THE DETERMINATION OF THE STATE AND CONTENT OF VITAMIN A IN EGGS

by

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INTRODUCTION AND REVIEW OF LITERATURE

Growth promoting properties of egg yolk fat, now known to be due to vitamin A seem first to have been recognized by McCollum and Davis (1) in 1913, and Osborne and Mendell (2) in 1914. Although Murphy and Jones (3) in 1924, and Bethke et al. (4) in 1927 developed procedures for the determination of vitamin A in egg yolk by rat assay, the detection of vitamin A in egg yolk was claimed by Euler and Klussmann (5) in 1932, using the Carr-Price (6) antimony trichloride color test as the criterion. Chromatographic methods of separation of carotenoid pigments and vitamin A offered additional proof of the existence of vitamin A per se in egg yolk, Gillam and Heilbron (7) and Mann (8).

Using colorimetric means, Sjollema and Donath (9), determined the vitamin A, carotene and xanthophyll content of eggs, finding the normal egg to contain 200-300 I.U. of vitamin A. They also reported that a hen would not lay if the vitamin A content dropped below two micrograms per gram of yolk and that the carotene content of the egg was about 10 percent of the total carotenoid pigments. Bauman et al. (10) applying spectophotometric methods found that eggs contained 6.6-9.2 micrograms of vitamin A per gram of yolk, depending on the ration fed. Bioassays of Koenig et al. (11) indicated that high producing hens deposited 20 units per gram of vitamin A in the yolk as compared to 33 units per gram for low producing hens.

Zaborowski (12) found that the mortality of the chicks during incubation appeared to be related to quantities of the vitamins in egg yolk. By use of the rat bioassay, Sherwood and Fraps (13) and Bethke et al. (4) pointed out that hatchability increased with increasing portions of vitamin A in the feed. Nestler (14) found that eggs laid by quail in the early part of the season contained more vitamin A than those laid during the later part of the season. He suggested differences in vitamin A content as the reason for a decrease in hatchability of the later eggs.

Thomas and Quackenbush (15) reported that the efficiency of the hens in transferring the vitamin A from the ration to the eggs decreased as the vitamin A intake was increased. Russell and Taylor (16) reported that 11-32 percent of the vitamin A consumed as cod liver oil was recovered in the eggs. Cruickshank and Moore (17) found only two percent of the vitamin A fed at high levels, in the form of cod liver oil, was transferred to the egg. An interesting report on the administration of high levels of vitamin A was that of Deuel et al. (18) who showed that large doses of vitamin A suppressed the pigment content of the egg yolk.

According to Bohren et al. (19) and Peterson et al. (20) the rate of decline of the carotenoid pigments in the eggs was very gradual after two to three weeks feeding of a basal ration which was low in carotenoid pigments.

The first methods of analyses, employed for the determination of the vitamin A content of eggs were rat bioassays

used by McCollum and Davis (1), Osborne and Mendel (2), Murphy and Jones (3), and Betzke et al. (4). Euler and Kluseman (5) apparently were the first workers to apply the antimony trichloride color test for vitamin A to the ether extract of egg yolk. After separation of vitamin A and carotenoids by chromatography, Mann (8) and Gillam and Heilbron (7) applied spectographic methods for determination of vitamin A by measuring ultra-violet light absorption. Bauman et al. (10) found that it was necessary to correct the vitamin A values due to carotenoid interferences. Thompson et al. (21) separated vitamin A by a chromatographic procedure before determining the vitamin A content by the Carr-Price colorimetric method, since attempts to apply a correction factor for the carotenoid interferences were unsuccessful.

Other physico-chemical methods have been proposed for the determination of vitamin A of dried egg products by Schrenk et al. (22), Gauge and Zscheile (25) and Klose et al. (24).

The present work was undertaken to study the variations of the vitamin A content of eggs in order to gain additional information on the utilization of vitamin A in egg formation. A group of White Leghorn laying hens, maintained on a diet adequate for good production but containing sub-optimum levels of vitamin A, was used in this study. Variations in the vitamin A content of eggs were compared on eggs within single clutches, on eggs from different clutches and on eggs from different hens. Methods of analysis adopted for this study were modifications of previously used procedures of analyses for vitamin A content

of eggs.

The state in which vitamin A occurs in egg yolk, vitamin A alcohol or vitamin A ester, apparently has not been determined. Such information is of interest in nutrition studies with the hen, in studies of physiology of egg formation, as well as offering possibilities of better understanding and improving methods of determination of vitamin A content of eggs. Consequently, a study was also made of the state in which vitamin A occurs in egg yolks. It was necessary to develop a method for the determination of the state of vitamin A in this product. The method used was an adaptation of the determination of the state of vitamin A in blood serum developed by Parrish et al. (25).

PROCEDURE

Feeding and Management of the Laying Flock

November 10, 1947, 30 White Leghorn pullets and two roosters were placed in a straw loft, open front, laying house and fed a low pigment ration. This ration was selected since it has been shown by Thompson et al. (21) and Schrenk et al. (22) that large amounts of carotenols in dried eggs complicated the determination of vitamin A by Carr-Price reaction.

The composition of the ration is as follows:

Corn, white, ground	120 lbs.
Shorts, wheat (16 percent)	20 lbs.

Oats, ground	20 lbs.
Bran, wheat	10 lbs.
Meat and bone scrap (50 percent)	7 lbs.
Soybean oil meal (42 percent)	9 lbs.
Fishmeal (65 percent)	3 lbs.
Brewer's yeast	3 lbs.
Dried skim milk	3 lbs.
Calcium carbonate	2 lbs.
Salt, common	1 lb.
Fish solubles (Fishtex)	2 lbs.
Vitamin A (Prot A)	300,000 I.U.
Vitamin D_3 (D-Sec)	80,000 I.U.
Riboflavin (#54)	100,000 micrograms

The mash of composition shown above, oyster shell, and fresh water were available to the chickens at all times. The ration contained a sub-optimum level of vitamin A (1800 I.U. per pound of feed) so that in another phase of the study an investigation could be made of the transfer or conversion of carotene, vitamin A alcohol and vitamin A ester to the egg, without an extended period of depletion. On this ration, the hens maintained an average daily production of 65 percent during the collection period, February to April, 1948.

Collection of Eggs

During the period of the trial the hens were trap nested and individual egg records kept. When eggs were desired for

analysis, all the eggs of a clutch were collected, marked, dated, and stored in a refrigerator at approximately 4° C. until analyses could be made. All the eggs were analysed within three weeks following the date they were laid.

Eggs were collected for analyses during the two following periods:

1. February 11-20, 1948. The eggs were utilized for studying variations of vitamin A from clutch to clutch. They were also used in developing the procedure for the determination of the state of vitamin A in egg yolk. These eggs could not be used for any other studies planned as the result of an error in feed mixing in which the vitamin A was replaced with a deficient level of carotene for a short period of time.

2. March 24-April 22, 1948. These eggs were used to study variations of vitamin A of eggs within the clutch from the same hen, variation in vitamin A of eggs from different hens, and variations of vitamin A in eggs from clutches laid during this and the earlier period. Studies also were made of the state of vitamin A in egg yolk.

Method for Determination of Total Vitamin A in Egg Yolk

The yolk of the egg to be analysed was dried on a towel in order to remove adhering egg white and was carefully transferred to a small funnel supported in a beaker. The weight of the whole yolk was determined by difference. The weighing funnel containing the unbroken egg yolk was placed over a glass-stopper-

ed graduated mixing cylinder, the membrane covering the yolk was broken, and the contents were allowed to drain into the cylinder which contained 10-15 ml of approximately 13 percent salt solution. This solution aided in preventing the yolk from sticking to the walls of the cylinder. The funnel containing the residue again was weighed in order to determine by difference the amount of yolk transferred to the cylinder. The cylinder was filled with sufficient salt solution so that three ml of the final emulsion contained one g of yolk. The cylinder was stoppered and shaken until the emulsion appeared homogeneous. A moderate amount of foaming occurred during shaking. Although it was not believed that adsorption by the foam materially interfered with the ability to obtain accurate aliquot samples, the effect of this factor requires further investigation.

A six ml aliquot of the yolk emulsion was pipetted into a 125 ml boiling flask and eight ml of alcoholic potash[1], was added to the mixture. After saponification by refluxing for 20 minutes, the mixture was transferred to a 250 ml separatory funnel, and the boiling flask was rinsed with eight ml of 95 percent alcohol and eight ml of water, and the rinsings also were added to the mixture in the separatory funnel.

A modification of the two-separatory-funnel extraction procedure, Boyer et al. (26) was employed; 30 ml of diethylether was used for the first extraction and 20 ml for the second

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[1]Prepared by using 20 g potassium hydroxide dissolved in 10 ml water and 100 ml of 95 percent alcohol, Boyer et al. (26). The alcohol was checked frequently for aldehydes which form artifacts and if present give erroneous results.

extraction. The extracts were washed first with 60 ml of cold water[2] (18-20° C.), then with 30 ml of cold acidified alcoholic wash[3], and finally with 20 ml of acidified alcoholic wash. Each wash solution was poured into the extract in the first separatory funnel, and the solutions were mixed by shaking. After each wash solution separated, it was drained into the second separatory funnel and mixed by shaking. Following separation of solutions in second separatory funnel the wash solutions were discarded. After the third washing, the extracts were combined in the first separatory funnel, five ml of Skellysolve B were added to decrease the solubility of the water in the ether layer, and the extracts were washed with 50 ml of cold water. The extracts were allowed to stand 15 minutes to permit more complete separation of water, which was carefully drained off.

The ether extracts were evaporated to dryness using reduced pressure and a hot water bath (65-70° C.). The cooled residue was taken up in 10.0 ml of Skellysolve F and transferred to Evelyn photometer absorption tubes. The carotenoid pigment was determined with the Evelyn photometer using the 440 mμ filter.

The Skellysolve F solution was evaporated to dryness by attaching two absorption tubes to stoppers (foil-covered) fitted to a Y-tube attached by flexible tube to water aspirator. Heat was supplied by a hot water bath (65-70° C.). The residue was cooled and dissolved in one ml of chloroform. In the deter-

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[2] If an emulsion formed it was broken by adding five ml alcohol.

[3] Prepared by using one ml hydrochloric acid, 100 ml alcohol diluted to 1000 ml with water, Boyer et al. (26).

mination of vitamin A, the Carr-Price (6) antimony trichloride reaction was used for developing the blue colored-complex which was read on Evelyn photometer using the 620 mμ filter. The galvanometer reading was made about five seconds after the Carr-Price reagent had been added. Methods for preparing reagents, calibration of the photometer and techniques employed in photometric measurements were taken from previously published reports, Koehn and Sherman (27), Dann and Evelyn (28), Boyer et al. (29) and Kimble (30). Reading on duplicate samples which were within one galvanometer unit or less of each other were considered to be within experimental error.

Method for Determination of the State of Vitamin A in Eggs

A homogeneous emulsion of the egg yolk was prepared as previously described. The procedure followed for the determination of the form of vitamin A is an adaptation of the determination of the state of vitamin A in cow blood, Parrish et al. (25).

Six ml of the yolk emulsion were transferred to a glass stoppered centrifuge tube. Eight ml of 95 percent alcohol was added and mixed with the yolk emulsion to denature the proteins present. Thirty ml of Skellysolve B were added and the tube was shaken for three minutes for extraction of fat soluble material. A second and a third two-minute extraction with 15 ml of Skellysolve B was made to insure completeness of extraction. The three extracts were combined in a 250 ml separatory funnel. Three cold water (15-20° C.) washings of 70 ml, 50 ml and 50 ml

respectively, were made to remove alcohol and each of these wash solutions was extracted successively by shaking with 20 ml of Skellysolve B in a second separatory funnel. The Skellysolve B solutions containing the lipin material were combined and evaporated to about 10-15 ml under reduced pressure using a hot water bath (65-70° C.) to hasten evaporation.

An alumina column (12 mm x 50 mm) was prepared and the Skellysolve B solution poured on the top of the column. The column was eluted with 100 ml of four percent acetone in Skellysolve B by the flowing chromatogram technique to remove the ester form of vitamin A, leaving the alcohol form behind as a diffused band near the top of the column. The vitamin A alcohol could be identified as a bright blue band by painting the column with antimony trichloride reagent.

The eluate containing the esterified vitamin A was transferred to a boiling flask and evaporated to one to two ml on the steam cone. Eight ml of alcoholic potassium hydroxide and one to two ml of water were added to the solution. This mixture was saponified by refluxing on a steam cone for 20 minutes. The remainder of the procedure followed was the same as for the determination of total vitamin A.

The vitamin A values obtained on samples treated as outlined above were assumed to represent the vitamin A ester content of the sample. A determination for total vitamin A content was run concurrently and the difference between values obtained in the two determinations was the vitamin A alcohol content.

Calibration Curves and Sample Calculations

The vitamin A content of samples was obtained from a standard curve prepared from photometric determinations of the blue color developed when antimony trichloride reacted with known amounts of vitamin A dissolved in chloroform, Carr and Price (6). Known amounts of vitamin A were plotted against the L_{620} values (2 - log G_{620}, where G is the observed galvanometer reading). The points were approximately on a straight line having a slope of 13.1. Calibration data were prepared by Caldwell (31).

Since carotenoid pigments react with the Carr-Price reagent, it is necessary to determine the amount of pigment present in order to correct for errors that they introduce. The concentration of carotenoid pigments were determined as carotene by use of the standard beta-carotene calibration curve. The calibration curve for beta-carotene related L_{440} values (2 - log G_{440}) to known amounts of pure beta-carotene. A straight line with the slope of 2.73 was obtained. The beta-carotene calibration curve was used since its absorption curve was similar to those of the various xanthophylls and related compounds which go to make up the carotenoid pigments of egg yolk.

The correction factor was obtained from the two curves, Fig. 1, one for the yellow color due to the pigment and the other for the blue color due the reaction of antimony trichloride with the same amount of pigment. The ratio of the

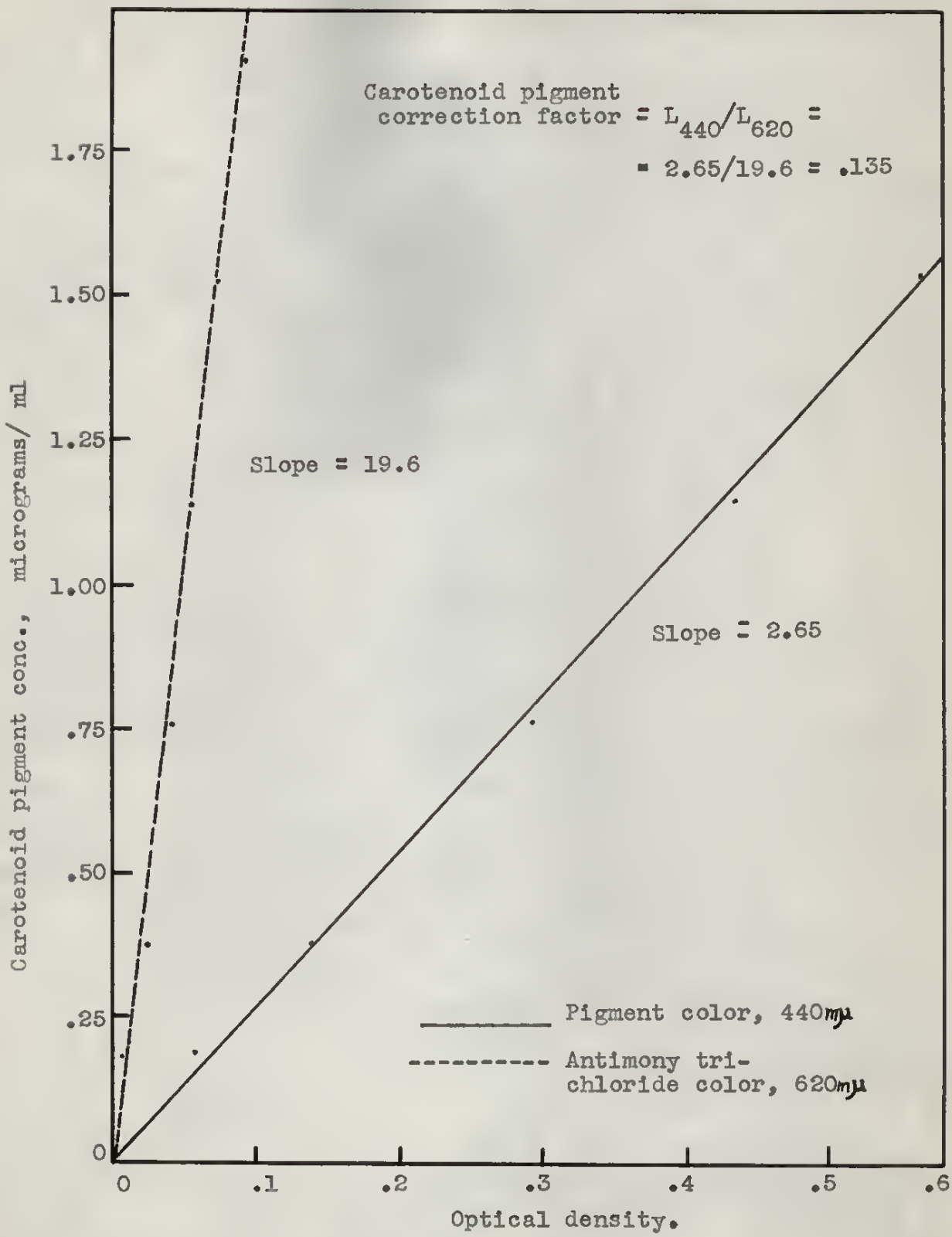

Fig. 1. The optical density of various concentrations of carotenoid pigments read as the yellow pigment and as the blue antimony trichloride color.

slopes of the yellow color curve / blue color curve was 0.135.

The amount of carotenoid pigments were obtained in this manner:

Micrograms carotenoid pigment/sample (as carotene) = L_{440}value x 2.73 x 10

(10 was in the above equation since the extract was made up to a final volume of 10 ml)

The amount of vitamin A in a sample was obtained by the following formula:

Micrograms vitamin A per sample = (L_{620}value - 0.135 x L_{440}value) x 13.1

The L-value is commonly obtained by looking up corresponding galvanometer readings in a table supplied with the photometer.

Results were desired expressed as micrograms per gram of yolk and as micrograms per total yolk. Since a two gram sample was used, and the final volume of Skellysolve F containing the dissolved non-saponifiable matter was 10 ml, the following equations applied:

Micrograms vitamin A per g of yolk = (L_{620}value - 0.135 x L_{440}value) x 13.1/2

Micrograms carotenoid pigment per g of yolk = (L_{440}value x 2.73 x 10)/2

Sample calculations:

If G_{620} = 35, L_{620} = 2 - log G_{620} = 0.456

If G_{440} = 72, L_{440} = 2 - log G_{440} = 0.143

If the egg yolk weighed 17 grams, then the following calculations apply:

(.456 - .135 x .143) x 6.55 = 2.86 micrograms vitamin A per g of yolk.

2.86 x 17 = 48.6 micrograms vitamin A per yolk.

(1.43 x 13.65) = 1.95 micrograms carotenoids per g of yolk.

1.95 x 17 = 33.1 micrograms carotenoids per yolk

The calculations for the determination of the form of vitamin A. It was assumed that the amount of vitamin A present in the eluate from the alumina column was all in the ester form. The difference between values obtained for total vitamin A and for esterified vitamin A was assumed to be the alcoholic vitamin A.

If the total vitamin A = 3.50 micrograms per g of yolk.

If the ester vitamin A = 0.35 micrograms per g of yolk.

Then alcohol vitamin A = 3.15 micrograms per g of yolk.

3.15 / 3.50 x 100% = 90% vitamin A alcohol.

CRITICAL STUDIES AND DISCUSSION OF CERTAIN STEPS OF PROCEDURES

Total Vitamin A Recoveries

Four aliquots were taken from an egg yolk emulsion prepared as previously described. A determination of total vitamin A was made on two samples. To the second pair, a known amount of vitamin A was added and the vitamin A content determined. Three duplicate analyses showed that 101-109 percent of the vitamin A added was recovered. These results were an indication that vitamin A was not lost in the course of the analysis.

Precision

Six identical samples of the same egg were analyzed for determination of precision. The average deviation from the average was 1.4 percent. The maximum deviation was 2.5 percent. This precision was considered to be satisfactory for the present investigation.

Carotenoid Pigment Interferences

It was suggested by Baumann et al. (10) that the colorimetric method might give results which were too low due to certain substances which reduce the intensity of the blue color. On the other hand Thompson et al. (21) found that their results on dried eggs were high when using the blue color test compared to the rat bioassay values and they could not apply a suitable correction factor for the presence of the pigment. In both cases the investigators were using eggs with normal content of carotenoid pigments.

Since the present work was carried out on eggs low in carotenoid pigments it offered the opportunity to study the effect of the addition of various levels of carotenoid pigments for determining the extent of error attributable to these pigments.

Two pigment preparations were used. The first was an extract prepared from dehydrated alfalfa by saponification with alcoholic potash, extraction with Skellysolve B, and evaporation to concentrate the pigments, followed by a chromatographic

separation. Only the pigments strongly absorbed on the chromatogram were saved. These pigments were the xanthophylls, with perhaps some oxidized products. The second pigment preparation was that extracted with ether from eggs using a method similar to the procedure for obtaining the fat-soluble material in the determination of vitamin A (described in a previous section). The extract was chromatographed on a column of alumina using six percent acetone and two percent alcohol in Skellysolve B as the eluting solvent for the removal of all the vitamin A alcohol as well as some weakly absorbed pigments such as carotene and cryptoxanthol. The column was extruded and sectioned and the pigment-containing section eluted with 100 ml of 20 percent alcohol in Skellysolve B in order to obtain the desired pigments. This solution, washed with water to remove alcohol and concentrated by evaporation, contained mostly luteol plus small amounts of other pigments.

The carotenoid pigment was added to samples of egg emulsion in amounts ranging from 1-44 micrograms per sample. Vitamin A was determined and corrections for the presence of pigments were applied in the usual manner. Recovery of the pigment averaged 99 percent. The data in Table 1, indicated that the vitamin A deviations frequently increase sharply for any addition of carotenoids above 15-20 micrograms. However, analysis of the pigment preparation alone gave a blue color value which was essentially canceled out by the correction (.135) applied to the yellow color (100 micrograms of pigment gave a net vitamin A activity of only .92 micrograms of vitamin A).

Table 1. Deviations in vitamin A determinations as related to increased amounts of added carotenoid pigments in the sample.

Pigment in micrograms			Vitamin A in micrograms		
Added to sample	Recovered from sample	Percent recovery of pigment	In normal sample	In sample containing added pigment	Percent deviation from normal sample
1.08*	1.06	98	3.07	3.09	0.7
1.20	1.36	112	3.51	3.88	10.5
1.61	1.86	115	3.51	3.82	8.9
2.41	2.08	87	3.51	3.81	8.6
3.36*	3.46	106	3.07	3.29	7.2
5.42*	5.42	100	2.95	3.07	4.1
7.96	8.07	101	3.63	3.97	9.4
8.71*	8.36	96	2.23	2.49	11.6
8.71*	8.18	94	2.26	2.43	7.6
10.83	10.84	100	3.10	3.30	6.5
11.94	11.17	94	3.63	3.94	8.5
12.24*	11.80	97	3.91	3.98	1.8
14.44	14.12	98	3.10	3.40	9.7
17.42*	15.62	90	2.26	2.44	8.1
17.64	14.73	83	2.46	2.95	19.7
26.13*	26.88	103	2.23	2.96	32.0
26.13*	27.08	104	2.26	2.83	25.6
27.42	28.85	106	2.62	3.13	19.4
30.60*	32.60	105	3.97	4.19	7.2
34.84*	36.58	105	2.23	2.92	31.0
34.84*	35.50	102	2.26	3.31	46.5
36.56	37.05	101	2.62	3.22	22.5
44.10	45.90	104	2.46	3.07	24.8

*Egg extract pigment. The remaining samples were alfalfa pigment extracts.

The additional intensity caused by pigments added to the yolk emulsion was possibly due to the removal of some type of an inhibiting factor when the pigment and extract are mixed, or to some change in the carotenoid pigment similar to the deterioration that takes place when the carotenoid pigment is evaporated from its solution and exposed to heat, light and air, Johnson and Baumann (32).

Since the deviation was not directly proportional to the amount of carotenoid pigments added, a correction could not be determined. Further investigation with low-pigment eggs may reveal an explanation of this unexpected increase of blue color due to the added carotenoid pigment.

Carotenoid Pigment Stability Test. It had been noted by Johnson and Baumann (32), that the vitamin A activity of carotenoid pigments were higher when the pigment had been allowed to stand for some time. In order to determine whether age or state of oxidation might affect the correction that should be applied for the presence of pigments in determination of vitamin A of egg, it seemed advisable to carry out the following experiment.

Four samples of carotenoid pigments in Skellysolve F were analyzed for apparent vitamin A content. Four identical samples were exposed to light in laboratory and were allowed to evaporate over a period of 24 hours. The samples were then made up to the original volume with Skellysolve F and analyzed for carotenoid pigments and vitamin A in the usual manner. As a result of treatment the yellow pigment values decreased while the vita-

min A values increased shown in Table 2.

Table 2. Deterioration of carotenoid pigments.

Sample	Before treatment: Carotenoid pigment in sample (micrograms)	Before treatment: Apparent vitamin A in sample (micrograms)	After treatment: Carotenoid pigment in sample (micrograms)	After treatment: Decrease of carotenoid (percent)	After treatment: Vitamin A in sample (micrograms)	After treatment: Increase of apparent vitamin A (percent)
1	5.11	.091	3.51	31	.120	32
2	10.80	.023	5.82	46	.111	79

If some of these oxidized carotenoid pigments were present in the hen's feed, they might be excreted in the eggs and would tend to give high results similar to findings described in the previous section when high concentrations of pigments were added to egg extracts.

Extraction of Vitamin A Prior to Saponification

This procedure was tested by analyzing similar samples from which vitamin A was extracted before and after saponification. The results, Table 3, indicated that total shaking time should be increased from three to seven minutes for three extractions. The seven minute shaking resulted in an average of 100.7 percent extraction of vitamin A. The residue from the samples extracted before saponification was also analyzed for vitamin A yielding an average of 0.16 micrograms or 5.4 percent vitamin A remaining in the residue. However, a blank containing casein which was analyzed to check for the formation of artifacts during the

procedure indicated 0.15 micrograms or 5.1 percent vitamin A activity per sample. The blank thus tended to cancel the vitamin A activity found in the residue. Small amounts of artifacts might have been formed from aldehydes in the saponification mixture, Parrish et al. (25). The final assumption was that the extraction of vitamin A was approximately 97-99 percent complete.

Table 3. Comparison of results of the extraction of vitamin A before and after saponification and the analysis of the residues for vitamin A.

Shaking time*	Extraction before saponification	Extraction after saponification	Completeness of extraction before saponification	Vitamin A in residue	
min.	micrograms of vitamin A		percent	micrograms	percent
3	2.32	2.22	95.7		
3	3.40	3.07	90.3		
3	3.27	3.12	95.5		
3	3.27	3.10	94.8	0.23	7.4
7	3.24	3.15	97.3	0.17	5.3
7	2.96	3.09	104.0	0.16	5.4
Blank**				0.15	5.1

*Total time for the three extractions.

**Analysis of a blank containing casein for apparent vitamin A due to artifacts.

Absorption of Added Vitamin A Alcohol by an Alumina Column

An egg sample was divided into four aliquots each containing the equivalent of two grams of yolk. The first pair were analyzed for vitamin A ester. A known amount of vitamin A

alcohol was added to a second pair prior to passing the Skelly-solve B solution through the adsorption column. The determinations for vitamin A were completed in the usual manner. The results, Table 4, indicated that not more than one to two percent of the alcohol vitamin A added came through the column. The concentration of the vitamin A alcohol added was many times the concentration of the vitamin A alcohol found in normal egg samples. Thus it was indicated that at least 98-99 percent of the vitamin A alcohol was removed from the solution. These results appeared satisfactory for use of the method in the investigation of the state of vitamin A in eggs.

Table 4. The absorption of added vitamin A alcohol on an alumina absorption column.

Vitamin A				
alcohol added to sample	ester and alcohol recovered*	ester recovered from sample	alcohol obtained by difference	alcohol absorbed
micrograms				percent
38.9	1.35	1.00	0.35	99.1
38.9	1.32	0.71	0.61	98.4
72.2	1.14	0.47	0.67	99.1

*Obtained by analysis of duplicate aliquot samples for total vitamin A.

Recovery of Added Vitamin A Ester From an Alumina Column

An attempt was made to check the efficiency of the absorption column by adding vitamin A ester to the extract prior to passing the solution through the column. The recovery of 40-67 percent

of the added vitamin A ester indicated that further study would be necessary to find the reason for the low results. Although the stock solution of the vitamin A ester was put through the alumina column to remove the vitamin A alcohol which might have been present, it was possible that the ester may have partially deteriorated before it was chromatographed the second time. The first absorption on the column might have made the ester sensitive to later change.

Recovery of Absorbed Vitamin A Alcohol from an Alumina Column

Attempts were made to recover vitamin A alcohol of the egg yolk extracts absorbed on the alumina column in the procedure for separation of alcoholic and esterified vitamin A. The amount of vitamin A alcohol thus recovered plus the vitamin A ester eluted from the column were added and the sum compared with the amount of total vitamin A determined on aliquots of the same sample.

The vitamin A alcohol was recovered by removing the upper two-thirds of the column and eluting it with 100 ml of 20 per-cent alcohol in Skellysolve B. The eluate was filtered off into a 250 ml separatory funnel and washed with water. Fifteen to twenty ml of alcohol were used to break emulsions which formed in the Skellysolve B layer. A final washing was made with water to remove the alcohol. The solution of vitamin A alcohol in Skellysolve B was evaporated to dryness using a hot water bath and reduced pressure. The residue was made up to a 10.0 ml

volume and the yellow and blue color determined in the usual manner and vitamin A content calculated.

Results, Table 5, indicated that on the average 98 percent of the two forms of vitamin A could be accounted for, which appears satisfactory for investigations of the state of vitamin A in eggs.

Table 5. Vitamin A recovered after absorption on an alumina column.

Vitamin A ester in sample		Vitamin A alcohol recovered		Total vitamin A in sample	Total recovery as vit. A ester plus vit. A alc.
micrograms	percent	micrograms	percent	micrograms	percent
.286	13.7	1.66	79.5	2.09	93.2
.292	12.8	1.85	81.1	2.28	93.9
.411	17.2	2.01	84.1	2.39	101.3
.334	14.0	2.19	92.0	2.38	106.0

VARIATIONS IN VITAMIN A LEVELS IN YOLK OF EGGS FROM HENS RECEIVING EXPERIMENTAL RATION

Experimental Results

The variations of the vitamin A and carotenoid pigment in eggs from hens of the experimental ration are presented in Tables 6, 7, 8, 9, and in Figs. 2, 3, 4. The results are expressed in terms of both micrograms per gram of yolk and per whole yolk. Since the ration was low in pigments and carotenoid content of eggs is unimportant for purposes of this study, no

special consideration will be given to the carotenoid pigment content.

The average vitamin A content of all eggs (112 eggs) analyzed during the March 24-April 14 period was 41.4 micrograms per egg (2.53 micrograms/g).

Variations within the Clutches laid by Individual Birds. The total amount of vitamin A deposited in the eggs tended to be less for each subsequent egg laid, Table 6, 7, 8, 9. Trends are more evident when the results are expressed as micrograms of vitamin A per yolk. The graphs, Figs. 2, 3, 4, indicate a tendency toward a gradual decrease throughout the duration of the clutch, but some of the variations from the trends are observed which are probably attributable to the individuality of the hens. The rate of decrease of vitamin A in eggs was approximated by taking the difference between vitamin A values per yolk of the first and last eggs of the clutch and dividing by the number of eggs in the clutch. The change per egg within the clutch ranged from a 2.3 microgram decrease to a 0.3 microgram increase, with an over all average of 1.1 micrograms decrease. Hens 3981 and 3993 were the only ones to deposit increasing amounts of vitamin A in the yolk. Examination of the data indicated an 80 percent greater rate of decrease of vitamin A per egg within the 4-, 5-, and 6-egg clutches (1.21 micrograms/egg) than in the 10-29-egg clutches (0.67 micrograms/egg).

Analysis of the first egg laid of the subsequent clutch revealed a vitamin A content approximately five percent higher, Tables 7, 8, 9, than that of the last egg of the previous

Table 6. The vitamin A and carotenoid pigment content of yolks of eggs laid in four egg clutches.

Hen no.	Egg in clutch no.	Yolk weight g	Vitamin A per g	Vitamin A per yolk	Carotenoid pigments per g	Carotenoid pigments per yolk
			micrograms			
3968	1	17.47	1.98	34.6	1.59	27.8
	2	17.52	1.83	31.9	1.59	27.8
	3	16.33	1.78	29.0	1.64	26.8
	4	16.75	1.80	30.2	1.72	28.8
3964	1	17.99	2.04	36.7	1.55	27.9
	2	18.41	1.87	34.5	1.50	27.6
	3	18.82	1.74	32.7	1.29	24.3
	4	16.90	1.68	27.4	1.39	23.5
3977	1	17.42	2.46	42.9	1.88	32.8
	2	17.96	2.47	44.4	1.81	32.5
	3	15.67	2.12	33.2	1.77	27.8
	4	15.15	2.32	35.1	1.90	28.6
3985	1	16.76	2.56	42.9	1.82	30.4
	2	17.54	2.51	44.1	1.73	30.4
	3	17.19	2.46	42.3	1.86	32.0
	4	15.53	2.31	35.9	1.69	26.3
3989	1	19.32	2.42	46.8	1.49	28.9
	2	-----	----	----	----	----
	3	16.72	2.26	37.8	1.50	25.1
	4	16.04	2.32	37.2	1.51	24.2
3981	1	16.87	2.60	43.8	2.09	35.2
	2	17.39	2.77	48.2	2.13	37.0
	3	17.50	2.69	47.1	2.11	36.9
	4	17.26	2.61	45.0	2.13	36.9
3975	1	18.69	2.83	52.8	1.90	35.4
	2	18.37	2.81	53.0	1.90	35.8
	3	17.94	2.91	52.3	1.94	34.8
	4	18.27	2.70	49.4	1.78	32.5

Table 7. The vitamin A and carotenoid pigment content of yolks of eggs laid in five egg clutches.

Hen no.	Egg in clutch no.	Yolk weight g	Vitamin A per g (micrograms)	Vitamin A per yolk (micrograms)	Carotenoid pigments per g (micrograms)	Carotenoid pigments per yolk (micrograms)
3993	1	16.66	1.92	32.0	1.55	25.8
	2	16.50	2.10	34.7	1.51	25.0
	3	16.30	2.06	33.6	1.66	27.0
	4	16.12	2.09	33.7	1.60	25.8
	5	16.07	2.03	32.7	1.65	26.5
	1[a]	16.84	2.23	37.6	1.70	28.6
3970	1	17.41	2.50	43.5	1.33	23.2
	2	16.89	2.40	40.5	1.14	19.3
	3	15.71	2.38	37.4	1.11	17.4
	4	15.95	2.23	35.5	1.08	17.3
	5	15.73	2.26	35.5	1.10	17.2
3982	1	18.57	3.48	64.6	2.25	41.7
	2	18.09	3.40	61.4	1.96	35.4
	3	18.46	3.27	60.5	1.82	33.5
	4	18.22	3.27	59.6	1.71	31.1
	5	17.18	3.24	55.7	1.61	27.6
3980	1	17.33	2.23	38.7	1.30	22.5
	2	16.74	2.26	37.8	1.45	24.3
	3	16.80	2.09	35.1	1.30	21.8
	4	-----	----	----	----	----
	5	16.89	2.28	38.5	1.46	24.1

[a]First egg of the following clutch.

Table 8. The vitamin A and carotenoid pigment content of yolks of eggs laid in 6-16-egg clutches.

Hen no.	Egg in clutch no.	Yolk weight g	Vitamin A per g	Vitamin A per yolk	Carotenoid pigments per g	Carotenoid pigments per yolk
			micrograms			
3972	1	20.35	2.17	44.2	1.36	27.7
	2	18.18	2.48	45.1	1.72	31.3
	3	18.61	1.97	36.6	1.37	25.5
	4	17.63	2.07	36.5	1.74	20.6
	5	18.99	1.91	36.6	1.50	28.5
	6	19.79	1.88	36.9	1.36	26.9
	1*	18.91	1.89	35.6	1.43	27.1
3971	1	17.45	2.33	40.6	1.50	26.2
	3	17.48	2.30	40.1	1.42	24.9
	5	17.33	2.29	39.7	1.50	26.0
	7	17.21	2.36	40.6	1.63	28.1
	9	16.71	2.28	38.1	1.78	29.8
	10	16.14	2.21	35.7	1.96	30.0
3996	1	18.30	2.36	44.6	1.80	34.0
	3	19.53	2.41	47.0	1.62	31.7
	5	19.72	2.34	45.8	1.54	30.3
	7	19.18	2.33	44.6	1.55	29.7
	11	18.55	2.09	38.7	1.45	27.0
	1*	19.11	2.00	38.4	1.33	25.4
3969	1	18.07	2.76	49.9	1.64	29.6
	2	19.20	2.60	49.8	1.48	28.4
	3	17.79	2.79	48.0	1.52	27.0
	4	18.80	2.71	50.9	1.55	29.2

Table 8. (concl.).

Hen no.	Egg in clutch no.	Yolk weight g	Vitamin A per g	Vitamin A per yolk	Carotenoid pigments per g	Carotenoid pigments per yolk
			micrograms			
	5	16.49	2.66	47.0	1.43	23.6
	6	17.40	2.57	45.5	1.50	26.1
	7	16.71	2.59	43.2	1.57	26.3
	8	17.49	2.40	42.0	1.49	26.1
	9	17.01	2.35	40.0	1.30	22.0
	10	17.18	2.50	42.8	1.34	22.9
	11	16.47	2.44	40.1	1.29	21.2
	1*	17.32	2.40	41.6	1.30	22.4
3992	1	16.25	2.45	39.8	2.41	39.1
	3	15.40	2.03	31.2	2.07	31.9
	5	15.70	1.92	30.2	1.82	28.5
	7	17.54	2.03	35.5	1.96	34.4
	10	16.28	1.87	30.5	2.10	34.2
	12	16.18	1.69	27.3	1.70	27.5
	14	15.75	1.39	21.9	1.31	20.5
	16	15.05	1.27	19.2	1.23	13.5
	1*	15.40	1.37	21.1	1.43	21.6

*First egg of the following clutch.

Table 9. The vitamin A and carotenoid pigment content of eggs laid in a 29-egg clutch.

Hen no.	Egg in clutch no.	Yolk weight g	Vitamin A per g	Vitamin A per yolk	Carotenoid pigments per g	Carotenoid pigments per yolk
			micrograms			
3979	1	16.31	3.80	62.0	2.04	33.3
	2	16.93	3.71	62.9	2.02	34.3
	3	16.65	3.79	63.2	2.14	35.6
	4	15.70	3.62	56.8	2.16	33.9
	5	15.77	3.72	58.7	2.21	34.9
	6	16.78	3.69	61.9	2.30	38.5
	7	15.83	3.45	54.7	2.16	34.2
	8	15.98	3.62	57.5	2.19	34.8
	9	16.23	3.50	56.8	2.10	34.1
	10	16.53	3.44	56.8	1.89	31.2
	11	16.93	3.45	58.5	1.85	31.4
	12	16.30	3.41	55.6	1.96	31.9
	13	16.72	3.34	55.8	1.84	30.6
	14	15.02	3.21	48.4	1.79	26.9
	15	14.93	3.01	45.1	1.66	24.8
	16	14.74	3.00	44.2	1.55	22.9
	17	16.34	3.42	52.6	1.67	17.2
	18	15.35	3.18	48.8	1.59	24.4
	19	14.68	3.03	44.5	1.55	22.8
	20	15.19	3.07	46.6	1.55	23.5
	22	16.71	2.98	49.5	1.70	28.4
	23	16.19	2.95	47.6	1.49	24.1
	25	16.08	2.90	46.7	1.72	27.7
	27	14.36	2.85	40.8	1.63	23.3
	29	14.73	2.39	36.3	1.34	19.8
	1*	15.75	2.38	37.5	1.31	20.5

*First egg of the following clutch.

clutch, except in two cases the vitamin A content continued to decrease slightly.

The largest decrease within a clutch and the smallest amount of vitamin A found in any egg was noted in the eggs from hen 3992, which decreased from 39.8 to 19.2 micrograms per yolk (2.41 to 1.29 micrograms/g) over a 16 day period.

Variations of Vitamin A Levels Among Hens. The clutch average vitamin A content of eggs from different hens varied from 29-55 micrograms per egg (Tables 6, 7, 8, 9, and Figs. 2, 3, 4). No apparent correlation seemed to exist between percent production or the size of clutch and the vitamin A content of the eggs. This may be illustrated in the case of two high producing hens, 3992 and 3979. Hen 3992 laid a 16-egg clutch and averaged 29.3 micrograms vitamin A per egg, while hen 3979 laid a 29-egg clutch and averaged 55 micrograms vitamin A per egg.

On an average, eggs in the 4-egg clutches contained 40.5 micrograms vitamin A per yolk, while those of the 5-and 6-egg clutches contained 42.3 micrograms per yolk and those of the 10-29-egg clutches contained 43.2 micrograms per yolk. These data indicated that hens laying longer clutches deposited somewhat more vitamin A in the eggs than the birds laying the shorter clutches.

Variations in the Vitamin A Level from Clutch to Clutch Laid by the Same Hen. Data on the analyses of eggs from clutches laid in the period February 11 - 20, as compared to that of the period March 24 - April 14, Table 10, indicated that the amount

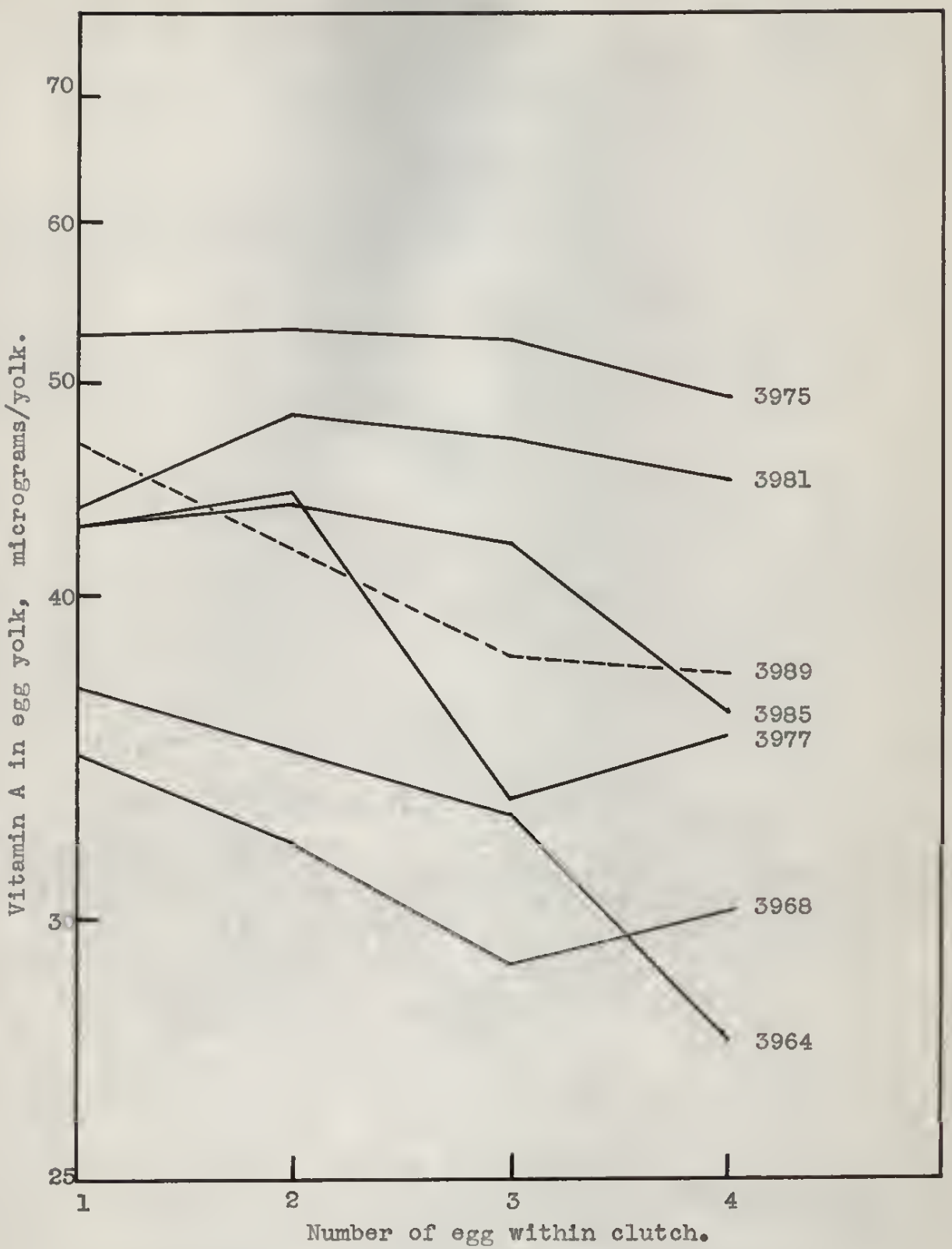

Fig. 2. The rate of decrease of vitamin A in eggs from hens laying four egg-clutches.

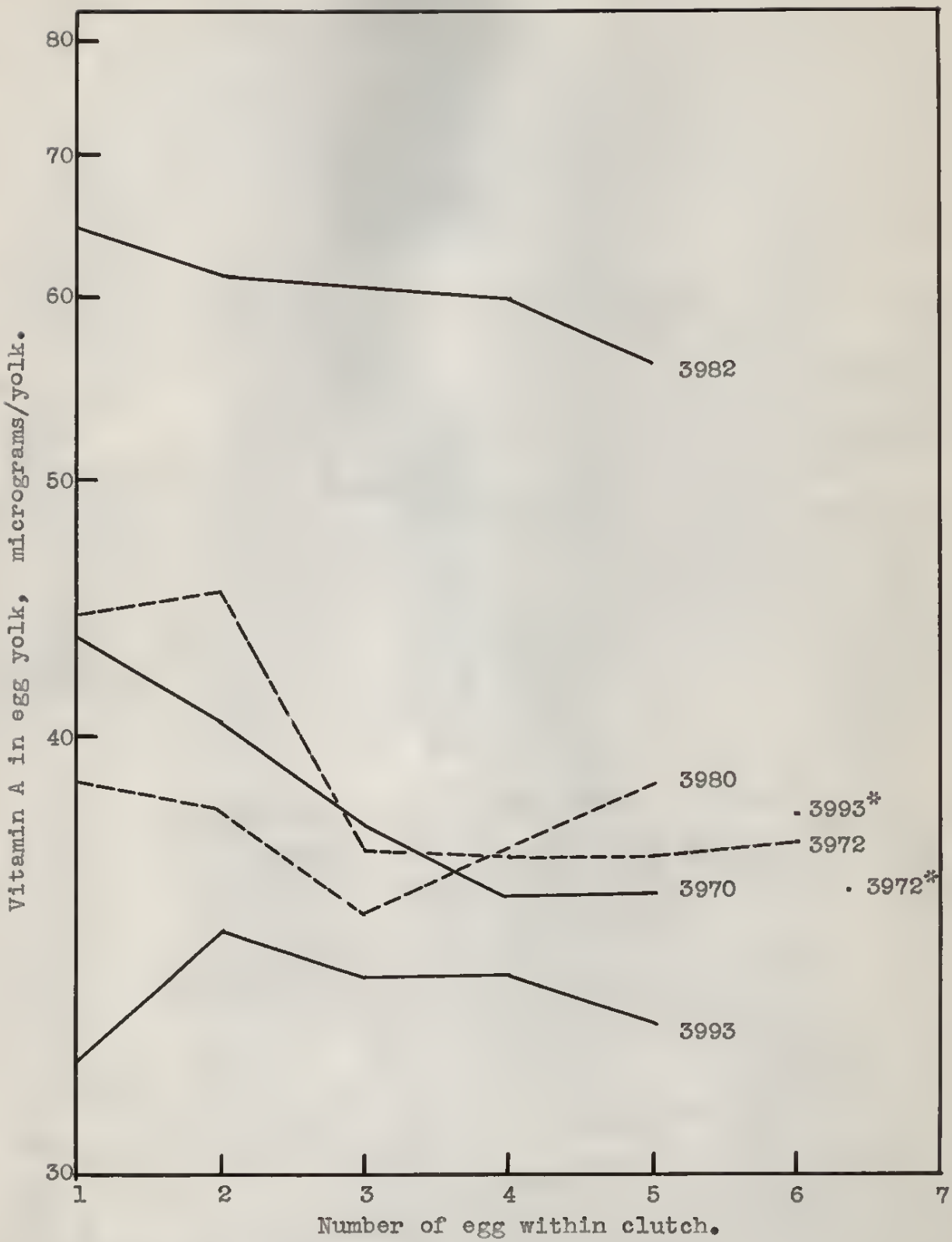

Fig. 3. The rate of decrease of vitamin A in eggs from hens laying five and six-egg clutches.

*First egg from the following clutch.

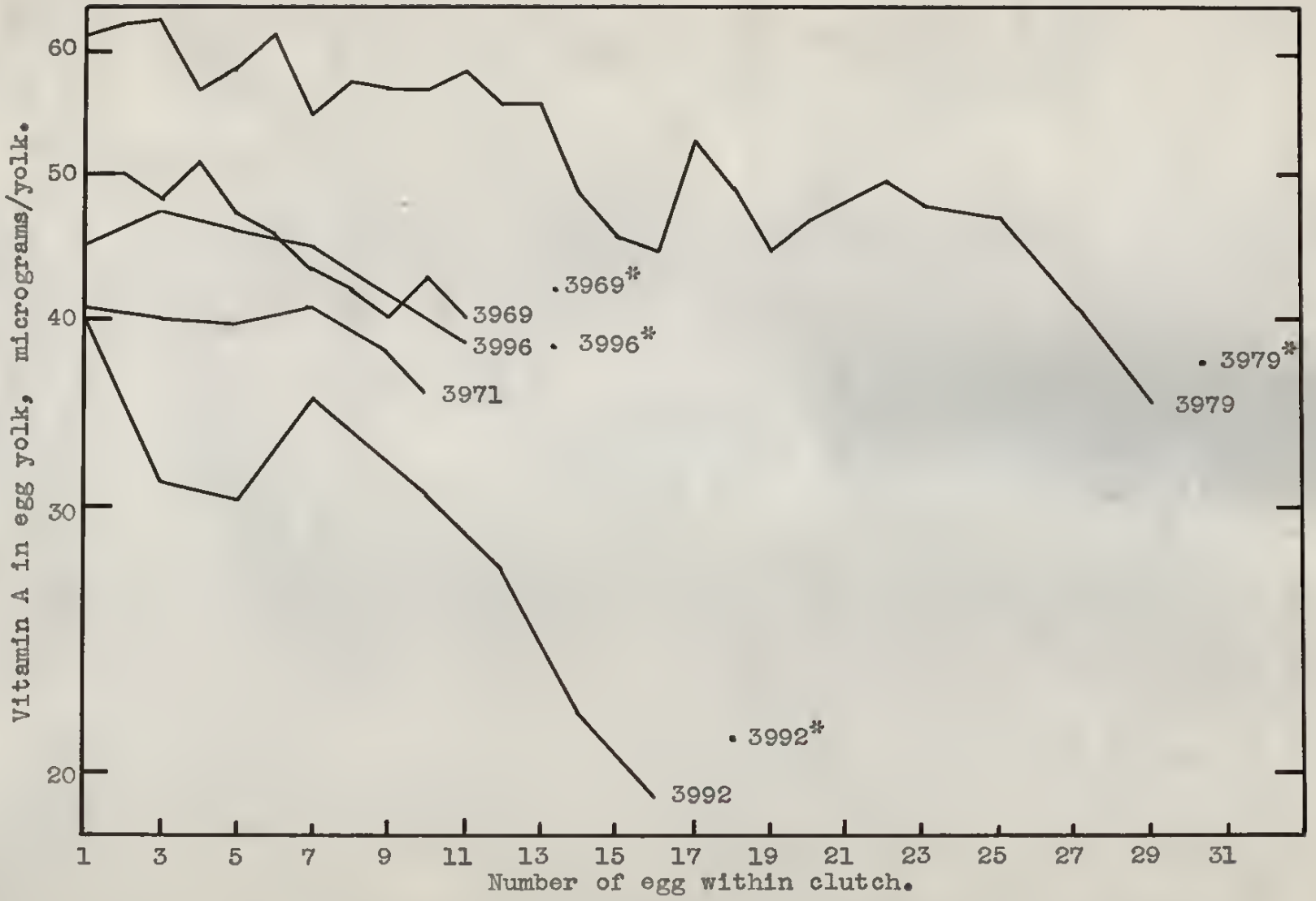

Fig. 4. The rate of decrease of vitamin A in eggs from hens laying 10-29-egg clutches.

*First egg from the following clutch.

Table 10. Average yolk weights and vitamin A levels of eggs laid at two different periods during the laying season.

Hen no.	Period	Eggs per clutch	Av. yolk weight	Vitamin A per g of yolk	Vitamin A per yolk	Drop from periods 1 to 2
		no.	g	micrograms		percent
3992	1*	6	15.57	3.84	59.8	50.0
	2**	16	16.02	1.83	29.3	
3964	1	4	16.51	3.18	52.5	37.1
	2	4	18.03	1.83	33.0	
3985	1	3	15.92	3.92	62.4	34.0
	2	4	16.75	2.46	41.2	
3971	1	3	16.73	3.42	57.2	31.5
	2	10	17.05	2.30	39.2	
3975	1	3	17.16	4.39	75.4	31.3
	2	4	18.44	2.31	51.8	
3989	1	4	17.63	3.13	55.2	26.6
	2	4	17.36	2.33	40.5	
3983	1	3	15.54	2.50	38.9	15.2
	2	5	16.31	2.02	33.0	

*Period 1 dates from February 11 - 20.

**Period 2 dates from March 24 - April 14.

of vitamin A deposited in the eggs decreased 15-50 percent, with an average decrease of 32 percent during the five to six intervening weeks. A point of interest was that in most cases the average yolk weight increased, while the concentration of vitamin A per gram of yolk decreased enough to give an over all decrease in the total vitamin A content per yolk. The average rate of decrease of vitamin A in each succeeding egg was 10 percent greater during the later clutches than during the early clutches.

Discussion

It was noted that the vitamin A content reported herein (an average of 2.53 micrograms/g of yolk) is lower than values reported by other workers. Vermes et al. (33) found 5.55-11.3 micrograms/g; Baumann et al. (10), 6.6-9.2 micrograms/g; Koenig et al. (11), 4.45-6.67 micrograms/g; Russell and Taylor (16), 4.17-5.91 micrograms/g; Mann (8), 4.1-4.5 micrograms/g, and Russell (34), 2.78-3.61 micrograms/g/ Gillam and Heilbron (7), however found only 2 micrograms of vitamin A/g of yolk. The difference is possibly ascribable to the fact that the flock used in the present study was fed a sub-optimum level of vitamin A which tend to deplete reserves that the birds may have had prior to the start of the trial.

It was noted that a decreasing trend in the vitamin A content of succeeding eggs within the clutch prevailed, and that the 4-, 5-, and 6-egg clutches exhibited a sharper average

daily rate of decrease than the 10-29-egg clutches. Since the birds laying the longer clutches deposit more vitamin A in the yolk and at a smaller rate of decrease from egg to egg, they must provide vitamin A for the eggs in one, or more, of three ways: draw upon reserves; eat more feed; or be more efficient in the absorption, utilization, and secretion of vitamin A into the egg than the hens laying the short clutches.

The variations in the size of clutch (4-29 eggs), in the vitamin A content of the eggs (29-55 micrograms vitamin A/yolk), and in the rate of decline of the vitamin A content (0.67-1.21 micrograms vitamin A/day), all point to a high degree of individuality of the laying hen, each having its own capacity to produce a certain number of eggs containing a certain quantity of vitamin A.

Comparison of the variations in the vitamin A level of eggs from clutch to clutch laid by the same hen owes its origin to an opportunity which occurred due to an error made in feeding an abnormally low level of carotene as the vitamin A supplement to the laying flock. It is not believed that any serious damage was done to the birds since they were fed this ration only a relatively short time and the production of the individual hens was between 73-90 percent. A 15-50 percent decrease of the amount of vitamin A stored in the eggs from the early as compared to those of the later clutches might be due to the fact that some of the hens had just started production before the eggs in the earlier clutches were collected. This supposition could not be confirmed since there no production records kept

prior to February 1. Another possibility is that stored vitamin A might have been available for secretion into the eggs of the earlier clutches but was exhausted before the eggs of the later clutch were laid. It is common knowledge that the carotenoid pigments in the beak and legs are resorbed as the hen goes into heavy egg production. A similar mechanism might have functioned in the case of vitamin A.

Continuation of the rate of decrease revealed by this study probably would lower the vitamin A level in the eggs to a level that might affect hatchability of the eggs and the health of the newly hatched chicks. To what extent the vitamin A content of egg eventually would decrease is not known. Nestler (14) found that the eggs laid by quail in the early part of the season had better hatchability than those laid in the later part of the season. He reasoned that the vitamin A content of eggs played a part in the hatchability of eggs, since the content of this vitamin also decreased in the eggs as the season progressed. Studies have been made of the requirements and utilization of vitamin A for hatchability and growth by various investigators. Holmes et al. (35), reported six-hour chick livers contained 12 blue units of vitamin A compared to 58 blue units of vitamin A in the unabsorbed yolk. Bearse and Miller (36) found that 500 Sherman-Munsell vitamin A units per 100 grams of feed in the breeding hen ration sufficient vitamin A for maximum hatchability. Suomalainen (37) reported that the chick embryo used 350 I.U. or 80 percent of the vitamin A in the yolk during the last two weeks of incubation. If this amount of

vitamin A is required for chick development, it is probable that the eggs collected in the present study which contained low amounts of vitamin A might not hatch. Studies now in progress should provide valuable information on hatchability of eggs with low vitamin A content.

DETERMINATION OF STATE OF VITAMIN A IN EGG YOLK

Experimental Results

By means of the chromatographic procedure previously described, the determination of the distribution of vitamin A alcohol and vitamin A ester in the yolks of eggs from hens on the low pigment feed containing sub-optimum levels of vitamin A indicated that the vitamin A ester content of eggs ranged from 9-20 percent (average 15 percent) of the total vitamin A, Table 11. These data indicated that, for the most part, the egg contains the vitamin A in the form of the alcohol.

Discussion

It is of interest to compare the average vitamin A ester content of egg yolk (15 percent) with values obtained on some other animal products. Hoch and Hoch (38) reported that human serum contains 10-17 percent vitamin A ester; Parrish et al. (25) found normally blood serum of the dairy cow contained 10 percent and calf liver, 70-96 percent vitamin A ester; Parrish

Table 11. The vitamin A ester and vitamin A alcohol content of egg yolk.

Hen no.	Total vitamin A per g of egg yolk	Vitamin A ester per g of egg yolk	Vitamin A alcohol in egg yolk
	micrograms		percent
3981	2.77	.236	91.5
3981	2.56	.230	91.0
3980	2.20	.292	87.2
3980	2.09	.286	86.3
----	3.13	.434	86.1
3979	2.38	.334	86.0
3982	3.48	.514	85.2
3976	3.01	.471	84.4
3972	1.91	.319	83.3
3995	4.72	.790	83.3
3979	2.39	.411	82.8
3977	2.46	.426	82.7
3982	3.40	.615	81.9
----	2.32	.480	79.3

et al. (39) found colostrum contained 97 percent of the vitamin A as the ester. The vitamin A of fish liver oil and fish liver oil concentrates is about 93-98 percent vitamin A ester, Reed et al. (40).

Although chromatographic separation indicated that the vitamin A in egg yolk was predominately in the alcohol form, there exists that possibility that this alcohol was conjugated with protein in the egg yolk; the alcoholic form of vitamin A was freed in the process of denaturation, which proceeded extraction of the vitamin.

The distribution of the alcoholic and esterified forms of vitamin A in the egg may be compared with that of carotenoids. About 10 percent of the total carotenoids of eggs has been shown to be the hydrocarbon, carotene, Gillam and Heilbron (7) and Peterson et al. (20), while the rest is made of the carotenols, luteol, zeaxanthol, and cryptoxanthol, plus small amounts of other hydroxy- and oxy- containing pigments. The principal form of vitamin A in the yolk, the alcoholic form, likewise contains a hydroxy group, whereas the esterified form which has a solubility similar to carotene, occurred to the extent of 9-20 percent in the yolk. The similarity of carotene and vitamin A ester in physical and chemical properties might be the explanation for their being in the same ratio in the egg.

The work of Parrish et al. (25) with cattle and Hoch and Hoch (38) with human blood serum indicated that the vitamin A ester increased in the blood when large doses of the vitamin A of either the alcoholic or esterified form was administered.

From this it might be suspected that vitamin A in the blood of the hen, and possibly also in the egg, would increase in proportion of vitamin A ester when high levels of vitamin A are fed. The literature did not reveal any data that indicates the state of vitamin A in the blood serum of the hen. Further work is required to establish the state of vitamin A in blood of the hen and how the state of vitamin A in the blood is related to that of the egg. The feeding of vitamin A ester, vitamin A alcohol and carotene as the only source of vitamin A to individual hens and concurrent determinations of the form of vitamin A in the egg, in the blood, and in the storage organs, might indicate the manner in which these various forms of vitamin A are utilized.

SUMMARY

1. The range of vitamin A in eggs from hens on experimental ration was from 19.2 to 64.4 micrograms per yolk.

2. In most cases, the amount of vitamin A deposited in the egg tended to be less for each succeeding egg within the clutch.

3. Vitamin A content of eggs expressed as micrograms per yolk in many cases indicated the variations better than when expressed as micrograms per gram.

4. The data indicated that the vitamin A content of eggs tended to decrease at a more rapid rate in short clutches than in long clutches.

5. Analysis of the first egg of the subsequent clutch usually indicated a slight increase of vitamin A content over that of the last egg of the previous clutch.

6. Data on the analyses of eggs from clutches laid February 11-20, and from March 24-April 14, showed 15-50 percent decreases in the vitamin A content of eggs.

7. On an average, the hens laying the longer clutches deposited more vitamin A in the egg than hens laying the shorter clutch.

8. Analyses of eggs from hens on the experimental ration indicated a vitamin A ester content of 9-20 percent (average 15 percent) of the total vitamin A present.

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LITERATURE CITED

(1) McCollum, E. V. and M. Davis.
The necessity of certain lipins in the diet during growth. Jour. Biol. Chem. 15:167-75. 1913.

(2) Osborne, T. B. and L. B. Mendel.
The influence of cod liver oil and some other fats on growth. Jour. Biol. Chem. 17:401-8. 1914.

(3) Murphy, J. C. and D. B. Jones.
Vitamin A content of fresh eggs. Jour. Agr. Res. 29: 253-7. 1924.

(4) Bethke, R. M., D. C. Kennard, and H. L. Sassman.
The fat-soluble vitamin content of hen's egg yolk as affected by the ration and management of the layers. Jour. Biol. Chem. 72:695-706. 1927.

(5) Euler, H. V. and E. Klussman.
Vitamin A and growth action of egg yolk. Z. physiol. Chem. 208:50-4. 1932. (From Chem. Abs. 26:4360. 1932)

(6) Carr, F. H. and E. A. Price.
Color reactions attributable to vitamin A. Biochem. Jour. 20:497-501. 1926.

(7) Gillam, A. E. and I. M. Heilbron.
Vitamin A active substances in egg yolk. Biochem. Jour. 29:1064-7. 1935.

(8) Mann, B. T.
Separation of vitamin A from xanthophyll in the presence of egg sterols. Analyst 68:233-8. 1943. (From Chem. Abs. 38:5318. 1944)

(9) Sjollema, B. and W. F. Donath.
The vitamin A, carotene, and xanthophyll contents of the yolk of hen eggs. Biochem. Jour. 34:736-48. 1940.

(10) Baumann, C. A., J. Semb, C. E. Holmes, and J. G. Halpin.
The determination of vitamin A in the hen egg. Poultry Sci. 18:48-53. 1939.

(11) Koenig, M. C., M. M. Kramer, and L. F. Payne.
Vitamin A content of eggs as related to rate of production. Poultry Sci. 14:178-82. 1935.

(12) Zaborowski, G. M.
Vitamins and the hatching of chicks. Jour. Agr. prat., 97:69-71. 1933. (From Chem. Abs. 27:3239. 1933)

(13) Sherwood, R. M. and G. S. Fraps.
Amount of vitamin A potency required by hens for egg production. Tex. Agr. Expt. Sta. Bull. 493:5-22. 1934.

(14) Nestler, R. B.
Vitamin A in diet of Bob Whites. Nutr. Rev. 5:287. 1947.

(15) Thomas, B. H. and F. W. Quackenbush.
The effect of diet on the quantity of vitamin A and D occurring in hen eggs. Ia. Agr. Expt. Sta. Rept. 27. 1933.

(16) Russell, W. C. and M. W. Taylor.
The relation between vitamin A and D intake by the hen and the output in eggs. Jour. Nutr. 10:613-23. 1935.

(17) Cruickshank, E. M. and T. Moore.
The effect of the administration of large amounts of vitamin A on the vitamin A content of hen eggs. Biochem. Jour. 31:179-87. 1937.

(18) Deuel, H. J., M. C. Hrubetz, F. H. Mattson, M. G. Morehouse, and A. Richardson.
The effect of vitamin A intake on the carotenoid and vitamin A content of the eggs, liver, blood and body fat of hens. Jour. Nutr. 26:673-85. 1943.

(19) Bohren, B. B., C. R. Thompson, and C. W. Carrick.
The transfer of carotenoid pigments to the egg yolk. Poultry Sci. 24:356-62. 1945.

(20) Peterson, W. J., J. S. Hughes, and L. F. Payne.
The carotenoid pigments. Kan. Agr. Expt. Sta. Tech. Bull. 46. 1939.

(21) Thompson, C. R., M. A. Swan, S. M. Hauge, B. B. Bohren, and F. W. Quackenbush.
Chemical determination of vitamin A in dried whole eggs. Ind. Eng. Chem. 18:113-5. 1946.

(22) Schrenk, W. G., D. S. Chapin, and R. M. Conrad.
Determination of vitamin A in dehydrated eggs. Ind. Eng. Chem., Anal. Ed. 16:632-4. 1944.

(23) Hauge, S. M. and F. P. Zscheile.
Effect of dehydration on the vitamin A content of eggs. Science 96:536. 1942.

(24) Klose, A. A., G. I. Jones, and H. L. Fevold.
Vitamin content of spray-dried whole egg. Ind. Eng. Chem. 35:1203-5. 1943.

(25) Parrish, D. B., J. [illegible]. Wise, and J. S. Hughes.
Effect of vitamin A supplements upon the state of vitamin A in blood serum of dairy cow and in blood serum and liver of its neonatal calf. Jour. Biol. Chem. 172: 355-65. 1948.

(26) Boyer, P. D., R. Spitzer, C. Jensen, and P. H. Phillips.
Determination of vitamin A and carotene in milk. Ind. Eng. Chem., Anal. Ed. 16:101-2. 1944.

(27) Koehn, C. J. and W. C. Sherman.
The determination of vitamin A and carotene with photoelectric colorimeter. Jour. Biol. Chem. 132:527-538. 1940.

(28) Dann, W. J. and K. A. Evelyn.
The determination of vitamin A with the photoelectric colorimetor. Biochem. Jour. 32:1008-17. 1938.

(29) Boyer, P. D., P. H. Phillips and J. K. Smith.
Separation of carotene from vitamin A for the determination of vitamin A in blood plasma. Jour. Biol. Chem. 152:455-62. 1944.

(30) Kimble, M. S.
The photoelectric determination of vitamin A and carotene in human plasma. Jour. Lab. and Clin. Med. 24: 1055-65. 1939.

(31) Caldwell, M. J. (Unpublished data) 1943.

(32) Johnson, R. M. and C. A. Baumann.
Studies on the reaction of certain carotenoids with antimony trichloride. Jour. Biol. Chem. 169:83-90. 1947.

(33) Vermes, M., P. Meunier, and Y. Raoul.
Poor reserves of vitamin A in hens eggs and in the liver of young chickens. Compt. rend. 209:578-81. 1939. (From Chem. Abs. 34:4768. 1940)

(34) Russell, W. C.
Vitamin A in eggs. New Jersey Agr. 16, No. 2. 1934. (From Chem. Abs. 29:1465. 1935)

(35) Holmes, A. D., F. Tripp, and P. A. Campbell.
Vitamin A reserves of embryo and baby chicks. Jour. Nutr. 11:119-28. 1936.

(36) Bearse, G. E. and M. W. Miller.
The effect of varying levels of vitamin A in the hen ration on the vitamin A content of the egg yolk, on hatchability, and on chick livability. Poultry Sci. 16:39-43. 1937.

(37) Suomalainen, P.
Vitamin A and carotene in the hen egg during hatching. Suomen Kemistilehti 12B:30. 1939. (From Chem. Abs. 34:145. 1940)

(38) Hoch, H. and R. Hoch.
The state of vitamin A in human serum. British Jour. Expt. Path. 27:316-28. 1946.

(39) Parrish, D. B., G. H. Wise, and J. S. Hughes.
The state of vitamin A in colostrum and in milk. Jour. Biol. Chem. 167:673-8. 1947.

(40) Reed, G., E. C. Wise, and R. J. L. Frundt.
Quantitative separation of alcohol and ester forms of vitamin A. Ind. Eng. Chem. 16:509-10. 1944.