossible, to interpret the results in terms of Sherman's units. It does, however, appear that the results indicate that the eggs used in this experiment contained as much or probably more vitamin A than is indicated by the estimate of 45 to 60 units given in Sherman's text, "The Vitamins." At any rate, the data probably indicates that where cod-liver oil or some other material containing liberal amounts of vitamin A is fed, it is possible to produce reasonably light colored egg yolks that are rich in vitamin A.

Conclusion

While the results in this experiment show that the ration containing the light egg yolks produced slightly higher average weekly gains than that containing dark egg yolks, the writer does not feel that a significant difference between the vitamin A content of light and dark egg yolks as tested under the conditions described herein has been demonstrated.

Table 1.

Group 1.__Data on growth and food consumption of rats fed on ration containing 0.3% fresh egg yolk. Letters (L) and (D) indicate light and dark yolks respectively.

Rat Number	Deplet-ion Period Days	Wt. at End of Deplet-ion Grams	Period On egg Yolks Weeks	Aver. Weekly Food Intake Grams	Final Weight Grams	Aver. Weekly Gain Grams
19-(L)-	31	120	4	85	143	5.5
21-(D)-	31	129	5	62	175	9.2
18-(L)-	31	166	2	95	181	11.5
24-(D)-	31	168	2	95	189	10.5
27-(L)-	31	155	2	85	176	10.5
20-(D)-	31	147	2	92	185	18
26-(D)-	31	105	5	82	144	7.8

The total average weekly gain for (L) 9.16

The total average weekly gain for (D) 11.3

Table 2.

Group 2.__Data on growth and food consumption of rats fed on ration containing 0.5% fresh egg yolk. Letters (L) and (D) indicate light and dark yolks respectively.

Rat Number	Depletion Period Days	Wt. at End of Depletion Grams	Period On egg Yolks Weeks	Aver. Weekly Food Intake Grams	Final Weight Grams	Aver. Weekly Gain Grams
18-(L)-	30	145	5	107	202	11.4
21-(D)-	30	134	5	108	150	3.2
20-(L)-	31	170	5	125	214	8.8
26-(D)-	31	133	5	103	161	5.6
32-(L)-	34	181	5	153	227	9.2
27-(D)-	30	172	4	105	180	2.4
33-(L)-	30	172	5	132	250	15.6
19-(D)-	34	124	5	106	150	5.2

The total average weekly gain for (L) 11.15

The total average weekly gain for (D) 4.1

Table 2.

Group 3.__Data on growth and food consumption of rats fed on ration containing 0.5% fresh egg yolk. Letters (L) and (D) indicate light and dark yolks respectively.

Rat Number	Depletion Period Days	Wt. at End of Depletion Grams	Period On egg Yolks Weeks	Aver. Weekly Food Intake Grams	Final Weight Grams	Aver. Weekly Gain Grams
22-(L)-	34	105	4	106	130	6.25
23-(D)-	34	105	4	106	128	5.6
16-(L)-	34	131	5	106	169	7.8
31-(D)-	34	133	4	122	163	7.5
24-(L)-	34	107	4	103	142	8.75
28-(D)-	37	109	5	106	124	3
34-(L)-	34	103	4	107	124	5.25
30-(D)-	34	106	5	107	130	4.8
29-(L)-	34	98	4	105	114	4
36-(D)-	34	108	5	107	140	6.4
17-(L)-	37	158	5	106	188	6

The total average weekly gain for (L) ... 6.35

The total average weekly gain for (D) ... 5.4

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