THE INFLUENCE OF CAROTENE FEEDING ON THE VITAMIN A CONTENT OF THE YOLKS OF EGGS AND OF THE LIVERS OF CHICKENS

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by

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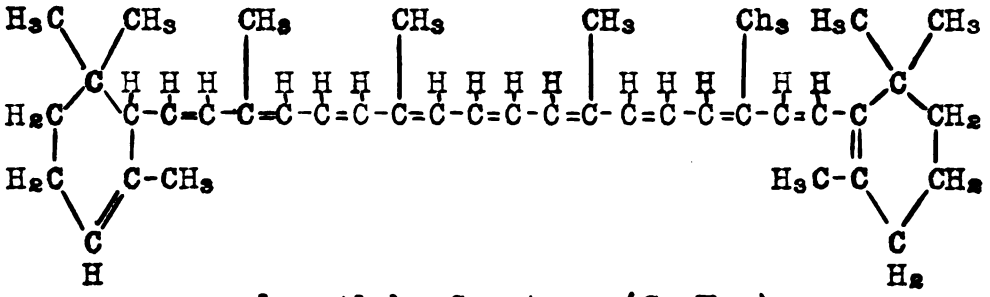
Introduction

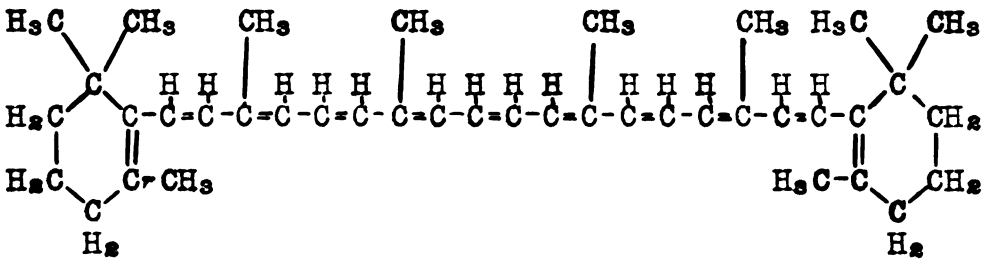
During the past decade, a great deal of emphasis has been placed on the importance of vitamin A in the nutrition of man and animals. In recent years this trend has been especially strong in poultry nutrition.

Fraps, Treichler and associates (1, 2) working at the Texas experiment station, have contributed a great deal in the study of the vitamin A content of foods and feeds in conjunction with a rather comprehensive study of the vitamin A requirements of poultry for various physiological functions. Their work and that of other investigators (3,4,5) in this field has shown in a general way what the vitamin A requirements in poultry are and that these requirements may be met by the feeding of vitamin A containing concentrates such as cod liver oil or by the use of feeds containing certain pigments which are convertible to vitamin A in the animal organism.

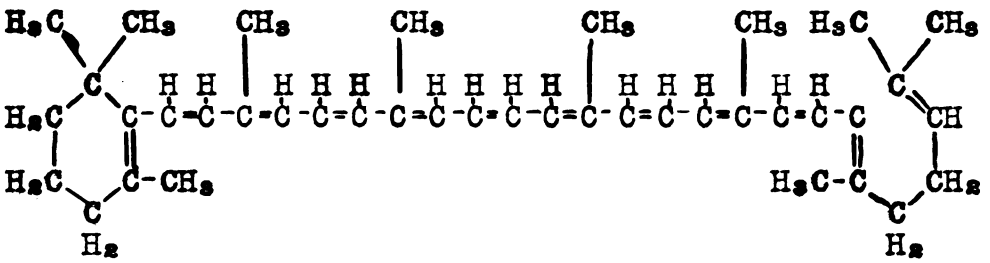
Carotene has been known for several years to take the place of vitamin A in the diet. At the present time three different forms of carotene are recognized, each of which is convertible to vitamin A. As can be seen from the structural formulae given below, the alpha form gives rise to one molecule of vitamin A, the beta form, two, and the gamma form one.



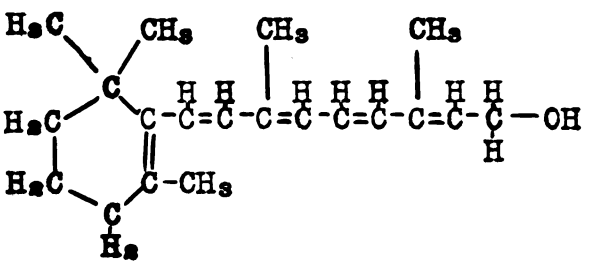
1. Alpha Carotene ($C_{40}H_{56}$)



2. Beta Carotene ($C_{40}H_{56}$)

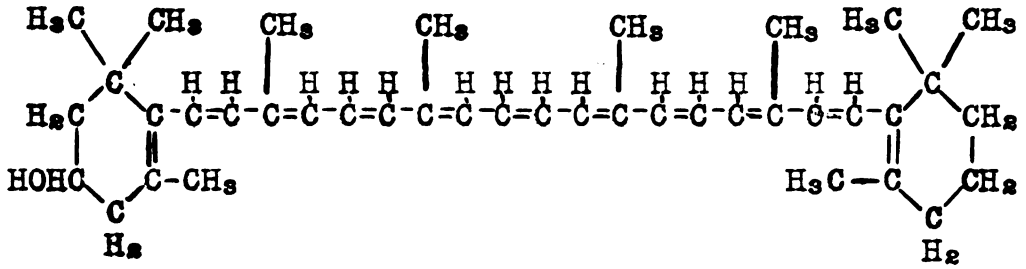


3. Gamma Carotene ($C_{40}H_{56}$)



4. Vitamin A ($C_{20}H_{30}O$)

Recently (6) another pigment has been isolated from yellow corn, which from its structural configuration is capable of forming vitamin A, molecule for molecule.



5. Kryptoxanthin ($C_{40}H_{56}O$)

This pigment called kryptoxanthin, the formula for which is given above, apparently lies intermediate between beta carotene and zeaxanthin, the latter having no vitamin A value at all. Whether there are still other pigments capable of forming vitamin A is not definitely known. It is, however, quite likely that others may be found.

While it has been shown that certain pigments from various plant materials are an important source of vitamin A, relatively little has been done in the study of the value and effectiveness of feeding carotene concentrates. This then becomes the object of the present study, the criteria being the vitamin A content of the eggs and livers of chickens.

Experimental

In this work the problem involved was to make a study of the vitamin A content of the yolks of the eggs and of the livers of hens that had been fed a carotene supplement in conjunction with an adequate ration. In determining the amount of vitamin A in the egg yolk, the Sherman-Todhunter single feeding method was used, whereas in the case of the livers, the Carr-Price antimony trichloride reaction was employed.

Management of the chickens

The birds used were from the Michigan State College "White Leghorn" flock. The flock was range raised on pasture and the birds used were pullets, six months old at the beginning of this experiment. The two general rations used in feeding the chicken had the following composition:

Corn Ration 6

Ground yellow corn(Mich,)	18%
Bran (Mich.)	20%
Ground oats	13%
Middlings	20%
Meat scrap(50% protein)	10%
Dried skim milk	5%
White fish meal	3%
Alfalfa meal (Mich.)	8%
Sodium chloride	1%
Sardine oil (Source of vitamin D)	2%

Grain

Whole corn ad lib (in hoppers)

Whole oats ad lib (in hoppers)

40 lbs. of wheat per month scattered in the litter

Ration 10 was the same as ration 6, except that barley was used in the place of corn in the dry mash and in the whole grain supplement. Rations 4 and 8 were supplemented with carotene of an amount calculated to supply each bird with the equivalent of 11,200 U.S.P.X.(1934) vitamin A units per month. The carotene was obtained from the S.M.A. corporation in the form of a solution in oil and was incorporated in the dry mash. The monthly allowance of carotene was fed in such a way that it would be consumed in the course of a week. This practice seemed desirable because of the uncertainty of the stability of the carotene in the feed mixture. To check on the keeping quality of the mixture a determination was made of the vitamin A equivalence of the ration after a weeks storage. The results of these tests indicated that there was no appreciable loss.

Analysis of Egg Yolks

The technique used in determining the amount of vitamin A in the egg yolks was the Sherman-Todhunter single feeding method (7). This method was selected because of several appreciable advantages which it possesses over the official biological method. First of all there is a considerable saving of time, because

a single feeding replaces the daily feeding over a period of from six to eight weeks. Then there is the elimination of the danger of the loss of vitamin potency incidental to the storage of the material to be tested. Finally the number of failures is likely to be reduced because of the fact that a relatively large amount of the material is fed at one time. This brings about a more uniform response to the vitamin A supplement than would be the case with very small daily doses.

The animals selected were white and striped rats of the chemistry department colony. They were at least 21 days old and weighed between 35 and 45 grams. The usual care was observed in having a uniform distribution of the rats with regard to size and sex in the various series. The animals were placed on a basal ration consisting of the following:

Dextrin.....	60%
Casein(Vitamin free).....	18%
Yeast	7%
Yeast(irradiated).....	1%
Salt mixture.....	4%
Crisco	10%

The animals were weighed weekly for three weeks and after that every other day until the weights became constant. This constancy in weight marked the end of the depletion period. At this stage

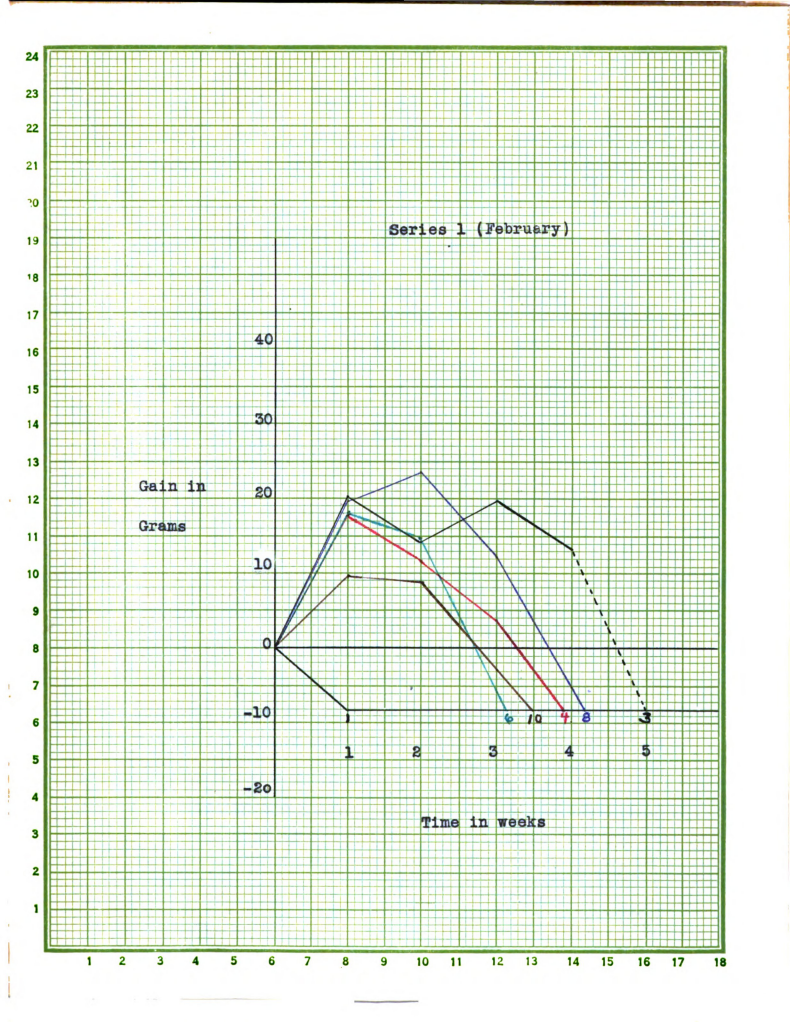
the rats were put into separate cages and fed the various supplements.

The arrangement of the groups was as follows:

Group 1	Controls
Group 3	60 U.S.P.X.(1934)A units
Group 4	1 gram of yolk 4
Group 6	1 gram of yolk 6
Group 8	1 gram of yolk 8
Group 10	1 gram of yolk 10

The supplement was placed in a china cup and was consumed usually in less than a day. After the feeding of the supplements the rats were weighed at weekly intervals. The average gains and losses in weight were plotted against time. Using the average loss in weight of the control animals for the first week following the depletion period as a base line, the areas under the growth curve were measured by the use of a planimeter and the amount of vitamin A calculated by comparing the areas with that given by the animals receiving the 60 U.S.P.X. (1934) vitamin A units of standard reference oil.

The curves for the first series only are given to illustrate the manner of obtaining the areas mentioned above. In the subsequent series were obtained in a similar manner, but the results are presented in tabular form.


Series 1 (February)
Gain in
Grams
40
30
20
10
0
-10
-20
1
2
3
4
5
Time in weeks

Series 1 (February)

Group	Supplement	Area in sq. in.	Calculated vitamin A units per gram
3	60 U.S.P.X. 1934 vit. A units	6.16	
4	1 gm. of yolk 4	3.53	34.38
6	1 gm. of yolk 6	3.15	30.68
8	1 gm. of yolk 8	5.04	49.08
10	1 gm. of yolk 10	2.40	23.38

Series 2 (April)

Group	Supplement	Area in sq. in.	Calculated vitamin A units per gram
3	60 U.S.P.X. 1934 vit. A units	5.45	
4	1 gm. of yolk 4	2.21	24.33
6	1 gm. of yolk 6	1.13	12.44
8	1 gm of yolk 8	1.42	15.63
10	1 gm. of yolk 10	.25	2.75

Series 3 (May)

Group	Supplement	Area in sq. in.	Calculated Vitamin A units per gram
3	60 U.S.P.X. 1934 Vit A units	3.44	
4	1 gm. of yolk 4	3.57	62.26
6	1 gm. of yolk 6	1.90	33.12
8	1 gm. of yolk 8	2.81	49.01
10	1 gm. of yolk 10	.79	13.78

An examination of the above tables indicates that the feeding of carotene definitely and appreciably increases the vitamin A potency of the eggs. This was true in the case of the corn ration as well as the barley ration. In general the eggs from the corn groups were somewhat richer in vitamin A than those from the barley groups. This would be expected because yellow corn is a better source of the vitamin A producing pigments than barley. The inconsistencies where they occur are due in part to the limitations of the biological methods of assay when a relatively small number of rats is used and in part to the fact that a small number of eggs was used in the study. Undoubtedly the data would have been more satisfactory if more complete assays with a greater number of eggs had been made.

Analysis of Livers

In determining the vitamin A potency of the livers it was decided to use the chemical method because of its rather general adoption during the past few years. The method used was that accredited to Carr and Price and involves the measurement of the blue color produced when vitamin A is treated with antimony tri-chloride.

The livers were obtained from the same group of hens from which the eggs were derived. In those cases in which the livers could not be analyzed soon after the hens were killed, the livers were stored in an electric refrigerator. The period of storage in no case exceeded two or three days so that there was little opportunity for loss in vitamin A potency.

In determining the vitamin A content of the livers, the Guilbert and Hart (8) procedure was used. This involved the following steps: The livers were weighed to the nearest tenth of a gram and 10 grams of the liver used for each determination. 20 to 40 c.c. of 10 percent potassium hydroxide was added to the liver in a 300 c.c. erlenmeyer flask and the mixture heated on a boiling water bath until the liver went into solution. The contents of the flask was then cooled and removed to a sepratory funnel. After the addition of 20 to 30 c.c. of ethyl alcohol,

two extractions were made with ethyl ether. The alkaline layer was drawn off and the ether solution washed free from alkali with distilled water. The ether solution was then transferred to a flask containing 5 to 10 grams of anhydrous sodium sulphate and the flask shaken vigorously to remove any water that might be present. The ether solution was decanted off and the sodium sulphate residue washed twice with anhydrous ether. The ether was distilled off under reduced pressure at 50 ° C. and the residue dissolved in 10 c.c. of anhydrous chloroform.

For the determination of vitamin A, 1 c.c. of the chloroform solution obtained by the above method was treated with 2 c.c. of a saturated solution of antimony tri-chloride in anhydrous chloroform. The blue color which developed was measured by the use of the Lovibond tintometer. The number of Lovibond blue units were calculated by the use of the following formula:

$$\text{Blue units per gram} = \frac{B\ V\ V_1}{W\ N}$$

Where:

B = Blue units on the tintometer

V = Number of c.c. in the cell

V_1 = Total volume of chloroform solution

W = Grams of liver sample

N. = Number of c.c. of chloroform solution used

The values are given in terms of Lovibond blue units as well as U.S.P.X. 1934 vitamin A units, it being assumed that 1 Lovibond blue unit is equivalent to 2 1/2 U.S.P.X. 1934 vitamin A units. This conversion factor was determined by Mr. Moore of the dairy department in an extensive comparison between the biological and chemical methods.

Chicken number	Pen number	Weight of liver in grams	Lovibond blue units per liver	Blue units per gram	U.S.P.X 1934 vit A units per gram
482	4	32.1	818.00	25.50	63.75
467	4	27.3	640.00	23.45	58.63
421	4	29.8	809.00	27.15	67.88
Ave.			755.60	23.36	63.40
688	6	29.2	76.35	2.63	6.58
623	6	27.4	131.50	4.80	12.00
Ave.			103.95	3.72	9.30
845	8	26.2	153.10	5.85	14.63
847	8	39.0	269.00	6.90	17.25
Ave.			211.05	6.38	15.95
1096	10	26.5	79.50	3.00	7.50
1010	10	20.8	30.00	1.50	3.75
Ave.			54.75	2.25	5.63

The data obtained on the livers indicates that a considerable storage of vitamin A is effected by supplementing poultry rations with a carotene concentrate. In the case of the corn ration the increase in vitamin A potency appeared to be considerably greater than was observed in the case of the barley group. In view of the limited scope of the experiment no explanation for this difference can be offered.

A determination of the yellow units was made, but the results were rather inconsistent and therefore are not included. The results however, do suggest that very little carotene is stored in the liver.

Conclusions

1. Feeding of carotene concentrates, definitely increases the vitamin A potency of yolks of eggs.

2. A considerable storage of vitamin A in the livers results from feeding a carotene concentrate. Apparently very little carotene is stored as such in the liver.

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