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The Vitamins A and D Activity of Egg Yolks of Different Color Concentrations

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This study was undertaken in collaboration with Professor Harry L. Kempster of the Poultry Department of the University of Missouri.

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ABSTRACT

A comparison has been made of the vitamins A and D activity of egg yolks of definite and uniform color concentrations produced by hens under carefully controlled conditions as to rations and housing, and an attempt made to correlate such activity with the carotinoid pigments. A method is described for the determination of the carotene and xanthophyll content of egg yolks. The vitamin A tests have shown: (1) that while there seems to be some relationship between color and growth, the vitamin A activity of egg yolks cannot be explained on the basis of the carotinoid pigments they contain; (2) and that the vitamin A activity of egg yolks is directly dependent upon the rations of the hens. The vitamin D tests have shown that the rations of the hens used in these experiments had but little effect upon the deposition of calcium in the bones of the rats.

The Vitamins A and D Activity of Egg Yolks of Different Color Concentrations

BERTHA BISBEY, VIRGINIA APPLEBY*, ADELIA WEIS*, SYLVIA COVER

Eggs are important sources of protein, fat and minerals as well as vitamins and since they are available at all seasons of the year they should constitute an important item in family dietaries. They are of primary importance in special diets and in the diets of infants and young children. They are also an important source of income and anything that can be done to enhance their value is of interest to producer and consumer alike. The standards that have been set up for the grading of eggs are based upon such qualities as freshness and physical characteristics. Bethke, Kennard and Sassaman[1]† report a relationship between the vitamins A and D content of the ration of the hen and the occurrence of these vitamins in egg yolks, and in discussing the qualities which determine the relative market price, make the following observation: "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 egg 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 adequate ration and having access to suitable outdoor range or its equivalent throughout the year."

Consumers differ as to their preferences for light, medium or dark colored egg yolks. In this connection, the suggestion made by Kempster[2] that "There may be a close relationship between the degree of yellow color in the yolk and the vitamin A content of the egg," is worthy of investigation.

The experiments described here were designed to determine: (1) the vitamins A and D potency of egg yolks of different color concentrations, produced by hens under carefully controlled conditions; (2) the carotene and xanthophyll content of such egg yolks.

*A part of the data contained in this paper was submitted by Virginia Appleby and Adelia Weis in partial fulfillment of the requirements for the degree of Master of Arts in the Graduate School of the University of Missouri, 1933.

†Numerals refer to Bibliography, Page 32.

PART I. VITAMIN A EXPERIMENTS

Introduction

Since Steenbock[3], in 1919, reported a close correlation between yellow pigments and vitamin A, much attention has been directed to study of plant and animal pigments in relation to their vitamin A activity. These investigations have been excellently reviewed by Sherman and Smith in their monograph "The Vitamins."[4] Accordingly reference will be made to such papers, only, as have direct connection with the study here reported.

Palmer and Kempster[5] showed that chicks could be raised to maturity on a carotinoid-free diet and yet be normal in all respects, except for the lack of color in the egg yolks, body fat, and skin. Palmer, Kennedy and Kempster[6] secured normal growth and reproduction in rats on a diet rich in vitamin A, but practically devoid of yellow pigments. They agreed however, with Steenbock, that yellow pigments and vitamin A are in some way related, but are not the same substance.

Hauge and Trost[7]; Russell[8]; and Fraps[9] have reported that the vitamin A content of yellow corn seems to be directly related to the amount of pigmentation present.

Moore[10] suggested and presented evidence to show that carotene is a precursor substance synthesized in plants, changed to vitamin A in the animal body and stored. His rats received graded portions of carotene in addition to a basal diet adequate in all respects other than vitamin A. Colorimetric and spectrographic examinations, at the close of the experimental period, showed that the greater part of the carotene had been converted into colorless vitamin A and stored in the liver of the animal. The results of these experiments led Capper, McKibbin, and Prentice[11] to determine the possibility of a similar conversion in fowls. Their results showed that chickens as well as rats have the power of converting carotene into vitamin A.

The presence of xanthophyll has been observed in green leaves, yellow corn, and egg yolk. Carotene and xanthophyll are closely associated in plants and are present in the ratio of 0.6 to 1 in green plants according to von Loesecke[12]. The amount of yellow color deposited in egg yolks is directly dependent on the amount of xanthophyll in the ration of the hen, therefore the amount of yellow corn or green, leafy vegetables in the ration controls the depth of yolk color.

Experiments in which xanthophyll was fed to rats as the sole source of vitamin A gave negative results[13]. The xanthophyll used in these feeding experiments was from plant sources. No study has been found of the vitamin A potencies of egg yolks of definite and uniform colors which also shows the relative quantities of carotinoids in the egg yolks tested. In this investigation egg yolks of three distinct color concentrations, light, medium and dark were used as sources of vitamin A and an attempt was made to correlate the vitamin A activity with the amounts of carotinoid pigments they contained.

Experimental Procedure

In general the method developed and described by Sherman and his co-workers[14] for the quantitative determination of vitamin A was used.

Experimental Animals.—The animals used in the experiments herein reported were young, growing, albino rats reared by mothers on a diet consisting of one-third, by weight, whole milk powder, two-thirds ground whole wheat, with sodium chloride, two per cent of the weight of the wheat.

Such young animals, separated from their mothers at 28 to 29 days of age have been shown to have a considerable store of vitamin A. In order to free the bodies of such reserves a fore-period in which the animals received the vitamin-A-free diet alone was observed. When the rats had reached constant weight and symptoms of ophthalmia had appeared they were divided into groups, care being taken to distribute them as uniformly as possible in relation to weight and sex, and placed in individual wire cages with raised buttons to prevent coprophagy. Only those weighing from 65 to 100 grams were used in the tests. One rat of each litter was continued on the vitamin-A-free diet as a negative control. The others received graded portions of egg-yolks as a source of vitamin A. All received the vitamin-A-free diet and distilled water ad libitum. The test period was continued for eight weeks beyond the depletion period. At the end of the experimental period the surviving animals were killed and autopsied.

The Basal Diet.—The basal diet as used in this investigation was of the following composition:

Casein (freed from fat-soluble vitamins by triple extraction with boiling 95% alcohol)	18%
Salt mixture (Osborne and Mendel)	4%
Sodium chloride	1%
Dried brewers' yeast	10%
Corn starch	67%

Viosterol* was used as a source of vitamin D. Cover[15] had shown that a 0.5 per cent solution of viosterol (250 D) in cottonseed oil when fed at a level of 1 drop (0.0203 gm.) per rat per day was ample to protect from vitamin D deficiency. She dropped the viosterol from a microburette directly into the mouths of the rats. In the preparation of the basal diet the viosterol was dropped from a microburette upon a small portion of the salt mixture, triturated until the mixture was homogeneous and then added to the remaining components of the diet and mixed thoroughly. It was estimated that a rat would eat an average of 7 grams of the basal diet per day.

Sources of Vitamin A.—The eggs used in these experiments were supplied by the Poultry Department of the University of Missouri. They were produced by White Rock hens, of known dietary history, under carefully controlled conditions. The hens were fed the following all-mash rations:

	Pen 25 lbs.	Pen 26 lbs.	Pen 27 lbs.	Pen 15 lbs.
Ground wheat	15.0†	15.0	15.0	15.0
Wheat shorts	4.5	4.5	4.5	4.5
Bran	----	5.0	5.0	5.0
Meat scrap	8.0	8.0	8.0	8.0
Dried skim milk	2.0	2.0	2.0	2.0
Sodium chloride	0.5	0.5	0.5	0.5
Yellow corn	65.0	65.0	25.0‡	25.0
White corn	----	----	40.0‡	40.0
Cod liver oil	----	----	----	0.5
Alfalfa leaf meal	5.0†	----	----	----

†Changed from 15 to 10 per cent whole wheat and from 5 to 10 per cent alfalfa leaf meal January first.

‡Changed from 25 to 35 per cent yellow corn and from 40 to 30 per cent white corn January first.

A weekly supply of eggs was delivered at the laboratory regularly. They were stored in tightly closed containers in a refrigerator at a temperature of about 35°-40° F. until needed for feeding. A few eggs from each delivery were dipped in paraffin oil and stored for use in the determination of carotene and xanthophyll.

The band number of the hen was placed on the egg before she was released from the trap nest. This was important since care was taken to use eggs from different hens in the same group in making up composite samples of yolks.

*The Viosterol used in these experiments was obtained through the courtesy of Mead Johnson and Company.

Feeding the Supplements.—Since vitamin A is stored in the body of the rat and the amount of egg yolk needed is so small, it was fed twice weekly. On the days of feeding three eggs from each group were removed from the refrigerator and allowed to come to room temperature. The yolks were separated from the whites, care being taken to remove all visible traces of whites. A representative part of each yolk was removed by means of a pipette and transferred to a small container.

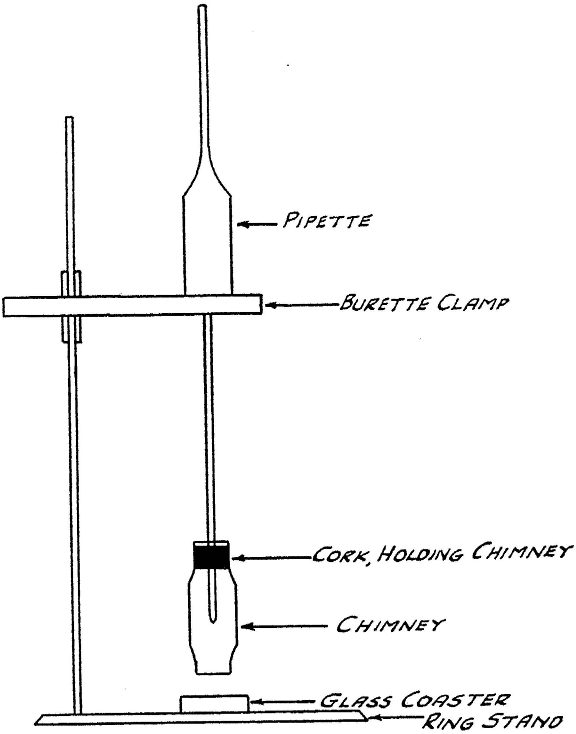

Fig. 1.—Diagram of device used for obtaining drops of egg yolks of near-constant weight, for use as supplements.

The composite sample was drawn into a standardized pipette which delivered a drop weighing 0.0312 ± 0.0001 gram. Drops were collected on individual glass coasters and fed immediately. The pipette tube was fitted through a glass chimney, thus forming a dead air pocket around the tip of the pipette. This chamber lessened the drying of egg yolk as it dropped from the pipette and the weight of the drop was much more uniform than was found in preliminary tests without the chimney. A diagram of the device is shown in Figure 1.

Depth of color and weight of representative drops were obtained when the supplement was fed.

The color of a composite sample of egg yolk was compared with ten milliliter samples of potassium dichromate solutions, each containing 0.1 gram of talc. A range of color was obtained by using solutions of the following concentrations: 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.12 normal.

The Determination of the Carotene* and Xanthophyll Content of Egg Yolks.†—[16-17-18-19-20] The method here described is a modification of the method developed by Schertz [21, 22] for the extraction and separation of the green and yellow pigments in plants.

The yolks of three eggs were carefully separated from the whites, the membranes removed and the yolks thoroughly mixed. One to two milliliters of methyl alcohol were poured over a 5-gram sample of the mixture and stirred vigorously until the yolk had coagulated into small particles. Acetone was used to extract the pigments from the sample. Several 20 milliliter portions were used before the yolk became colorless. Ethyl ether was used for the final washing. Ten milliliters of 20 per cent potassium hydroxide in methyl alcohol were added to the total volume and the mixture refluxed slowly for one hour. The solution was cooled and transferred to a separatory funnel. 50 to 75 milliliters of ethyl ether were added and then water of a volume somewhat greater than that of the alkaline acetone solution. After washing with water and filtering through anhydrous sodium sulfate, the solution was evaporated under reduced pressure until no further evaporation took place below 50° C.

At this stage, sufficient unsaponifiable substances were present to cause the appearance of a viscous yellow oil. The success of the method depends upon the assumption that substances present, other than carotene and xanthophyll, are either colorless and do not interfere with colorimetric comparisons or are constant in the amount of color they introduce, so that comparative, if not strictly quantitative, results may be obtained.

The residue was taken up in 50 to 75 milliliters of 90 per cent methyl alcohol and 50 milliliters of petroleum ether. It was trans-

*It is recognized that the yellow pigment referred to here as carotene may not be identical with the carotene of plant pigments.

†Acknowledgment is made to Laurence Ryden for assistance in the determination of the carotene and xanthophyll content of the egg yolks.

ferred to a separatory funnel and the methyl alcohol layer, containing xanthophyll was drawn off. The petroleum ether layer containing carotene was washed with 90 per cent methyl alcohol until the last washing was colorless. The combined alcohol solution and washings were washed with petroleum ether. The petroleum ether washings were added to the carotene fraction and washed with water. It was evaporated under reduced pressure until only a few oily drops remained. Ten milliliters of ethyl alcohol, containing 10 per cent petroleum ether, were added and the solution compared colorimetrically with a standard carotene solution, containing 2 milligrams of carotene per liter. The standard was set at 15 millimeters.

The xanthophyll fraction in methyl alcohol was transferred to a separatory funnel. About 50 milliliters of ethyl ether were added and enough water to cause the ether solution to separate readily. One extraction was usually sufficient. The ether solution was washed with water to remove any remaining alcohol, filtered through anhydrous sodium sulfate and evaporated under reduced pressure. The oily residue containing a few crystals was dissolved in 10 or 20 milliliters of ethyl alcohol and compared colorimetrically with a standard containing 10 milligrams of xanthophyll per liter. The standard was set at 10 millimeters.

To determine the effect of refluxing an alkaline acetone solution on carotene, samples were removed, from a mixture of 10 milliliters of a standard carotene solution (10 milligrams per liter), 40 milliliters of acetone, and 10 milliliters of 20 per cent potassium hydroxide in methyl alcohol, as it slowly refluxed. No significant differences were noted in samples taken after 0, 30, 60 and 80 minutes had elapsed.

Results and Discussion

The average color of composite samples of egg yolks used in these tests corresponds to the following concentrations of potassium dichromate solutions: Dark (Pen 25, 65 per cent yellow corn plus 10 per cent alfalfa leaf meal) 0.1 N; medium (Pen 26, 65 per cent yellow corn) 0.07 N; light-1 (Pen 27, 35 per cent yellow corn) 0.05 N; light-2 (Pen 15, 25 per cent yellow corn plus 0.5 per cent cod liver oil) 0.04 N.

The different colored egg yolks, used in the tests reported here, will be designated as dark, medium, light-1 and light-2. The dark were from Pen 25, the medium from Pen 26, the light-1 from Pen 27, and the light-2 from Pen 15.

The weights of drops of composite samples of yolks were determined at frequent intervals throughout the tests, when the pipette was full, half full, and about empty. The weights were exceedingly uniform, the average being 0.0312 ± 0.0001 gram per drop.

The average length of time required to deplete the rats of their stores of vitamin A was 36 days. This is in close agreement with the time 38.8 days, required for the depletion of rats in the vitamin A experiments reported by Munsell.[23]

The average survival period of 51 negative controls was 24 days, during which time the average loss in weight was 26 grams. the results are shown in Figures 2, 3, and 4, Table I, Groups 1 and 2, and Table IV (page 20).

Munsell[23] reported that the yolks of market eggs, fed at a level of 0.02 gram per rat per day 6 days per week corresponding to 0.12 gram per rat per week, furnished sufficient vitamin A for unit growth. Preliminary experiments, in this investigation, in which light-1 and medium egg yolks were fed at levels of 4 drops (0.125 gm.), 6 drops (0.19 gm.), 8 drops (0.25 gm.) and 12 drops (0.37 gm.), per rat per week, respectively, showed; (1) that these amounts are insufficient to support unit growth; (2) and that medium yolks are a more potent source of vitamin A than light-1 yolks. The results (8 drops and 12 drops) are given in Figure 2, Tables II and III, Groups 1 and 2.

Accordingly the medium yolks were fed at levels of 16 drops (0.50 gm.), 24 drops (0.75 gm.) and 36 drops (1.12 gm.) and the light-1 yolks 32 drops (1.0 gm.), 48 drops (1.50 gm.) and 72 drops (2.25 gm.), per rat per week, respectively. The results show that 24 drops (0.75 gm.) per week or 0.107 gram per rat per day of medium yolks induced an average gain of 29 ± 1.8 grams in 8 weeks and 32 drops (1.0 gm.) per week or 0.143 gram per rat per day of light-1 yolks induced an average gain of 28 ± 1.4 grams in 8 weeks. The results are given in Figure 3, Tables II and III, Groups 3, 4 and 5.

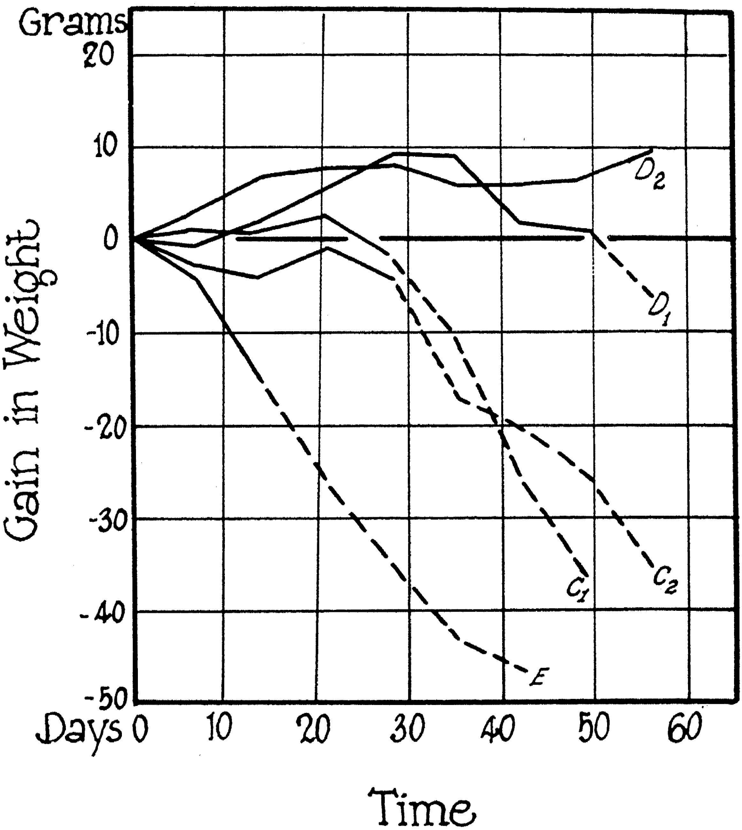

Fig. 2.*—Average gain curves of rats receiving the basal diet alone and the basal diet plus graded portions of either light-1 or medium egg yolks. January 1 to April 15.

1. Light-1 egg yolks, color corresponding to 0.05 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:
C_1, 8 drops (0.25 gm.) C_2, 12 drops (0.37 gm.).

2. Medium egg yolks, color corresponding to 0.07 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:
D_1, 8 drops (0.25 gm.) D_2, 12 drops (0.37 gm.).

3. Basal diet alone. E. Negative controls.

*Data for Figure 2 are shown in Table I, Group 1; Table II, Groups 1 and 2; and Table III, Groups 1 and 2. A broken line indicates the point at which one or more of the animals died.

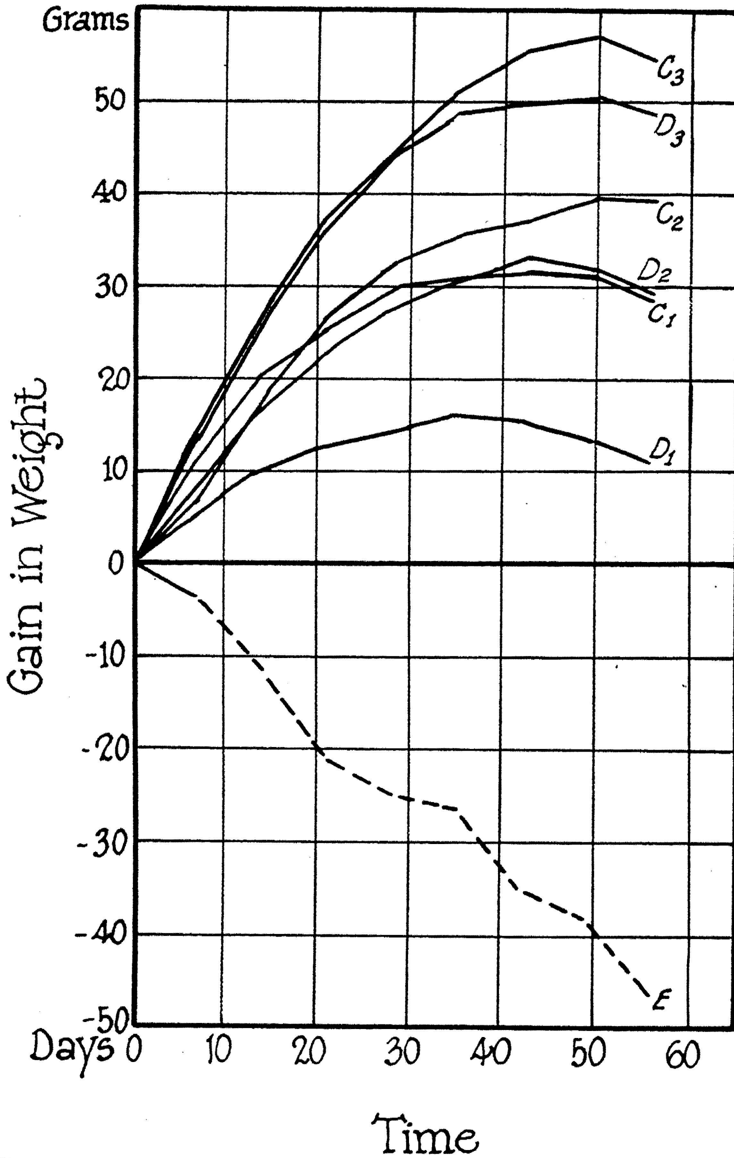

Fig. 3.*—Average gain curves of rats receiving the basal diet alone and the basal diet plus graded portions of either light-1 or medium egg yolks. March 15 to July 6.

1. Light-1 egg yolks, color corresponding to 0.05 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:

C_1, 32 drops (1.00 gm.) C_2, 48 drops (1.50 gm.). C_3, 72 drops (2.25 gm.).

2. Medium egg yolks, color corresponding to 0.07 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:

D_1, 16 drops (0.50 gm.) D_2, 24 drops (0.75 gm.) D_3, 36 drops (1.12 gm.).

3. Basal diet alone. E. Negative controls.

*Data for Figure 3 are shown in Table I, Group 2; Table II, Groups 3, 4, and 5; and in Table III, Groups 3, 4 and 5. A broken line indicates the point at which one or more of the animals died.

TABLE I.*—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET ALONE

Group 1. Negative Controls. January 1 to April 15

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams								Final wt. gm.	Total gain gm.	Total food intake gm.
			1	2	3	4	5	6	7	8			
5343♀	38	78	−4	−15	------	------	------	------	------	------	59	−19	84
5350♂	47	88	−7	−1	2	−16	−9	------	------	------	57	−31	137
5450♀	44	111	−3	−18	−14	------	------	------	------	------	76	−35	113
5453♂	43	90	2	−12	−11	------	------	------	------	------	69	−21	56
5464♀	59	100	−1	−20	------	------	------	------	------	------	79	−21	98
5468♂	46	100	−9	−11	------	------	------	------	------	------	80	−20	79
5517♂	38	107	−16	−15	------	------	------	------	------	------	76	−31	57
5514♀	40	100	±0	−3	−16	−11	------	------	------	------	70	−30	168
5591♂	33	70	−5	3	------	------	------	------	------	------	68	−2	39
5605♀	44	98	−3	−7	−11	------	------	------	------	------	77	−21	97
5608♂	41	113	−13	−21	------	------	------	------	------	------	79	−34	40
5634♀	40	81	−2	1	−8	−3	−6	−3	------	------	60	−21	217
5622♀	46	80	±0	−20	−11	------	------	------	------	------	49	−31	84
Av.	43	93.5	−4.7	−10.7	−9.8	−10	−7.5	−3.0	------	------	69.1	−24.4	98

Group 2. Negative Controls. March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5664♂	45	110	−8	−14	−15	------	------	------	------	------	73	−37	92
5673♀	46	102	6	−4	−24	−4	------	------	------	------	76	−26	154
5677♂	50	121	1	−4	−23	−17	------	------	------	------	78	−43	135
5710♂	50	92	−9	−18	------	------	------	------	------	------	65	−27	64
5721♂	53	124	−7	−24	−11	−12	------	------	------	------	70	−54	219
5732♀	38	85	−8	−9	−8	------	------	------	------	------	60	−25	79
5735♀	52	120	−12	−4	−5	8	−3	−22	------	------	82	−38	238
5760♀	33	72	1	−12	−11	------	------	------	------	------	50	−22	68
5790♀	37	68	−4	−5	−13	------	------	------	------	------	46	−22	84
5770♂	41	83	−6	−12	−8	−2	------	------	------	------	55	−28	97
5776♂	44	116	−8	−22	−8	------	------	------	------	------	78	−38	75
5798♀	28	70	−2	−3	−10	------	------	------	------	------	55	−15	83
5813♀	37	70	−4	−2	2	−6	±0	1	−5	−9	47	−23	210
5822♀	30	60	−1	3	−6	1	±0	−6	−6	−8	37	−23	224
5926♂	39	66	−4	−2	−1	−5	−6	------	------	------	48	−18	160
5955♂	55	94	6	3	−11	1	1	−8	−1	−10	75	−19	258
5969♂	32	72	−3	−6	−3	−7	------	------	------	------	53	−19	92
5934♂	27	73	−13	------	------	------	------	------	------	------	60	−13	21
5941♂	36	83	−4	−1	−12	−8	------	------	------	------	58	−25	140
5977♀	45	72	1	−4	−8	4	−3	−7	−2	−6	47	−25	235
Av.	40.9	87.6	−3.9	−7.4	−9.7	−4.0	−1.8	−8.4	−3.5	−8.2	60.6	−27	136
											P.E.	±1.50	
											C.V.	36.8%	

*Average gain curves for Group 1, Table I shown in Figure 2 and for Group 2, Table I in Figure 3.

TABLE II.—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET SUPPLEMENTED BY GRADED PORTIONS OF LIGHT-1 EGG YOLKS, COLOR CORRESPONDING TO 0.05 N POTASSIUM DICHROMATE SOLUTION

Group 1.* Basal Diet plus 8 Drops (0.25 gm.) Egg Yolk per Week
January 1 to April 15

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams: 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5336♂	41	82	−6	−7	2	−13	------	------	------	------	58	−24	146
5447♂	41	86	3	−1	−7	−8	------	------	------	------	73	−13	144
5463♂	56	94	7	−1	7	−6	−9	------	------	------	92	−2	261
5470♀	41	86	4	3	4	1	−8	−14	------	------	76	−10	132
5513♀	43	96	−5	8	12	4	−6	−36	−5	------	68	−28	287
5594♀	34	82	−3	5	−4	−5	−13	------	------	------	62	−20	177
5609♂	39	106	−6	1	2	−3	−12	−15	------	------	73	−33	210
5631♀	43	96	1	1	±0	−5	−3	−5	−15	------	70	−26	290
5621♂	43	92	−1	±0	2	−4	−10	−5	−13	------	61	−31	257
Av.	42.3	91.1	−0.7	1.0	2.0	−4.3	−8.7	−15.0	−11.0	------	70.3	−20.8	212

Group 2.—Basal Diet plus 12 Drops (0.37 gm.) Egg Yolk per Week
January 1 to April 15

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5588♂	37	91	−6	2	11	−16	------	------	------	------	82	−9	177
5610♀	38	102	−8	1	2	−6	−5	2	−3	−12	73	−29	270
5629♂	42	103	2	−9	−1	3	−15	−10	−5	------	68	−35	258
5633♀	42	89	1	2	−5	5	−2	−3	−8	−12	68	−21	276
5619♂	48	93	−3	−12	12	7	−29	------	------	------	68	−25	175
5623♀	42	79	−5	10	−1	−13	------	------	------	------	70	−9	161
Av.	41.5	92.8	−3.2	−1.0	3.0	−3.3	−12.7	−3.3	−5.3	−12.0	71.5	−21.3	220

*Average gain curves for Groups 1 and 2 shown in Figure 2.

Group 3.*—Basal Diet plus 32 Drops (1.0 gm.) Egg Yolk per Week
March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5672♀	47	107	6	2	6	3	−2	5	2	7	136	29	448
5685♀	41	103	10	5	2	1	3	2	7	±0	133	30	494
5711♂	48	100	7	2	5	3	−5	−5	±0	12	119	19	403
5724♀	47	114	−3	3	4	8	2	2	5	−2	133	19	493
5773♀	40	91	2	1	3	4	4	6	3	−4	110	19	347
5781♀	45	97	10	4	7	8	1	5	−1	−6	125	28	425
5808♂	44	90	8	11	4	1	2	−1	3	−3	115	25	353
5817♂	39	79	10	18	5	±0	5	−2	−2	±0	113	34	437
5928♂	34	77	18	15	11	3	3	−5	4	−11	115	38	476
5929♀	37	79	17	6	11	4	5	−5	5	−10	112	33	431
5960♀	47	64	12	10	6	1	2	2	−2	14	109	45	325
5964♂	39	82	22	18	9	3	3	−5	−1	−11	120	38	515
5936♀	26	70	17	10	10	1	−1	−1	−5	−6	95	25	403
5944♀	36	81	16	11	4	8	−7	5	−12	−12	94	13	465
5980♀	42	70	19	8	−2	7	5	1	−4	−11	93	23	370
5981♀	37	70	16	10	1	7	±0	7	−6	−2	103	33	392
Av.	40.6	85.9	11.7	8.4	5.4	3.9	1.2	0.7	−0.2	−2.8	114.1	28.2	424
											P.E.	±1.4	
											C.V.	29.3%	

*Average gain curves for Group 3, shown in Figure 3.

TABLE II.—(CONTINUED)

Group 4.*—Basal Diet plus 48 Drops (1.50 gm.) Egg Yolk per Week
March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5665♀	46	105	17	3	±0	3	6	6	±0	6	146	41	487
5671♂	43	95	−4	5	10	−1	6	3	−1	9	122	27	414
5682☿	48	93	8	7	7	2	7	3	8	6	141	48	398
5712♂	47	105	−7	−1	10	10	4	6	3	8	138	33	456
5722♀	50	115	−15	15	7	3	3	−4	1	14	139	24	465
5727♂	52	125	8	4	12	12	4	2	2	−2	167	42	521
5731☿	45	104	6	±0	8	4	5	6	3	−1	135	31	442
5734♂	47	112	−18	20	10	±0	11	15	4	−4	150	38	498
5736☿	49	113	±0	6	6	12	1	1	13	−9	143	30	476
5758☿	42	83	8	11	6	5	−5	−2	4	−3	107	24	358
5784♂	42	83	10	10	8	9	5	9	1	−1	134	51	403
5789♀	39	75	11	10	10	7	6	3	3	2	127	52	414
5774♀	39	85	10	6	5	5	5	2	5	−2	121	36	409
5780♀	42	98	9	9	9	9	4	−3	4	1	140	42	594
5816♂	41	83	23	25	15	11	3	2	−7	−1	154	71	532
5925♂	37	78	23	23	14	7	7	−3	7	−5	151	73	594
5961♀	44	62	11	16	8	2	3	±0	3	4	109	47	314
5966♂	35	74	9	15	16	4	4	−9	−1	−8	104	30	415
5935♂	26	89	13	12	16	3	−5	−15	−5	−3	105	16	403
5943♀	32	66	16	16	2	7	−3	3	2	−3	106	40	342
Av.	42.3	92	6.9	10.6	8.9	5.7	3.6	1.3	2.5	0.4	132	39.8	447
											P.E.	±2.1	
											C.V.	35.7%	

*Average gain curves for Group 4, shown in Figure 3.

Group 5.*—Basal Diet plus 72 Drops (2.25 gm.) Egg Yolk per Week
March 15 to July 6

5752♂	44	84	8	10	7	7	3	4	6	3	132	48	398
5757☿	42	78	17	15	12	9	5	0	7	−2	141	63	437
5786♂	40	72	13	15	3	3	5	3	4	1	119	47	364
5775☿	35	76	5	3	7	7	8	9	3	±0	118	42	387
5777♂	37	87	13	9	3	4	5	1	−5	−9	108	21	448
5809♂	43	102	17	14	13	16	13	7	−4	−11	167	65	538
5812☿	39	85	26	10	26	4	7	5	5	1	169	84	571
5819☿	38	70	20	20	13	6	7	5	−2	−5	134	64	566
Av.	39.7	81.7	14.9	12.0	10.5	7.0	6.6	4.2	1.8	−2.8	136	54.2	464
											P.E.	±4.2	
											C.V.	32.7	

*Average gain curves for Group 5, shown in Figure 3.

TABLE III.—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET SUPPLEMENTED BY GRADED PORTIONS OF MEDIUM EGG YOLKS, COLOR CORRESPONDING TO 0.07 N POTASSIUM DICHROMATE SOLUTION

Group 1.*—Basal Diet plus 8 Drops (0.25 gm.) Egg Yolk per Week
January 1 to April 15

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5340♂	35	73	3	−2	14	6	−5	6	2	−8	89	16	333
5351☿	52	93	5	3	−3	6	±0	4	4	−7	105	12	448
5446♂	44	97	−1	5	2	10	5	−9	±0	−15	94	−3	448
5460♂	57	94	1	5	5	−2	7	±0	−3	3	110	16	392
5469☿	42	76	2	±0	5	−5	4	3	2	−14	73	−3	358
5515☿	36	90	2	3	3	3	−3	−1	±0	−2	95	5	386
5606♀	42	105	−10	−2	4	6	−2	−29	−3	----	69	−36	224
5630♂	41	96	−3	5	−1	5	−6	−19	−6	------	71	−25	218
5626♀	37	84	−4	5	5	2	±0	−19	−4	−6	63	−21	285
Av.	42.9	89.8	−0.6	2.4	3.8	3.4	±0	−7.1	−0.9	−7	85.4	−4.3	344

TABLE III.—(CONTINUED)

Group 2.—Basal Diet plus 12 Drops (0.37 gm.) Egg Yolk per Week
January 1 to April 15

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5595♀	34	82	7	1	10	±0	−5	1	−3	3	96	14	435
5604♂	41	112	−1	3	±0	1	2	−5	−7	5	110	−2	348
5632♀	43	98	7	3	2	5	2	±0	5	2	124	26	324
5667♀	43	104	−6	10	−1	−5	−15	9	6	3	105	1	285
5670♂	44	98	5	±0	±0	−1	3	2	2	6	115	17	364
5684♀	43	100	6	3	−2	−2	2	−6	−1	1	101	1	380
Av.	41.3	99	3.0	3.3	1.5	−0.3	−1.8	0.2	0.3	3.3	108.5	9.5	356

*Average gain curves for Groups 1 and 2, shown in Figure 2.

Group 3.*—Basal Diet plus 16 Drops (0.50 gm.) Egg Yolk per Week
March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5662♂	48	114	7	3	±0	−4	±0	−4	7	7	130	16	392
5674♀	45	111	5	−1	4	−1	5	−2	2	7	130	19	437
5683♀	45	99	8	9	±0	1	3	7	±0	7	134	35	448
5715♀	46	96	−18	12	11	1	±0	−7	2	−1	96	±0	347
5723♀	48	107	3	5	7	2	4	−5	4	−1	126	19	426
5730♂	44	99	−6	−1	10	10	3	±0	−1	−1	113	14	358
5769♂	41	88	−1	±0	−4	2	7	−2	−4	−4	82	−6	291
5779♀	43	96	6	2	2	9	−4	4	±0	−5	110	14	397
5811♂	39	93	−4	14	2	−6	5	−2	−6	1	97	4	308
5821♀	34	75	11	6	5	7	1	−1	3	−4	103	28	448
5959♀	47	69	11	5	5	1	1	1	2	±0	95	26	280
5971♀	30	65	10	−3	2	±0	−1	−9	1	−8	57	−8	291
5938♀	27	80	8	10	±0	−1	−2	−10	−5	−7	73	−7	291
5940♂	37	80	2	±0	2	5	−1	−4	6	−5	85	5	291
5974♂	43	71	10	10	2	5	1	2	−8	−14	79	8	342
5976♀	46	76	15	6	2	±0	−3	1	1	−9	89	13	330
5972♂	49	80	23	3	1	−6	6	−1	−14	−10	82	2	347
Av.	41.9	88.2	5.3	4.7	3.0	1.5	1.5	−1.9	−0.6	−2.8	98.9	10.7	354
											P.E.	±2.0	
											C.V.	114.0%	

*Average gain curves for Group 3, shown in Figure 3.

Group 4.*—Basal Diet plus 24 Drops (0.75 gm.) Egg Yolk per Week
March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5666♀	44	94	7	9	−3	1	4	1	−2	4	115	21	381
5660♂	50	117	±0	3	4	3	5	9	9	5	155	38	431
5713♀	50	105	−8	15	5	2	6	1	±0	1	127	22	420
5725♀	43	115	3	7	7	±0	9	5	±0	−7	139	24	493
5726	53	123	8	−5	14	5	−2	8	−3	−1	147	24	403
5729♂	48	103	12	1	11	10	4	3	−7	−15	122	19	414
5756♀	43	89	9	7	6	6	1	−5	−3	−6	104	15	386
5788♀	39	73	6	9	6	9	2	4	1	1	111	38	381
5787♂	39	78	2	14	7	15	9	5	4	±0	134	56	386
5772♀	41	87	6	7	5	2	2	5	3	−5	112	25	370
5778♀	46	103	8	7	11	4	5	2	5	−4	141	38	571
5795♂	40	97	9	4	5	2	5	5	3	−4	126	29	381
5818♀	44	83	21	11	4	8	8	2	−2	−4	131	48	666
5927♂	34	70	16	17	2	1	2	−7	−10	−3	88	18	358
5967♂	33	69	16	11	4	1	1	1	−11	−5	87	18	453
5947♀	34	70	8	8	11	±0	−1	2	−2	4	92	22	386
5979♀	42	65	23	7	6	7	−6	5	−2	−5	100	35	370
Av.	42.5	90.6	8.6	7.8	6.2	4.5	3.2	2.7	−1.0	−3.1	119.5	28.8	426
											P.E.	±1.8	
											C.V.	38.9%	

*Average gain curves for Group 4, shown in Figure 3.

Group 5.*—Basal Diet plus 36 Drops (1.12 gm.) Egg Yolk per Week
March 15 to July 6

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Total food intake gm.
5759♀	37	75	7	10	9	10	6	−1	4	−5	115	40	392
5785♂	42	83	10	10	10	13	8	−2	3	2	137	54	454
5783♂	43	67	10	15	16	9	9	±0	−3	−3	120	53	375
5771♀	42	77	8	6	5	7	1	3	3	−2	108	31	330
5782♀	39	92	7	11	12	4	1	9	±0	−8	128	36	470
5797♀	39	92	12	5	12	10	4	9	1	−7	138	46	454
5807♂	45	99	10	23	11	10	7	−4	−2	−5	149	50	454
5810♂	42	86	27	4	11	17	−3	7	−6	6	149	63	487
5815♂	48	95	30	24	2	±0	5	−6	3	3	156	61	571
5820♀	34	68	14	16	10	4	2	1	2	−2	115	47	331
Av	41.1	83.4	13.5	12.4	9.8	8.4	4.0	1.6	0.5	−2.1	131.5	48.1	432
											P.E.	±2.1	
											C.V.	20.4%	

*Average gain curves for group 5, shown in Figure 3.

Dark and light-2 egg yolks were fed at levels of 4 drops (0.12 gm.), 6 drops (0.19 gm.), 8 drops (0.25 gm.) and 10 drops (0.31 gm.) per rat per week respectively. Neither 4 drops (0.12 gm.) nor 6 drops (0.19 gm.) per week were sufficient to support unit growth. The rats receiving 8 drops (0.25 gm.) per week or 0.036 gram per day of light-2 yolks gained 25 ± 1.51 grams; those receiving 8 drops (0.25 gm.) per week or 0.036 gram per day of dark yolk gained 26 ± 1.47 grams in 8 weeks. The rats receiving 10 drops (0.312 gm.) per week or 0.045 gram per day of light-2 yolks gained 37 ± 2.55 grams; those receiving 10 drops (0.312 gm.) per week or 0.045 gram per day of dark yolks gained 41 ± 2.33 grams in 8 weeks. The results are given in Figure 4, Tables V and VI. The data for the negative controls of these groups are given in Table IV.

TABLE IV.*—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET ALONE

Negative Controls

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Weekly food intake gm.
5311♂	47	100	−2	−17	−8	−1	------	------	------	------	72	−28	38
5326♀	39	80	−8	−13	−6	------	------	------	------	------	59	−21	37
5405♂	50	82	−9	−5	−11	------	------	------	------	------	57	−25	32
5403♀	51	99	−4	±0	−20	−8	------	------	------	------	67	−24	45
5474♀	37	97	−11	−4	−9	−14	------	------	------	------	59	−38	46
5523♂	43	103	−7	−17	−5	------	------	------	------	------	74	−29	21
5546♀	47	116	−10	−15	------	------	------	------	------	------	81	−35	26
5552♂	45	109	−6	−15	−10	------	------	------	------	------	78	−31	40
5565♂	37	85	−4	−3	−16	------	------	------	------	------	62	−23	40
5577♀	50	96	1	2	−4	−14	−5	------	------	------	76	−20	44
5613♀	40	80	5	−2	−7	−6	------	------	------	------	70	−10	42
5642♂	52	116	3	1	−13	−9	−17	------	------	------	81	−35	45
5658♀	46	101	−3	−15	------	------	------	------	------	------	83	−17	32
5690♀	51	111	−5	−1	−5	−8	−14	−10	------	------	69	−42	37
5739♂	33	84	−8	−8	±0	−6	------	------	------	------	62	−22	39
5750♀	50	82	2	±0	−4	−1	−4	−10	------	------	65	−17	49
5763♂	49	94	1	−2	−2	−16	−11	------	------	------	64	−30	38
5806♀	40	70	−1	−5	−1	−3	−5	−2	−4	------	49	−21	34
Av.	44.9	94.7	−3.7	−6.6	−7.6	−7.8	−9.3	−7.3	−4	------	68.7	−26	38
											P.E.	±1.27	
											C.V.	30.7%	

*Average gain curves for Table IV shown in Figure 4.

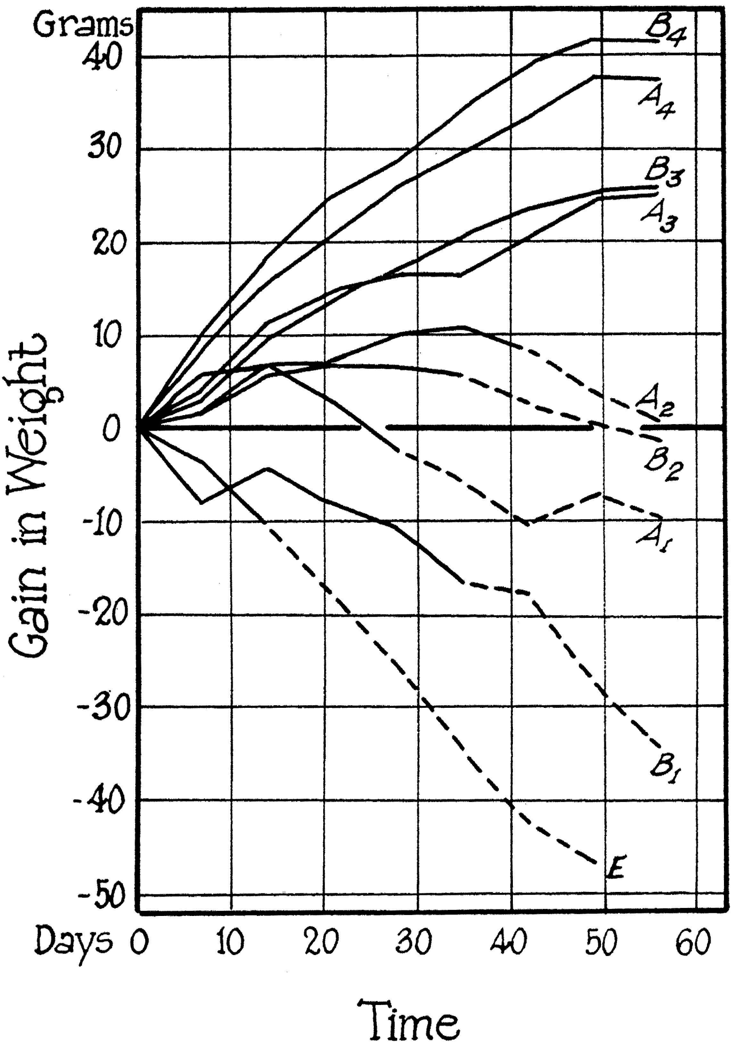

Fig. 4.*—Average gain curves of rats receiving the basal diet alone and the basal diet plus graded portions of either light-2 or dark egg yolks.

1. Light-2 egg yolks, color corresponding to 0.04 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:

A_1, 4 drops (0.12 gm.) A_2, 6 drops (0.19 gm.)
A_3, 8 drops (0.25 gm.) A_4, 10 drops (0.31 gm.).

2. Dark egg yolks, color corresponding to 0.1 N potassium dichromate solution. The amounts fed, per rat per week, were as follows:

B_1, 4 drops (0.12 gm.) B_2, 6 drops (0.19 gm.)
B_3, 8 drops (0.25 gm.) B_4, 10 drops (0.31 gm.).

3. Basal diet alone. E. Negative controls.

*Data for Figure 4 are shown in Tables IV, V, and VI. A broken line indicates the point at which one or more of the animals died.

TABLE V.*—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET SUPPLEMENTED BY GRADED PORTIONS OF LIGHT-2 EGG YOLKS, COLOR CORRESPONDING TO 0.04 N POTASSIUM DICHROMATE SOLUTION

Basal Diet plus 4 Drops (0.12 gm.) Egg Yolk per Week

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end of successive weeks, grams 1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Weekly food intake gm.
5324♀	42	86	±0	±0	−3	−3	−9	−7	------	------	64	−22	42
5406♂	46	85	4	−6	−2	−2	------	------	------	------	79	−6	42
5404♀	48	86	7	2	−10	−15	------	------	------	------	70	−16	50
5471♀	47	97	11	8	1	−1	2	−2	3	−2	107	10	56
Av.	45.8	88.5	5.5	1.0	−3.5	−5.2	−3.5	−4.5	3.0	−2.0	80	−8.5	47
											P.E.	±4.14	

Basal Diet plus 6 Drops (0.19 gm.) Egg Yolk per Week

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Weekly food intake gm.
5313♂	42	78	4	3	±0	9	±0	−5	3	−7	85	7	46
5410♂	40	88	2	4	3	4	−7	−19	------	------	75	−13	45
5413♀	41	72	−6	9	±0	5	−4	−6	4	3	77	5	39
5400♀	55	96	2	11	5	−2	6	6	−5	−5	114	18	53
5473♀	40	89	1	8	−3	±0	4	−3	−5	7	98	9	52
5525♀	41	96	−1	±0	−17	13	10	−5	−5	−4	97	1	44
5556♀	45	104	−1	4	−9	7	2	1	−30	------	78	−26	48
5578♀	50	94	5	10	6	±0	1	2	−7	−3	122	28	58
5611♂	42	108	6	5	6	−2	5	−3	−8	------	117	9	58
5652♂	51	114	5	−2	10	−4	−9	6	−10	−16	94	−20	43
Av.	44.7	93.9	1.7	5.2	0.1	3.0	0.8	−2.6	−4.3	−3.6	95.7	1.8	49
											P.E.	±3.41	
											C.V.	88.8%	

Basal Diet plus 8 Drops (0.25 gm.) Egg Yolk per Week

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Weekly food intake gm.
5315♀	48	83	−5	9	3	4	−3	2	6	2	101	18	43
5319♂	46	89	−1	5	−2	±0	−1	3	7	−2	98	9	43
5409♂	41	75	−8	12	2	2	−8	8	13	2	98	23	46
5547♀	38	93	2	12	4	1	2	6	1	10	131	38	53
5557♀	45	109	5	8	−1	−6	−4	10	3	−6	118	9	45
5562♂	39	77	1	8	5	−2	3	12	−9	5	100	23	43
5561♂	40	93	±0	3	2	2	±0	7	8	4	119	26	45
5580♀	47	92	3	6	2	4	2	7	8	−4	120	28	57
5615♀	39	85	9	−3	±0	9	6	3	±0	1	110	25	53
5650♀	49	86	8	9	8	4	±0	1	1	3	120	34	48
5659♀	45	103	12	6	9	−1	2	−4	4	±0	131	28	52
5686♂	53	121	10	−7	4	9	−6	3	3	2	139	18	55
5643♂	51	96	16	4	−2	1	±0	2	5	−2	120	24	48
5691♀	50	101	3	18	9	−6	7	2	6	−2	138	37	59
5745♂	58	111	11	7	10	−3	−13	11	9	−1	142	31	44
5737♂	37	102	−6	9	5	3	2	−3	±0	1	113	11	46
5766♀	46	97	5	12	4	±0	3	−1	5	−7	118	21	40
5801♂	45	98	6	9	4	11	5	4	8	−3	142	44	49
Av.	45.4	95.1	4.0	7.1	3.6	1.8	−0.2	4.1	4.3	0.2	120	24.9	48
											P.E.	±1.51	
											C.V.	38%	

Basal Diet plus 10 Drops (0.31 gm.) Egg Yolk per Week

Rat No.	Initial wt. gm.	Wt. after depletion gm.	1	2	3	4	5	6	7	8	Final wt. gm.	Total gain gm.	Weekly food intake gm.
5554♂	41	98	1	9	2	9	5	9	−2	−16	115	17	50
5568♀	37	82	2	9	1	3	±0	−5	8	6	106	24	47
5581♀	43	76	3	11	1	3	2	2	9	4	111	35	52
5614♀	40	76	5	3	4	4	6	±0	4	2	104	28	47
5617♀	38	82	5	1	8	5	5	4	4	3	117	35	52
5648♀	52	99	16	2	9	−3	12	3	4	1	143	44	59
5656♀	48	95	9	12	6	7	6	2	4	−1	140	45	54
5692♀	49	94	12	8	11	2	±0	4	4	−3	132	38	51
5747♂	50	100	13	−3	−16	15	8	4	6	2	129	29	47
5749♀	51	79	13	7	10	7	5	3	3	−3	124	45	50
5740♂	33	58	4	8	11	3	13	9	2	4	112	54	43
5761♂	53	108	11	−3	12	6	4	2	3	−7	136	28	54
5764♂	45	91	15	8	8	3	−18	13	8	±0	128	37	47
5768♀	43	88	9	9	±0	7	9	6	±0	3	131	43	47
5803♀	47	96	7	8	10	6	1	3	4	−1	134	38	55
5800♂	47	87	16	16	5	10	1	3	6	−1	143	56	54
Av.	44.8	88.1	8.8	6.6	5.1	5.4	3.7	3.9	4.2	−0.4	125.3	37.3	50
											P.E.	±2.55	
											C.V.	27.3%	

*Average gain curves for Table V shown in Figure 4.

TABLE VI.*—GROWTH RECORD OF RATS RECEIVING THE BASAL DIET SUPPLEMENTED BY GRADED PORTIONS OF DARK EGG YOLKS, COLOR CORRESPONDING TO 0.1 N POTASSIUM DICHROMATE SOLUTION

Rat No.	Initial wt. gm.	Wt. after depletion gm.	Gains at end o. successive weeks, grams								Final wt. gm.	Total gain gm.	Weekly food intake gm.
			1	2	3	4	5	6	7	8			
Basal Diet plus 4 Drops (0.12 gm.) Egg Yolk per Week													
5312♂	43	88	−13	11	−2	1	−13	−2	------	------	70	−18	44
5321♂	43	95	−3	5	−8	1	−12	−6	------	------	72	−23	43
5408♂	41	85	−12	3	−1	±0	±0	------	------	------	75	−10	38
5527♀	39	94	−4	−12	−3	−4	−2	−3	−10	------	56	−38	35
5545♀	51	115	−8	11	−4	−12	−2	7	−9	−7	91	−24	46
Av.	43.4	95.4	−8	3.6	−3.6	−2.8	−5.8	−1.0	−9.5	−7	75	−20.4	41
											P.E.	±2.83	
Basal Diet plus 6 Drops (0.19 gm.) Egg Yolk per Week													
5327♀	39	80	−3	9	−3	1	−3	±0	6	−2	85	5	45
5407♂	46	88	±0	5	4	2	−4	6	5	−5	101	13	43
5414♀	39	73	−2	7	−3	2	−3	−11	------	------	63	−10	43
5401♀	52	96	9	−1	3	−2	4	−2	−6	1	102	6	53
5472♀	44	95	−1	8	10	−4	10	±0	−3	2	117	22	55
5542♂	45	111	2	7	−17	11	−10	------	------	------	104	−7	50
5559♂	42	100	−3	−1	4	−4	−16	−3	------	------	75	−25	43
5575♂	52	109	11	−2	5	−7	2	4	−11	−1	110	1	54
5612♀	42	86	5	−3	3	1	10	−8	6	−15	85	−1	46
5654♀	52	110	−3	10	6	−1	−2	−12	−14	11	105	−5	39
Av.	45.3	94.8	1.5	3.9	1.2	−0.1	−1.2	−3.0	−2.4	−1.3	94.7	--0.1	47
											P.E.	±2.66	
											C.V.	123%	
Basal Diet plus 8 Drops (0.25 gm.) Egg Yolk per Week													
5316♀	45	81	4	−6	9	3	6	4	4	1	106	25	47
5320♂	46	84	−8	16	−1	−1	7	6	8	5	116	32	46
5411♀	44	71	1	11	5	8	−3	6	4	1	104	33	52
5412♀	43	88	5	13	6	4	−3	1	1	3	118	30	51
5558♀	43	92	2	7	1	4	2	−1	1	3	111	19	44
5563♂	39	97	−12	3	10	−9	−1	8	−2	4	98	1	43
5567♀	37	76	6	4	±0	−2	6	2	±0	6	98	22	47
5579♀	49	97	7	9	5	3	4	−1	5	−4	125	28	60
5616♀	39	85	9	−2	6	5	1	5	1	−1	109	24	49
5651♀	47	88	−1	13	1	−2	8	±0	6	−4	109	21	45
5655♀	49	104	5	9	5	3	6	5	1	2	140	36	46
5645♂	41	87	1	4	6	6	4	2	3	±0	113	26	47
5647♀	52	95	−1	6	8	5	1	2	−2	1	115	2)	45
5693♀	49	99	18	7	5	−2	4	2	2	−3	132	33	48
5746♂	52	105	18	1	8	7	3	5	−3	4	148	43	58
5738♂	35	88	−4	−7	2	15	12	−2	±0	−3	101	13	45
5767♀	46	94	8	10	±0	5	6	3	−2	−3	121	27	46
5802♂	44	94	±0	8	3	10	6	6	±0	2	129	35	45
Av.	44.4	90.2	3.2	5.9	4.4	3.5	3.8	2.9	1.5	−0.8	116.2	26	48
											P.E.	±1.47	
											C.V.	35.6%	
Basal Diet plus 10 Drops (0.31 gm.) Egg Yolk per Week													
5576♂	51	93	17	6	4	1	8	2	7	1	139	46	58
5618♀	37	76	8	−6	13	8	4	4	5	2	114	38	59
5644♂	47	88	12	7	13	4	3	9	1	5	142	54	52
5649♀	49	95	2	6	13	7	1	10	4	−10	128	33	54
5689♀	53	95	13	12	−3	−9	10	2	1	−11	110	15	49
5657♀	47	97	7	6	4	2	4	5	5	−3	127	30	48
5646♀	52	104	4	11	8	3	2	4	3	3	142	38	56
5748♂	50	101	22	8	7	8	8	7	−2	4	163	62	61
5743♀	28	83	8	7	2	4	3	3	1	−4	107	24	62
5762♂	52	103	13	11	4	5	2	5	5	−2	146	43	54
5765♀	47	100	9	14	5	6	4	4	7	3	152	52	59
5799♂	48	108	6	12	12	9	11	3	1	2	164	56	60
5804♀	42	82	13	16	9	2	8	6	−1	1	136	54	58
5805♀	41	89	5	6	4	5	6	4	±0	4	123	34	52
Av.	46	93.9	9.9	8.3	6.8	3.9	5.3	4.8	2.6	−0.3	135.2	41.3	56
											P.E.	±2.33	
											C.V.	31.2%	

*Average gain curves for Table VI shown in Figure 4.

From the results of these tests it would appear: (1) that the amounts of egg yolk necessary to induce approximately unit growth are for dark and light-2 each 0.036 gram, medium 0.107 gram, and light-1 0.143 gram per rat per day; (2) that dark and light-2 egg yolks are of equal vitamin A potency; (3) that they are about three times more potent than medium and four times more potent than light-1 yolks; (4) and that the medium are about one and three-tenths times more potent than light-1 yolks.

These experiments were designed to show quantitative differences in the vitamin A activity of the egg yolks used, but throughout the tests specific evidences of vitamin A deficiencies were observed in the experimental animals. Ophthalmia was observed in all of the negative controls and was common in other rats receiving the lower levels of supplements. Autopsies of negative controls showed pus in the glands at the base of the tongue; pus in one or both ears; an unhealthy condition of the intestinal tract, with the characteristic odor described by Munsell;[23] and a bloody exudate from the urinary tract.

The rats on the lowest levels of supplements exhibited the same symptoms and in addition a prevalence of bladder and urinary tract abnormalities such as were noted by Batchelder.[24] At levels which supported unit growth, 5 per cent showed a slight infection of one or both eyes, 22 per cent a little pus in the glands at the base of the tongue, and in a few cases gas in the intestinal tract.

Experiments to determine the carotene and xanthophyll content of the egg yolks used in these tests gave the following results.

Egg Yolks	Carotene milligrams per gram	Xanthophyll milligrams per gram	Ratio of Carotene to Xanthophyll
Dark (Pen 25)	0.0099	0.0340	1:3.32
Light-2 (Pen 15)	0.0045	0.0142	1:3.15
Medium (Pen 26)	0.0067	0.0251	1:3.74
Light-1 (Pen 27)	0.0044	0.0147	1:3.34

The ratio of carotene to xanthophyll in the yolks studied is fairly constant regardless of depth of color of the yolk.

The results are summarized in the following table:

Egg Yolks	Amount of egg yolk fed per day grams	Total gain in 8 weeks grams	Carotene in egg yolk fed per day milligrams	Xanthophyll in egg yolk fed per day milligrams	Units of* Vitamin A per gram
Dark	0.036	26	0.00036	0.00122	28
Light-2	0.036	25	0.00016	0.00051	28
Medium	0.107	29	0.00072	0.00269	9
Light-1	0.143	28	0.00073	0.00243	7

*The units of vitamin A were calculated on the basis of a 7-day week.

The results seem to show a definite relationship between the depth of color in the medium and light-1 yolks and vitamin A. It is of interest to note that the daily portion of the light-1 yolks necessary to induce an equal increment in weight contains approximately the same amount of carotene and xanthophyll as the medium yolks.

However, a comparison of daily portions of dark and medium yolks which induce similar gains in weight shows that the dark yolks contain only about one-half as much carotene and xanthophyll as the medium yolks.

The rats receiving equal amounts per day of light-2 and dark yolks made equal gains in 8 weeks but the light-2 yolks contained less than one-half as much carotene and xanthophyll as the dark.

The results of the experiments here reported show that the vitamin A activity in egg yolks cannot be explained on the basis of the carotinoid pigments they contain.

The results of the experiments upon the carotene and xanthophyll content of egg yolks are submitted as preliminary work only. As stated elsewhere in this paper, the method as modified by us may not be strictly quantitative, but the results are at least comparative. This work is being continued in this laboratory.

It is noteworthy that the egg production of the groups of hens on the different diets was closely parallel to the vitamin A concentration of the egg yolks as shown by the gains in the weights of the test animals. This seems to be of interest in view of the statement by Sherman and Smith[25] that, "Undoubtedly the fowl depends upon its food for the vitamin A which the egg contains; but to what extent a shortage of this vitamin in the food results in the production of vitamin-A-poor eggs, as against a simple diminution of egg production, remains to be investigated in detail."

PART II. VITAMIN D EXPERIMENTS

Historical

The antirachitic value of egg yolks was first noted by Mellanby[26] in her work with dogs in 1921.

Hess (1923)[27] found that egg yolks were effective in protecting infants against rickets in winter, but its curative value was less than that of cod liver oil. In experiments upon rats, receiving Sherman and Pappenheimer's Diet 84, he found that 5 drops (0.25 gm.) of egg yolk per week afforded protection against rickets. The amount of phosphorus in the egg yolk was not sufficient to account for such protection. In curative experiments 0.5 gram of egg yolk per day for 8 days resulted in calcification. Hess and Weinstock (1924)[28] reported that as little as 1 drop (0.05 gm.) of egg yolk per day fed by pipette was sufficient to protect rats which were on a ration deficient in phosphorous but that the protection was much less when the rachitic diet was of the low calcium type.

Casparis, Shipley, and Kramer (1923)[29] reported that the bones of rats, rendered rachitic on McCollum's Diet 3143, showed definite evidences of healing in 6 days, following the substitution of 10 per cent egg yolk for 10 per cent gelatin in the diet.

The effect of irradiation of hens with ultra-violet light or exposure to sunlight, on the vitamin D content of egg yolks has been studied by a number of investigators. Hart, Steenbock, Lepkovsky and Kletzien (1925)[30] reported experiments upon the vitamin D potency of egg yolks collected in March, from hens housed in an attic one group of which received the basal ration alone and the other group the basal ration and irradiation with ultra-violet light 10 minutes daily. The egg yolks were fed to rats and the antirachitic activity determined by means of the line test. They reported that "the ration fortified with 0.2 to 0.5 gm. of the egg yolks from irradiated hens was quite as potent antirachitically as the ration fortified with 5.0 gm. of the yolks from the non-irradiated hens. In other words, the yolks from the eggs of the irradiated hens were about ten-fold as potent in calcifying properties as those from the non-irradiated hens."

Hughes, Payne, Titus and Moore (1925)[31] tested the antirachitic activity of eggs from hens receiving varying degrees of ultra-violet light by feeding the eggs to young growing chicks. They found that "the amount of ultra-violet irradiation which a hen receives is an important factor in determining the antirachitic vitamin content of the eggs which she produces when her feed is low in the antirachitic vitamin."

Bethke, Kennard and Sassaman (1927)[1] studied the antirachitic content of egg yolks produced by hens under the following conditions: the hens were divided into four groups of which (1) confined indoors received the basal ration plus 2 parts cod liver oil; (2) confined indoors, received the basal ration alone; (3) confined indoors, received the basal ration and alfalfa hay ad libitum; (4) received the basal ration and had access to blue grass range. They reported (1) that the egg yolks produced by hens that had access to blue grass range were about ten times as active antirachitically as the egg yolks from hens confined indoors; (2) that alfalfa hay did not improve the calcifying properties of the egg yolks; (3) and that cod liver oil increased the antirachitic activity of the egg yolks about five fold. They have shown that the ration of the hen and the ultra-violet light she receives have a marked influence on the vitamin D activity of the egg yolks produced.

Experimental Procedure

The method developed by Sherman and Stiebeling[32] for the quantitative determination of vitamin D, with minor modifications, was used in this investigation.

Experimental Animals.—The selection and routine care of the animals was the same as that described for vitamin A experiments. One rat in each litter was given the basal diet alone and served as a negative control; another matched in sex and weight, was given the basal diet plus an adequate amount of vitamin D in the form of a solution of 0.5 per cent viosterol (250D) in cottonseed oil, and served as a positive control. The viosterol was dropped from a standardized microburette directly into the mouths of the rats. The amount of viosterol required, one drop (0.0203 gm.) per rat per day, had been determined previously by Cover.[15] The remaining rats in each litter were distributed among the several groups and fed graded portions of the four kinds of egg yolks as sources of vitamin D. Care was taken that the distribution of sexes and the averages of the weights of the rats were as nearly uniform as possible for all groups.

The Basal Diet.—The basal diet used has the following percentage composition:

Corn Starch	66%
Casein (freed from fat-soluble vitamins by triple extraction with boiling 95 per cent alcohol)	18%
Dried brewers' yeast	10%
Salt mixture (Osborne and Mendel)	4%
Sodium chloride	1%
Dried winter spinach	1%

Previous experience in this laboratory has shown that rats will separate out parts of this diet if it is fed in the dry state. When it was mixed with distilled water in about equal proportions, by weight, it was quite liquid at first but soon set to a stiff jelly. The rats ate it without separation. Records were kept of the dry food before mixing. The food for each rat was mixed separately as needed, usually every second day. The food consumption records were determined at the end of the experimental period by drying the food which was left and subtracting its weight from that of the total dry food given during the experiment.

Supplements to the Basal Diet.—The egg yolk used in the vitamin D experiments were from the same sources and usually from the same composite samples as those used in the vitamin A experiments. Dark yolks were from Pen 25 (65 per cent yellow corn plus 10 per cent alfalfa leaf meal); medium from Pen 26 (65 per cent yellow corn); light-1 from Pen 27 (35 per cent yellow corn); and light-2 from Pen 15 (25 per cent yellow corn plus 0.5 per cent cod liver oil). The technique of feeding was the same as that described for vitamin A experiments.

Two series of tests were made, one from April 17th to May 26th and the other from June 11th to July 13th. The hours of sunshine for the duration of each series were obtained and the effect upon the vitamin D activity of the egg yolks compared.

On the twenty-eighth day of the experimental period (fifty-sixth day of life), the rats were killed. After autopsy, the femurs were dissected out, freed from adhering tissue, and weighed in weighing bottles. They were ashed, weighed, and the percentage ash calculated on the basis of the weight of the fresh bone.

Results and Discussion

April 17th to May 26th Experiments.—Egg yolks were fed at the following levels: medium and light-1, 30 drops (0.94 gm.) and 60 drops (1.87 gm.) and dark and light-2, 6 drops (0.19 gm.) and 18 drops (0.56 gm.) respectively, per rat per week. A relative increase in percentage ash of 87 was obtained with 18 drops of light-2 egg yolks per week. About the same relative increase was obtained with 30 drops of medium and with 60 drops of light-1 egg yolks per week. Eighteen drops of dark egg yolks per week induced a relative increase in percentage ash of 63. Although the tests in this series were preliminary the results seem to indicate the following: (1) that light-2 are a somewhat better source of vitamin D than dark egg yolks; (2) that light-2 and dark egg yolks are better sources of vitamin D than either medium or light-1 egg yolks; (3) and that 65 per cent yellow corn in the ration of the hen seems to insure somewhat more vitamin D in the egg yolks than when 35 per cent only is used. The results are shown in Table VII.

TABLE VII.—SUMMARY OF RESULTS OBTAINED FROM EXPERIMENTS UPON THE VITAMIN D ACTIVITY OF EGG YOLKS PRODUCED FROM APRIL 10 TO MAY 15

Rats grouped according to (1) controls (2) source of egg yolks fed.*	Amounts of egg yolk fed per week		Number of rats	Average beginning body weight grams	Average gain per week grams	Ash in green femur per cent	Absolute increase over negative controls	Relative increase
	drops	grams						
Negative Controls	--	----	11	51	14	19.74	----	---
Dark (10% alfalfa)	6 18	0.19 0.56	9 8	52 47	16 18	22.53 25.14	2.79 5.40	33 63
Light-2 (0.5% cod liver oil)	6 18	0.19 0.56	8 7	50 50	16 19	23.10 27.18	3.36 7.44	39 87
Light-1 (35% yellow corn)	30 60	0.94 1.87	7 7	51 51	21 22	25.91 26.71	6.17 6.97	72 82
Medium (65% yellow corn)	30 60	0.94 1.87	9 8	52 52	20 25	27.10 28.25	7.36 8.51	86 100
Positive controls, 0.5% viosterol (250 D) 1 drop per day	--	----	11	51	22	28.27	8.53	100

*Complete rations of hens producing egg yolks shown on page 8.

June Experiments.—In this series dark and light-2 egg yolks were fed at levels of 3 drops (0.09 gm.) and 6 drops (0.19 gm.) and medium and light-1 at 6 drops (0.19 gm.) and 12 drops (0.37 gm.) respectively, per rat per week. Thus it is possible to compare the results obtained from feeding the different kinds of egg yolks at a level of 6 drops (0.19 gm.) per rat per week.

Six drops (0.19 gm.) of light-1, medium, or dark egg yolks, per week, induced a relative increase in percentage ash of about 50. There seems to be no difference in the vitamin D potency of the eggs produced in June by hens receiving 35 per cent yellow corn, 65 per cent yellow corn, or 65 per cent yellow corn plus 10 per cent alfalfa leaf meal. Six drops (0.19 gm.) of light-2 egg yolks per week induced a relative increase in percentage ash of 62. It would appear that 0.5 per cent of cod liver oil in the ration of the hen increased the vitamin D activity of the egg yolks used in these tests. However, this apparent difference is not significant when treated statistically. It was expected that the cod liver oil in the ration of the hens would have a greater effect in increasing the vitamin D activity of the egg yolks. However, the amount fed 0.5 per cent was only about one-fourth as much as was used by Bethke, Kennard and Sassaman.[1] The results are shown in Tables VIII and IX.

TABLE VIII.—SUMMARY OF RESULTS OBTAINED FROM EXPERIMENTS UPON THE VITAMIN D ACTIVITY OF EGG YOLKS PRODUCED IN JUNE

Rats grouped according to (1) controls (2) source of egg yolks fed.*	Amounts of egg yolk fed per week		Number of rats	Average beginning body weight grams	Average gain per week grams	Ash in green femur per cent	Absolute increase over negative controls	Relative increase
	drops	grams						
Negative controls	--	----	22	47	11	17.80	----	0
Dark (10% alfalfa)	3	0.09	13	47	12	19.63	1.83	20
	6	0.19	13	47	16	22.44	4.64	50
Light-2 (0.5% cod liver oil)	3	0.09	16	47	14	21.29	3.49	38
	6	0.19	14	47	16	23.54	5.74	62
Light-1 (35% yellow corn)	6	0.19	15	47	16	22.73	4.93	53
	12	0.37	13	47	18	25.34	7.54	82
Medium (65% yellow corn)	6	0.19	15	47	16	22.76	4.96	54
	12	0.37	14	48	18	25.13	7.33	79
Positive controls, 0.5% viosterol (250 D), 1 drop per day	--	----	22	47	20	27.04	9.24	100

*Completerations of hens producing egg yolks shown on page 8.

TABLE IX.—MEASUREMENTS OF RELIABILITY OF DIFFERENCES IN PERCENTAGE ASH OBTAINED FROM GREEN FEMURS OF RATS FED GRADED PORTIONS OF EGG YOLKS PRODUCED IN JUNE

Rats grouped according to source of egg yolks fed.*	Amounts of egg yolk fed per week		Ash in green femur			
	drops	grams	per cent	difference	probable† error of differences	critical‡ ratio
Light-2 (0.5% cod liver oil) Dark (10% alfalfa)	6 6	0.19 0.19	23.54 22.44	1.10	±0.41	3
Light-2 (0.5% cod liver oil) Light-1 (35% yellow corn)	6 6	0.19 0.19	23.54 22.73	0.81	±0.42	2
Light-2 (0.5% cod liver oil) Medium (65% yellow corn)	6 6	0.19 0.19	23.54 22.76	0.78	±0.41	2
Light-2 (0.5% cod liver oil)	6 3	0.19 0.09	23.54 21.29	2.25	±0.52	4
Dark (10% alfalfa)	6 3	0.19 0.09	22.44 19.63	2.81	±0.35	8
Light-1 (35% yellow corn)	12 6	0.37 0.19	25.34 22.73	2.61	±0.39	7
Medium (65% yellow corn)	12 6	0.37 0.19	25.13 22.76	2.37	±0.36	7

*Complete rations of hens producing egg yolks shown on page 8.

$$\dagger\text{P.E. of difference} = \frac{t\sqrt{\dfrac{\Sigma X_1^2 + \Sigma X_2^2}{N_1 + N_2 - 2}}}{\sqrt{\dfrac{N_1 N_2}{N_1 + N_2}}}$$

N = number of cases
X = deviation from mean
t is obtained from Fisher's tables

$$\ddagger\text{Critical ratio} = \frac{\text{Difference}}{\text{P. E. of Difference}}$$

Since a significant difference was obtained from the feeding of 3 drops and 6 drops of egg yolk per rat per week, it is evident that the Sherman-Stiebeling method is delicate enough to distinguish between the vitamin D activity of aproximately one-half drop and one drop of egg yolk per rat per day (Table VIII).

An indication was obtained as to the effect of an increasing number of hours of sunlight on the relative increase in percentage ash. The hours of sunlight from April 10th to May 15th were 254 and from May 29th to July 3rd were 470. There were, therefore, approximately twice as many hours of sunlight available for the hens which produced the eggs for the June tests as for those that produced the eggs for the April-May tests. A comparison of the vitamin D activity of dark and light-2 egg yolks, fed at the same level, in both series shows the following: six drops of dark egg yolk per week gave a relative increase in percentage ash of 33 in April-May tests and 50 in June tests; six drops of light-2 egg yolks gave a relative increase of 35 in April-May tests and 62 in June tests. It is obvious that the additional hours of sunlight increased the storage of vitamin D in the egg yolks.

SUMMARY

A comparison has been made of the vitamins A and D potency of egg yolks of different color concentrations, produced by hens under carefully controlled conditions as to rations and housing.

There was a distinct gradation of color in the egg yolks from the hens on the different rations and the color of the yolks on a given ration was very uniform. The color of a composite sample of egg yolk was compared with 10 milliliter samples of potassium dichromate solutions, each containing 0.1 gram of talc. The colors correspond to the following concentrations of potassium dichromate solutions: dark 0.1 N, medium 0.07 N, light-1 0.05 N and light-2 0.04 N.

A method is described for the determination of the carotene and xanthophyll content of egg yolks.

The tests for vitamin A activity have shown: (1) that while there seems to be some relationship between color and growth, the vitamin A activity of egg yolks cannot be explained on the basis of the carotinoid pigments they contain; (2) and that the vitamin A activity of egg yolks is directly dependent upon the rations of the hens.

The tests to determine the vitamin D activity were carried on in two series, one upon egg yolks produced from April 10th to May

15th and the other upon egg yolks produced in June. In the first series there appeared to be somewhat better relative calcification in rats receiving light-2 and dark yolks than in those receiving light-1 and medium yolks. However, this difference was not apparent in the June tests. The hours of sunshine available for the hens that produced the eggs for the June tests were approximately twice as many as for the April-May tests and the relative calcification was about in the ratio of 1.5 :1. These results furnish further evidence that the amount of sunshine available for the layers makes a difference in the vitamin D activity of egg yolks.

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