THE FAT-SOLUBLE VITAMIN CONTENT OF HEN'S EGG YOLK AS AFFECTED BY THE RATION AND MANAGEMENT OF THE LAYERS.*

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The value of a food product as a source of vitamins in the human dietary is largely determined by other inherent factors. Of two or more substances having a similar vitamin content the one which is the most available at a reasonable price to the greatest number of people, and at the same time is a product which is palatable, easily digestible, and possesses other food values, is the one which will finally prove the more important. In respect to these considerations hen's eggs probably take first place when compared with other natural food products as a source of vitamins A and D.

That egg yolk is a rich source of vitamin A was pointed out by McCollum and Davis (1) and by Osborne and Mendel (2) in their early work on this vitamin. More recently Murphy and Jones (3) working on the vitamin A content of fresh eggs found that about 0.25 gm. of whole egg was required daily to cure rats of xerophthalmia, and 0.5 to 0.75 gm. daily to restore normal weight.

Working with the yolk of fresh eggs, Hess (4) found that 0.25 gm. daily proved sufficient to protect rats from rickets. Similar results were obtained by Casparis, Shipley, and Kramer (5). Work at the Ohio station (6) also demonstrated that egg yolk possessed distinct antirachitic properties in preventing leg weakness in young growing chicks. Hart, Steenbock, and coworkers (7) in studying the effect of ultra-violet light on production, hatchability, and fertility of the egg, found that egg yolk from irradiated

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hens was approximately 10 times as potent in antirachitic properties as egg yolk from non-irradiated hens. Similar observations were reported by Hughes, Payne, and Latshaw (8).

The fact that milk very often is deficient in iron and the antirachitic vitamin has led many pediatricians to incorporate fresh egg yolk in the diet of the infant. Hess (4) reported that for 6 months about 50 babies were fed with excellent results a mixture consisting of milk (24 ounces), barley water (12 ounces), sugar (¾ ounce), and 1 egg yolk. He further states that egg yolk is well tolerated by babies and that it "possesses marked antirachitic properties . . . . . far more than any other natural food product."

The increasing use of eggs in the human dietary, especially for infants and invalids, makes it imperative that we have further information as to what extent the vitamin A and D content of hen’s egg may be affected by the ration and management of the respective laying flocks.

EXPERIMENTAL.

For this study eggs were gathered from several groups of White Leghorn hens which had been under the same practical management and had received the same basal ration supplemented in various ways for 9 months. The basal ration fed these hens was a dry mash composed of ground yellow corn 30 parts, ground wheat 20, ground oats 20, wheat middlings 10, wheat bran 10, meat scraps 10, and oyster shells and water ad libitum. No scratch grain was fed. Pen 12 was confined indoors and received the basal mash fortified with 2 parts cod liver oil. Pen 13 received the basal ration only and was confined indoors. Likewise Pen 15 was kept indoors and fed the basal mash plus chopped alfalfa hay ad libitum. Pen 16 received the same mash but had access to a blue-grass range. The only sunlight available to Pens 12, 13, and 15 was that which filtered through the closed windows on the southern exposure.

Vitamin A.

For the determination of vitamin A, 12 litters, of six each, of young healthy stock rats, 23 to 24 days of age and weighing from 45 to 60 gm., were confined in our standard laboratory cages and
fed a basal ration devoid of vitamin A. The ration had the following composition: casein 18 parts, starch 71, Crisco 5, salt mixture 4, and agar 2. Vitamin B was supplied by 0.25 gm. of a commercial yeast fed separately each day. All animals were irradiated 10 minutes, every other day, at a distance of 30 inches with light from a quartz mercury vapor lamp.

The casein was prepared by extracting the commercial product repeatedly with alcohol and ether for 5 days and then drying at 110–120°C. for 24 hours. Air was passed through the Crisco for 24 hours at 100°C. to insure the destruction of any vitamin A which may have been present. The starch was not specially treated, since it was not found to contain a detectable quantity of this vitamin. The salt mixture employed was that described by McCollum (9) with the addition of 0.25 per cent potassium iodide.

Preliminary experiments with young rats of our stock colony showed that the vitamin A reserves of their bodies were depleted between the 4th and 5th week. Accordingly, at the close of the 5th week the rats from the various litters were confined in individual cages and distributed so that no two animals from the same litter received the same quantity of any particular kind of egg yolk. They were continued on the same basal ration with 0.25 gm. of yeast fed separately each day and irradiated as before. Varying amounts of egg yolk diluted 1:1 from the four different lots of hens (designated as Egg Yolk 12, 13, 15, and 16 to correspond with the individual pen treatment) were fed separately each day for 7 weeks.

The egg yolks were prepared by carefully separating the yolk from the white of 2 dozen eggs and then adding an equal weight of distilled water and mixing thoroughly with an egg beater. The diluted yolks were then transferred to stoppered flasks and stored at 34°F. Fresh quantities were prepared every 2 weeks.

The results are recorded in Charts I and II. Chart I shows how young rats fed the vitamin A-deficient ration rapidly declined in weight after the 5th week with the development of xerophthalmia, and respiratory troubles between the 6th and 7th week. The feeding of increasing amounts of the diluted yolks from Pens 13 and 15 caused a progressive delay in the development of xerophthalmia with a prolongation of life and increased resumption of growth.
Chart I.

Feeding increasing amounts of Egg Yolk 13 & 15 diluted (1:1) daily

- 0.1 c.c. Egg Yolk 13
- 0.1 c.c. Egg Yolk 15
- 0.2 c.c. Egg Yolk 13
- 0.2 c.c. Egg Yolk 15
- 0.4 c.c. Egg Yolk 13
- 0.4 c.c. Egg Yolk 15
- 0.8 c.c. Egg Yolk 13
- 0.8 c.c. Egg Yolk 15
- 1.0 c.c. Egg Yolk 13
- 1.0 c.c. Egg Yolk 15

+ Death

- Start of Egg Yolk feeding
- Ophthalmia
- Respiratory trouble

4 weeks
Chart II. Feeding increasing amounts of Egg Yolk 12 & 16 diluted (1:1) daily.

- 0.1 c.c. Egg Yolk 12
- 0.1 c.c. Egg Yolk 16

- 0.2 c.c. Egg Yolk 12
- 0.2 c.c. Egg Yolk 16

- Start of Egg Yolk feeding
- Respiratory trouble
- Death

- 0.4 c.c. Egg Yolk 12
- 0.4 c.c. Egg Yolk 16

- 0.6 c.c. Egg Yolk 12
- 0.6 c.c. Egg Yolk 16

- 1.0 c.c. Egg Yolk 12
- 1.0 c.c. Egg Yolk 16

CHART II.
The comparative growth curves show no substantial difference in the vitamin A potency of the egg yolks from Pens 13 and 15, although the yolks from Pen 15 appear to be the better of the two in that apparently normal resumption of growth and behavior were obtained when 1.0 cc. of the diluted yolk material was fed daily, while an equivalent quantity of yolk from the basal pen (No. 13) caused slower gains with evidence of respiratory troubles.

Chart II shows the effect of feeding increasing amounts of diluted egg yolks from the cod liver oil (No. 12) and blue-grass range (No. 16) pens. Both of these yolk materials were considerably more potent in vitamin A than those from Pens 13 and 15. In comparing the growth curves of Charts I and II it is quite evident that eggs from Pens 12 and 16 were approximately 5 times more potent than yolks from Pens 13 and 15. Only 0.2 cc. of the diluted yolk from Pens 12 and 16 was required to produce apparently normal behavior in contrast to 1.0 cc. of egg yolk from Pen 15 and more than 1.0 cc. from the basal group (Pen 13). Rat 2473 which received 0.6 cc. of the diluted egg yolk from Pen 12 developed a severe respiratory trouble 2 days after egg yolk feeding was begun. This was followed by what appeared to be an infection of the brain from which the animal did not recover and consequently was killed after the 8th week.

No attempts were made to determine the comparative vitamin A content of the rations fed the hens. From work published by numerous investigators it is evident that the basal ration was low in this factor and was correspondingly low in the egg. The addition of alfalfa hay, fresh green grass, and cod liver oil to the dietary of a laying hen increased the vitamin A intake of the bird and accordingly the potency of the egg yolk. The alfalfa hay used in this trial was a commercial product and not of the best grade, which may in part account for the small difference in the vitamin A content of the eggs from Pens 13 and 15. Another interesting point is the fivefold increased vitamin content of the eggs from the pen on blue-grass range (Pen 16), in contrast to the alfalfa hay group—suggesting that fresh green grass is more potent in vitamin A than the product made into hay with subsequent exposure to variable weather conditions.
To determine the comparative antirachitic properties of the eggs from the variously treated groups of hens, young rats were fed the egg yolks at levels ranging from 0.5 to 7 parts added to a rickets-producing ration and then examined for the severity of the rickets produced. The rats used were raised in our laboratory under standardized conditions. These rats, weighing from 45 to 60 gm., were taken at an age of 24 to 25 days and distributed in groups of three according to litter and sex.

The rickets-producing ration employed was the one reported by Steenbock (10), consisting of yellow corn 76 parts, wheat gluten 20, calcium carbonate 3, sodium chloride 1. In our experience this ration has proved very satisfactory, producing incipient rickets in our young stock rats in a few weeks and very severe rickets in 4 to 5 weeks. The egg yolk was added to the rachitic ration in varying parts per hundred.

In preparing the yolks, 3 dozen eggs from a particular pen were taken, broken individually, and the yolk separated from the white as carefully as possible. All the yolks from a certain pen were then placed in a beaker, twice the weight of distilled water added, and the mixture thoroughly stirred. To 1 kilo of the ration was then added, on an undiluted basis, 0.5 to 7.0 gm. of the egg yolk. After thoroughly mixing the yolk material with the ration the mixture was dried for 36 hours at 65°C. and then ground to insure complete distribution.

After the groups had been on their respective rations for 4 weeks, note was taken of the severity of the rachitic condition in each animal as indicated by enlargement of the joints—particularly the wrists—and by general behavior, previous to autopsy. The wrists of all animals were removed and subsequently examined by silver-staining the distal ends of the ulnæ and radii after splitting with a razor blade. The femurs were also removed, freed from adhering tissue, and ashed after exhaustive extraction with hot alcohol and ether. The data are, in part, tabulated in Table I.

It is quite evident from the data secured that the yolks of the eggs from the pen fed cod liver oil (No. 12) and the blue-grass range group (No. 16) were much more antirachitically potent than those from the basal or alfalfa hay group. The rations forti-
fied with 1.0 and 2.0 parts of the egg yolks from Pens 12 and 16, respectively, possessed greater calcifying properties than the rations carrying 5.0 parts of the yolks from groups in Pens 13 and 15. In other words, the yolks from the eggs of the range pen were more than 5 times as potent, antirachitically, as those from the basal and alfalfa hay pens. While the yolks from the cod liver oil-fed group were not as rich in the antirachitic factor as the yolks from the birds on range, they still proved to be considerably more active antirachitically than any of the other two groups.

### TABLE I.
**Showing the Calcifying Properties of the Various Egg Yolks when Incorporated in a Rickets-Producing Ration.**

<table>
<thead>
<tr>
<th>Yolk added</th>
<th>Average ash in femurs (gm. per cent)</th>
<th>Metaphyses</th>
<th>Yolk added</th>
<th>Average ash in femurs (gm. per cent)</th>
<th>Metaphyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg Yolk 13 (basal pen).</td>
<td></td>
<td></td>
<td>Egg Yolk 12 (cod liver oil pen).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>29.79 About 2 mm. wide.</td>
<td>0.50</td>
<td>27.02 About 2 mm. wide.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>34.71 1.5-2 &quot; &quot;</td>
<td>1.00</td>
<td>33.08 1.5-2 &quot; &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>36.13 About 1.5 &quot; &quot;</td>
<td>2.00</td>
<td>40.91 0.5-1.0 &quot; &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>37.98 1.0-1.5 &quot; &quot;</td>
<td>3.00</td>
<td>43.61 About 0.5 &quot; &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.00</td>
<td>43.64 About 0.5 &quot; &quot;</td>
<td>5.00</td>
<td>47.69 Normal.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg Yolk 15 (alfalfa hay pen).</td>
<td></td>
<td></td>
<td>Egg Yolk 16 (outdoor range pen).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>30.89 About 2 mm. wide.</td>
<td>0.50</td>
<td>36.25 1.0-1.5 mm. wide.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>32.47 1.5-2 &quot; &quot;</td>
<td>1.00</td>
<td>40.53 0.5-1.0 &quot; &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>34.01 1.5-2 &quot; &quot;</td>
<td>2.00</td>
<td>48.06 Normal.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>36.85 1.0-1.5 &quot; &quot;</td>
<td>3.00</td>
<td>48.91 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>38.33 About 1.0 &quot; &quot;</td>
<td>5.00</td>
<td>57.10 &quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results represent an average of three rats per lot.

Although the data in Table I show a rather convincing difference in calcifying properties between the various egg yolks, we thought it advisable to check these probable differences in another manner. For this purpose the line test method as described by McCollum and coworkers (11) was employed. In its essentials this procedure consists in inducing a rachitic condition in the growing rat by using a ration relatively high in calcium as compared to its phosphorus content. Steenbock's ration, as previously de-
TABLE II.
Calcium Deposition in Rachitic Rats after Feeding Egg Yolks from Various Treated Hens.

<table>
<thead>
<tr>
<th>Egg Yolk 13 (basal pen)</th>
<th>Egg Yolk 15 (alfalfa hay pen)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yolk added.</strong></td>
<td><strong>Rat No.</strong></td>
</tr>
<tr>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>1.00</td>
<td>1675</td>
</tr>
<tr>
<td>1686</td>
<td>75-74</td>
</tr>
<tr>
<td>2.00</td>
<td>1676</td>
</tr>
<tr>
<td>1688</td>
<td>79-85</td>
</tr>
<tr>
<td>3.00</td>
<td>1681</td>
</tr>
<tr>
<td>1694</td>
<td>73-80</td>
</tr>
<tr>
<td>5.00</td>
<td>1682</td>
</tr>
<tr>
<td>1693</td>
<td>92-101</td>
</tr>
</tbody>
</table>

**Egg Yolk 12 (cod liver oil pen).**

<table>
<thead>
<tr>
<th>Egg Yolk 16 (outdoor range pen).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yolk added.</strong></td>
</tr>
<tr>
<td>gm.</td>
</tr>
<tr>
<td>0.20</td>
</tr>
<tr>
<td>1683</td>
</tr>
<tr>
<td>0.50</td>
</tr>
<tr>
<td>1701</td>
</tr>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>1687</td>
</tr>
<tr>
<td>2.00</td>
</tr>
<tr>
<td>1696</td>
</tr>
<tr>
<td>3.00</td>
</tr>
<tr>
<td>1707</td>
</tr>
</tbody>
</table>

- No calcium deposition.
+ Evidence of calcium deposition.
+++ Narrow line of calcium.
++++ Wide line of calcium.
+++++ Very wide line of calcium.
++ Union with diaphysis.
scribed, was used. When the rats showed evidence of a rachitic condition at the end of the 3rd week they were confined in individual cages and the egg yolk from eggs of the four different groups of hens was incorporated into the ration at varying levels. The material represented a mixture of the yolks of 2 dozen eggs from each lot, prepared as previously described.

The yolk-bearing rations were fed to the rachitic rats for a 10 day period. Records were kept of their daily feed consumption. At the end of this time the rats were killed, the radii and ulnae removed and examined for calcium deposition by the silver nitrate method.

The results, in part, are summarized in Table II. The data confirm the results of the previous study (Table I); namely, that the yolks of eggs from the cod liver oil pen, No. 12, and the range group, No. 16, were very much more antirachitically potent than those from the basal pen, No. 13, or alfalfa hay pen, No. 15. The ration fortified with 0.2 part of Egg Yolk 16 (range) was as potent antirachitically as the ration fortified with 2.0 parts of Egg Yolk 13 (basal). Likewise, 0.5 part of Egg Yolk 12 (cod liver oil) proved to be better from an antirachitic standpoint than 2.0 parts of Egg Yolk 13. Further evidence is presented that the yolks of the eggs from the alfalfa hay group were no better than the yolks from the eggs of the birds receiving the basal ration only. Apparently the alfalfa hay, a commercial product, was not only low in vitamin A but also in the antirachitic factor.

DISCUSSION.

It is clearly evident from the data available that the vitamin A and D content of egg yolk is largely, if not entirely, determined by the amount of these substances present in the ration and environment of the hen. Hart, Steenbock, and coworkers (7) as well as Hughes and coworkers (8) have shown that the amount of ultraviolet irradiation a hen receives is an important factor in determining the antirachitic vitamin content of the eggs which it produces. The results presented substantiate these findings. Cod liver oil feeding likewise increases the antirachitic as well as the fat-soluble A vitamin content of eggs. It is of interest to note that hens having access to a luxuriant blue-grass range produce eggs which possess as great or greater vitamin A and D content
than similar hens receiving the same ration fortified with 2 parts of medicinal cod liver oil. Thus, from a nutritional view-point of the egg, it would appear unnecessary and uneconomical to provide laying hens with additional vitamin A- and D-containing substances in their ration to make the eggs more potent in these factors, when they have access to a good outdoor range. Alfalfa hay, as used in this experiment, did not increase the antirachitic potency of the egg yolk and exerted but a small effect in increasing the fat-soluble A content.

The practical poultryman is at the present very much concerned with the feeding of cod liver oil and the use of ultra-violet light as they affect egg production and hatchability. Reports from the Wisconsin (7) and Kansas stations (8) indicate that hatchability is related to the antirachitic vitamin content of the egg. In this connection it is interesting to speculate why alfalfa hay, as used in this experiment, did not influence the antirachitic vitamin content of the egg yolks and yet accounted for an increase in hatchability over the basal ration. Cod liver oil, on the other hand, caused a marked increase in vitamin A and the antirachitic factor of the yolks without affecting the hatching qualities of the eggs. Apparently, other factors aside from the antirachitic and fat-soluble A vitamin content of eggs exert some influence on hatchability, etc. These relations will be discussed in a subsequent paper.

We consider it significant that our data have shown that yolks from hen's eggs vary greatly in their antirachitic and fat-soluble A vitamin content—depending upon the ration and management of the layers. The poultryman and the consumer should recognize the misconception of the idea that "an egg is an egg" in respect to both its vitamin content and its quality. An inadequate ration may yield impoverished eggs as well as animals. The true nutritional value of eggs, like that of milk, is chiefly determined by the feed and management of the flock.

The relative price of market eggs is now determined by their freshness or fulness and other physical properties. May not an additional qualification, their vitamin content, based largely on the ration and management employed with the layers, be required for the highest class of eggs in the near future? Even in the light of present information it would not be unreasonable to secure eggs for use in hospitals and for infants from flocks receiving an ade-
quate ration and having access to a suitable outdoor range or its equivalent throughout the year.

**SUMMARY.**

The fat-soluble vitamin content of hen's egg yolk is greatly influenced by the amount of these substances present in the ration and by environment of the laying hen.

Yolks of eggs laid by hens which had access to a blue-grass range were approximately 5 times as potent in vitamin A, and 10 times as active antirachitically as the yolks of eggs laid by hens which received the same basal mash but were confined indoors.

The feeding of 2 parts of cod liver oil in the mash accounted for an approximate fivefold increase in the antirachitic and fat-soluble A vitamin content of the egg yolks.

The addition of alfalfa hay to the basal mash did not improve the calcifying properties of the egg yolks, but proved of some benefit in increasing the vitamin A content.

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