THE VITAMIN A CONTENT OF THE YOLK OF EGGS AS RELATED TO RATE OF PRODUCTION

by

MYRA CAROLINE KOENIG

B. S., Kansas State Teachers College
Pittsburg, Kansas, 1930

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1934

TABLE OF CONTENTS

INTRODUCTION

Contemporary vitamin A studies have included many investigations of food products of animal origin. Variations in vitamin content due to ration, to breed, to season, and to rate of production have received considerable attention. Eggs as a source of vitamin A have been studied in this connection.

A cooperative project (Department of Poultry Husbandry and Department of Home Economics) for the study of the vitamin A content of eggs was instituted in 1933. The present investigation is concerned with the vitamin A content of the yolks of eggs with reference to differences between eggs produced by good and poor laying pullets, some beginning and some completing the first year of production.

REVIEW OF LITERATURE

Eggs have been recognized as a good source of a growth promoting substance (vitamin A) from the time of the first studies of this nutritive factor. McCollum and Davis (1) in 1913 first observed that the failure of rats to grow after a certain point was reached was due to a lack of ether-soluble substances in the diet. These were supplied by ether extracts of egg or butter. In discussing the fact the

authers state, "These extracts contain some organic complex without which the animals cannot make further increase in body weight, but may maintain themselves in fairly good nutritive state for a prolonged period." A year later Osborne and Mendel (2) reported that the addition of egg fat to the diet of rats after cessation of growth, speedily cured the animals of accompanying pathological manifestations and led to a resumption of growth at a normal rate.

In 1924 Murphy and Jones (3) reported the results of experiments to study the vitamin A content of fresh eggs, finding 0.25 gram of whole egg required daily to cure rats of xerophthalmia and 0.5 to 0.75 gram daily to restore normal weight. Later, Jones, Murphy and Moeller (4) concluded from experimental data that whole egg which had been stored for 9 years, frozen, was as effective as fresh egg in curing xerophthalmia.

Bethke, Kennard and Sassaman (5) in 1926 reported that the ration of the hen has a marked influence on the vitamin A content of the egg yolk. Their results were obtained from experiments with rats, comparisons based upon amounts required to produce normal behavior. Yolks of eggs laid by hens which had access to a blue grass range were approximately five times as potent in vitamin A. The feeding of 2 parts of cod liver oil in the mash accounted for an approximate

five fold increase in the vitamin A content of egg yolks. The addition of alfalfa hay to the basal mash proved of some benefit in increasing the vitamin A content. Sherwood and Fraps (6) in 1932 reported on "The quantities of vitamin A required by pullets for maintenance and for egg production." It appeared that laying hens require large amounts of vitamin A, often lacking unless there is access to green feed. Low egg production, low vitamin content of the egg, undernourishment and eventual death of the hen may result from this shortage. More recently in 1933 Fraps and Trieehler (7) again stated that the vitamin A in eggs depends upon the quantity in the feed. Normal eggs, produced by hens receiving a complete diet plus green food, contained 20 to 30 Sherman units per gram of yolk. "When the pullets were kept on a ration where the sole source of vitamin A was yellow corn there was a gradual decrease in the vitamin A content (of the egg yolk) from 20.0 units per gram to 7 units per gram over a period of approximately five months."

Ellis, Miller, Titus and Beyerly (8) studied the vitamin A content of egg yolk produced in connection with a vitamin B and G experiment, reporting that "the vitamin A content of the eggs was maintained at a high level by the addition of cod liver oil to both groups of diets although the basal diet of Group B produced eggs which were low in

vitamin B and strikingly deficient in the yellow pigments usually associated with vitamin A."

This investigation did not deal directly with yolk color. However, it should be noted that the color of the yolk is now generally recognized as due largely to the ration. Palmer (9) in 1915 studied xanthophyll, the important yellow pigment in egg yolk, reporting that "The natural pigment characterizing the egg yolk, body fat, and blood serum of the hen is physiologically identical with the carotin and xanthophyll pigments of plants, with the latter class of pigments present in by far the greater proportion ----." Feeding tests with laying hens in which the pigment of the feed was carotin to the relative exclusion of xanthophyll were without appreciable influence upon the amount of pigment carried by the blood serum and deposited in the egg yolk. The feeding of rations relatively free from both carotin and xanthophyll to laying hens resulted in a marked reduction of the amount of this pigment carried by the blood serum and deposited in the egg yolk."

Carotene (carotin) has been found to be a precusor of vitamin A in the animal body. Moore has written extensively concerning vitamin A and carotene, pointing out in one paper (10) that carotene is intensely yellow and is snythesized by the plant while vitamin A is almost colorless. Egg yolk may be a good source of vitamin A if the ration of the hen

contained vitamin A or its precusor carotene. The amount of the yellow pigment xanthophyll is not necessarily an indication of the vitamin A content. This was shown by Bethke, Kennard and Sassman (5) who reported an approximate five fold increase in the vitamin A content of egg yolk after adding cod liver oil to the ration, although this did not add to the pigment in the yolk. Ellis (11) stated in a recent article "Hens on diets nearly devoid of yellow pigments but with cod liver oil supplement produced nearly colorless eggs which were equal to or possibly richer in vitamin A than highly pigmented eggs produced on normal diet containing yellow corn, alfalfa leaf meal and cod liver oil."

In discussing "Quantity and quality of egg yield," Thompson, Philpott and Page (12) divided the year as follows:

1. The winter season comprising the first 4 months of the pullet egg production year. High production during this so called winter season is indicative of inherited capacity for high annual total.

2. The spring season or the following 4-month period. It is during this period that egg production usually reaches its highest peak.

3. The summer-fall period or the last 4 months of production. Production during the latter months of the year is proof of the possession of "persistency of production."

For the purpose of distinguishing good layers from poor layers, poultrymen have made detailed studies of effects of heavy egg production upon the hen. Jull (13) stated that "The yellow pigment in the beak and shanks of breeds that normally have yellow skins tends to disappear as egg production continues. -----. Yellow corn and green feed contain xanthophyll but if rations are fed that do not contain this pigment, the beaks and shanks may be relatively pale though the hens may have laid but few eggs." According to Palmer and Kempster (14) when normally yellow skinned breeds are given feeds rich in the pigment the commencement of laying diverts the pigment received in the feed to the yolks. After continuous production over a period the pigment contained in the beak and shanks become depleted, first demonstrated by Blakeslee and Warner (15).

EXPERIMENTAL PROCEDURE

Albino rats of Wistar stock were used for this investigation. The stock animals were fed a diet used by Sherman and Crocker (16). The constituents are:

Dried Whole Milk	1/3
Ground Whole Wheat	2/3
Sodium Chloride	2% of weight of wheat.

This diet was fed ad libitum and distilled water was supplied at all times. The young rats were weaned at 4 weeks of age

or at the time the weight reached 40 grams.

These animals were then placed on the A-free diet as recommended by Sherman and Munsell (17) until the body store of vitamin A had been depleted. During this period the animals were kept in groups of 5 and 6 in cages with raised wire floors and weighed at frequent intervals. As the end of the depletion period approached they were placed in individual cages and were weighed daily. When their weights remained stationary for one week or xerophthalmia developed the animals were considered ready for experiment. Animals exceeding 106 grams in weight were not used in this study.

The modified A-free diet [Sherman and Munsell, (17)] was made as follows:

	Original diet per cent	Modified diet per cent
A-free Casein (extracted)	20	18
Starch	70	67
Yeast	5	10
Osborne and Mendel Salt Mixture	4	4
Sodium Chloride	1	1
Viosterol	0	(as described below)

As recommended by the U. S. P. and Druggists Committee (18), Squibbs' viosterol was used to furnish the vitamin D. The amount fed was 0.9 gram viosterol to 1000 grams of food.

This amount is equivalent to 3 per cent cod liver oil, having a vitamin D value of 100 units per gram and is calculated from the units as stated on the label of the bottle. The viosterol was accurately weighed, dissolved in ether, and added to the A-free casein. Other ingredients were added and the whole thoroughly mixed.

After growth had ceased the young rats were divided into experimental groups of not less than 10 individuals. These groups were regulated as to size and sex to secure well matched series.

Individual cages with removable raised wire bottoms were used for each animal in the experimental group. A-free diet and distilled water were supplied ad libitum. Weighed portions of the egg yolk to be tested were fed individually for an 8-weeks' period as the sole source of vitamin A. The daily portions customarily fed 6 times weekly were doubled and fed only 3 times each week in order to minimize the inaccuracies in weighing these small amounts. At the end of the experimental period or upon the death of the animal the weight and details of physical condition were noted. The animals were weighed weekly during the 8-week period.

An effort was made to determine the weight of egg yolk necessary to provide one unit of vitamin A. The unit as recommended by Sherman and Munsell (17) for numerical expression of results is that amount of vitamin A which when

fed daily induces an average gain of 3 grams per week in a standard test animal under the conditions described.

Control animals were maintained throughout the experiment. After the customary depletion period the negative controls were continued on the basal A-free diet until death. The positive controls received 0.5 gram daily of the egg yolk.

The study consisted of a fall series and a winter series. All eggs used were White Leghorn and were supplied by the Department of Poultry Husbandry. With one exception, a group with pale yolks, the eggs were produced by flocks receiving the regular Poultry Farm ration. The actual ration which the hens received was as follows:

Ground yellow corn	100 lbs.
Ground wheat	100 lbs.
Ground oats	100 lbs.
Meat and bone scraps	50 lbs.
Dried buttermilk	25 lbs.
Alfalfa leaf meal	25 lbs.
Salt	4 lbs.
Cod liver oil	4 lbs.

This mash mixture was before the birds continuously. In addition to this the hens had access to shelled yellow corn in one hopper and wheat in another. The feed consumption

from October 1, 1933 to May 12, 1934 inclusive per bird was as follows:

Shelled corn	6.48 lbs.
Wheat	24.54 lbs.
Mash	12.51 lbs.
Total	43.53 lbs.

The pale eggs (eggs with pale yolks) were produced by hens receiving the following ration:

White corn	70 lbs.
Dried buttermilk	20 lbs.
Wood pulp fiber	4 lbs.
Brewers yeast	3 lbs.
Cod liver oil	2 lbs.
Salt (Na Cl)	1 lb.

The hens producing pale eggs received 2 per cent cod liver oil while the regular Poultry Farm ration contained cod liver oil in the mash only, at the rate of 1 per cent.

The eggs were prepared for storage by dipping in hot mineral oil mixture, thus partially sealing the pores of the shell. This process largely prevents the loss of moisture and materially slows the rate of loss of carbon dioxide. The eggs were then packed in labeled cartons and the several cartons placed in a waxed paper container ready for storage at 35 to 45° F. until needed. Each egg was cooked for 20 minutes in a double boiler. The yolk was removed and mashed

thoroughly. The hard cooked yolk when not used immediately was stored in the freezing unit of an electric refrigerator.

Eggs of 3 kinds were used in the fall series. Their description follows:

1. X eggs laid by low-producing hens. This group included eggs from individuals whose actual production for the first or pullet year did not exceed 100 eggs.
2. Y eggs laid by high-producing hens. These hens produced 200 eggs or more during their pullet year. The eggs in both group X and group Y produced by hens near the close of their first year of production.
3. Pale eggs (eggs with pale yolks) were produced on a special pigment-poor ration containing cod liver oil as a source of vitamin A.

The winter series included the following:

1. No. 1 eggs laid by producing hens about the 4th month of their pullet year. Records of the hens showed a production of 53 eggs or less for the period from October 1 to March 1, 1933-34.
2. No. 2 eggs laid by high-producing hens about the 4th month of their pullet year. Any hen producing 60 eggs for the period from October 1 to March 1, 1933-34 may be considered a high

producer. This group included eggs from no individual that produced less than 83 eggs for the same period.

TABLE I

EGGS USED IN EXPERIMENT

Kind of Egg	No. of Hen	Production Oct. 1 - March 1	No. of Eggs Used
Egg No. 1	2895	53	3
	2885	44	3
laid by hens	2677	37	3
	2639	29	1
of low	2716	9	2
	2714	40	2
production	2912	43	2
	2910	32	2
Egg No. 2	2631	119	5
laid by hens	2685	120	5
of high	2676	83	4
production	2830	114	4

Kind of Egg	No. of Hen	Pullet Year Production	No. of Eggs Used
X egg laid	2172	99	4
by hens	2201	96	2
of low	2225	97	3
production	2423	81	1
Y egg laid	2173	228	1
by hens	2204	227	5
of high	2233	233	4
production	2244	237	3

TABLE II

RATS ON VITAMIN A-FREE DIET PLUS 0.03 GRAM YOLK OF X EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8062	35	37	79	73	79	80	87	87	91	88	89	56
8079	43	39	72	80	74	80	86	87	80	84	85	56
8077	47	39	89	99	90	88	92	84 D				35
8066	43	39	79	95	101	105	99	99	91	92	96	56
8067	43	39	73	80	89	98	108	109	105	100	85 D	52
8073	42	39	82	97	99	103	105	105	105	100	103	56
8097	47	34	77	87	104	111	121	124	125	124	122	56
8076	46	37	86	91	85	90	92	93	86	93	95	56
8063	44	39	78	94	100	106	112	109	114	118	121	56
8090	44	29	80	90	88	92	105	100	100	89 D		45
8091	43	29	76	90	94	80	81	92	102	86 D		47
8088	47	29	76	91	81	88	87	94	78	70 D		43
8089	45	29	78	94	99	102	96	87	82	84	70 D	56
Average	43.8	35.2	78.8	89.3	91.0	94.1	97.8	97.7	96.6	94.0	96.2	51.5
Average Live Wt.			78.8	89.3	91.0	94.1	97.8	98.3	96.6	98.1	101.6	

TABLE III

RATS ON VITAMIN A-FREE DIET PLUS 0.03 GRAM YOLK OF Y EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8061	35	36	65	60	61	63	62	59	51 D			38
8072	43	37	89	87	83	79	77	72	69	74	69	56
8107	42	36	67	76	75	80	68 D					28
8071	44	39	92	94	96	93	92	82	81	87	88	56
8081	45	39	79	80	81	70	69 D					22
8075	39	39	75	77	83	81	80	72	75	60 D		49
8084	43	34	76	97	100	103	102	81	83 D			36
8058	41	36	87	76	67	64	67	64 D				33
8070	41	36	78	80	75	75	79	77 D				34
8086	44	34	73	91	95	83	83	80	80	77	82	56
8092	40	29	59	78	76	77	64 D					27
Average	41.5	35.9	76.4	81.4	81.1	78.9	76.6	73.4	73.2	74.5	79.7	39.5
Average Live Wt.			76.4	81.4	81.1	78.9	80.2	74.3	76.2	79.3	79.7	

TABLE IV

RATS ON VITAMIN A-FREE DIET PLUS 0.04 GRAM YOLK OF Y EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8160	40	31	76	74	76	83	81	75	67	68	69	56
8185	45	34	101	96	97	94	97	84	98	101	95	56
8158	47	31	74	68	73	81	86	81	86	77	76	56
8171	41	37	96	95	99	91	96	98	85 D			39
8181	42	36	78	82	86	90	89	81	87	87	88	56
8172	46	39	99	97	107	110	111	102	106	99	91	56
8182	41	36	82	85	75	79	85	86	85	92	97	56
8156	45	31	83	87	87	94	95	98	99	97	88	56
8170	44	33	91	108	122	127	124	120 D				33
8194	42	31	79	81	90	84	93	84 D				33
8148	48	32	74	75	71	78	80	85	66	67 D		44
8196	40	31	83	86	96	92	78 D					28
8197	42	31	94	100	100	97	90	92	86	94	93 D	55
Average	43.3	33.3	85.4	87.2	90.7	92.3	92.7	90.5	86.5	86.9	87.1	48
Average Live Wt.			85.4	87.2	90.7	92.3	93.9	88.2	86.7	89.4	86.3	

TABLE V

RATS ON VITAMIN A-FREE DIET PLUS 0.03 GRAM YOLK OF PALE EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8116	45	34	91	100	104	100	104	109	110	110	108	56
8162	41	25	66	75	78	84	63	56 D				30
8111	47	29	81	98	105	115	108	108	109	97 D		46
8106	50	33	88	97	110	102	98	91 D				32
8120	42	26	82	97	100	80	79	76	79	80	75	56
8142	42	33	73	76	79	77	79	63 D				32
8115	44	36	102	102	105	107	112	88	84	72 D		47
8118	45	33	91	104	108	94	103	99	103	95 D		47
8144	44	26	82	96	101	99	111	111	115	119	122	56
8143	44	26	85	99	99	99	106	105	98	97	100	56
8122	43	30	81	93	97	99	92	88	89	62 D		45
8135	45	33	77	90	84	88	80	85	96	88 D		48
age	44.3	30.3	83.2	93.9	97.5	95.3	94.6	89.9	95.9	91.1	101.2	45.9
age wt.			83.2	93.9	97.5	95.3	94.6	96.6	95.9	101.5	101.2	

TABLE VI

RATS ON VITAMIN A-FREE DIET PLUS 0.04 GRAM YOLK OF PALE EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8125	44	29	80	94	108	106	112	113	109	102	101	56
8123	49	29	79	94	106	103	84	87	85	88	73 D	55
8124	45	30	81	91	98	100	97	101	103	97	97	56
8133	43	38	77	86	87	91	96	96	101	104	101	56
8146	40	31	73	87	83	89	94	89	63 D			42
8141	43	28	65	78	78	79	74	80	85	89	90	56
8140	42	28	67	79	80	86	87	93	98	100	106	56
8145	42	28	85	100	105	111	113	115	120	123	125	56
8136	42	31	74	76	78	82	85	91	98	104	98	56
8139	36	31	62	63	68	70	77	80	85	89	87	56
8134	49	31	79	82	83	86	91	89	93	96	91	56
Average	43.2	30.4	74.7	84.5	88.5	91.2	91.8	94.0	94.5	99.2	96.9	54.6
Average Live wt.			74.7	84.5	88.5	91.2	91.8	94.0	97.7	99.2	99.5	

TABLE VII

RATS ON VITAMIN A-FREE DIET - NEGATIVE CONTROLS

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8126	43	29	85	63 D								7
8065	44	39	77	75	72 D							8
8060	42	37	81	74	61 D							14
8112	45	29	85	76	77	68 D						15
8093	43	29	63	64	61	57 D						20
8184	40	34	96	89	70 D							13
8183	41	31	92	93	90	69	65 D					22
8068	45	39	80	83	78	68	60 D					27
8069	45	39	76	67	60	55 D						17
8152	46	33	77	76	64 D							11
8177	45	29	94	88	66 D							14
Average	43.5	33.5	82.4	77.1	69.9	63.4	62.5					15.3
Average Live Wt.			82.4	78.5	73.2	68.5						

TABLE VIII

RATS ON VITAMIN A-FREE DIET PLUS 0.50 GRAM YOLK OF EGG

Positive Controls

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
8101	42	33	72	99	113	125	132	142	150	155	157	56
8099	47	32	74	86	103	115	127	140	150	160	167	56
8100	44	33	73	91	103	115	120	128	135	139	142	56
8198	43	30	99	119	133	157	167	180	189	201	208	56
8195	42	31	94	117	130	145	166	170	188	196	187	56
8103	43	33	77	104	129	150	170	192	205	221	233	56
8109	45	29	72	93	111	122	131	148	160	167	184	56
8095	48	35	74	99	120	134	149	168	183	194	201	56
Average	44.2	32.0	79.4	101.0	117.7	132.9	145.2	158.5	170.0	179.1	184.9	56
Average Live Wt.			79.4	101.0	117.7	132.9	145.2	158.5	170.0	179.1	184.9	

TABLE IX

RATS ON VITAMIN A-FREE DIET PLUS 0.04 GRAM YOLK OF NO. 1 EGG

Rat No.	Wt. at 4 weeks gram	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
9152	44	34	91	100	113	119	126	132	134	133	138	56
9153	43	34	97	96	109	122	122	126	130	130	134	56
9175	42	24	92	93	106	111	116	117	123	124	97 D	54
9181	40	35	104	101	108	120	124	114	125	114	102 D	51
9179	42	34	84	91	110	113	116	117	116	110 D		44
9178	43	36	100	100	103	112	117	114	119	126	129	56
9203	42	31	92	102	115	123	129	132	137	139	141	56
9193	42	33	100	125	129	127	141	142	141	150	138	56
9151	41	34	103	107	120	130	124	126	133	120	113	56
9172	45	24	98	105	116	94	86 D					23
9140	42	38	105	109	109	117	121	126	130	136	134	56
9201	41	31	101	102	116	116	115	118	127	126	128	56
Average	42.2	32.3	97.2	102.6	112.8	117.0	119.7	124.0	128.6	128.0	125.4	51.7
Average Live Wt.			97.2	102.6	112.8	117.0	122.8	124.0	128.6	129.8	131.9	

TABLE X

RATS ON VITAMIN A-FREE DIET PLUS 0.05 GRAM YOLK OF NO. 1 EGG

Rat No.	Wt. at 4 weeks gram	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
9112	43	33	101	99	101	107	108	113	111	120	112	56
9104	42	35	80	70	75	84	92	103	106	110	117	56
9113	43	34	97	103	110	120	127	129	130	132	137	56
9147	41	30	90	89	103	111	117	124	123	130	130	56
9123	43	39	103	111	113	121	124	134	140	148	149	56
9105	45	36	100	91	107	112	118	116	104	112	116	56
9107	41	36	100	99	101	107	109	110	107	121	127	56
9128	44	31	99	100	120	127	125	138	137	145	145	56
9135	43	29	99	96	114	123	119	89	85 D			36
9132	41	30	97	105	119	129	129	129	138	133	134	56
9142	44	30	86	96	107	116	117	130	143	155	158	56
Average	42.7	33.0	95.6	96.3	106.4	114.3	116.8	119.5	120.4	130.6	132.5	54.2
Average Live Wt.			95.6	96.3	106.4	114.3	116.8	119.5	123.9	130.6	132.5	

TABLE XI

RATS ON VITAMIN A-FREE DIET PLUS 0.04 GRAM YOLK OF NO. 2 EGG

Rat No.	Wt. at 4 weeks gram	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
9174	43	27	88	101	112	122	132	133	136	129	133	56
9159	43	32	97	106	117	119	129	128	131	137	132	56
9158	45	33	105	100	110	116	118	124	120	124	125	56
9180	41	36	97	102	99	104	101	112	118	127	134	56
9103	44	30	94	105	100	116	118	117	120	124	128	56
9095	41	24	98	96	99	100	85	96	94	94	103	56
9196	41	32	102	112	117	126	130	132	146	148	150	56
9188	42	32	98	101	98	102	116	120	114	100	98 D	50
9157	43	32	95	105	108	100	113	116	105	89	88	56
9093	46	29	89	70	78	74	72	66 D				33
9185	43	25	88	114	121	119	124	125	127	135	135	56
9176	40	34	98	89	97	95	97	82 D				32
Average	42.7	30.5	95.7	100.1	104.7	107.7	111.2	112.6	121.1	120.7	122.6	51.6
Average Live Wt.			95.7	100.1	104.7	107.7	111.2	120.3	121.1	120.7	123.1	

TABLE XII

RATS ON VITAMIN A-FREE DIET PLUS 0.05 GRAM YOLK OF NO. 2 EGG

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
9129	43	32	93	99	110	117	116	129	136	130	135	56
9114	41	39	78	87	96	100	103	115	117	111	119	56
9133	42	32	103	116	122	126	132	145	145	146	148	56
9136	42	26	90	106	107	120	124	129	133	138	143	56
9124	40	32	90	99	105	110	113	105	115	121	131	56
9130	44	36	103	120	129	133	144	149	157	161	164	56
9134	41	35	102	103	110	118	122	112	121	126	140	56
9101	46	40	94	97	107	112	124	124	131	124	134	56
9102	41	39	83	82	91	92	91	99	93	95	98	56
9121	41	32	102	104	114	118	114	136	144	142	156	56
9122	40	32	92	100	112	121	127	129	130	139	140	56
9117	40	36	94	90	106	109	106	129	130	125	132	56
9143	41	30	86	90	97	107	114	125	133	137	144	56
Average	41.7	33.9	93.1	99.5	108.1	114.1	117.8	125.1	129.6	129.6	137.2	56
Average Live wt.			93.1	99.5	108.1	114.1	117.8	125.1	129.6	129.6	137.2	

TABLE XIII

RATS ON VITAMIN A-FREE DIET - NEGATIVE CONTROLS

Rat No.	Wt. at 4 weeks grams	Depletion days	Wt. at end of depletion grams	Weekly weights - grams 1	2	3	4	5	6	7	8	Survival days
9191	46	24	91	84	86	71	59 D					27
9110	41	43	96	79	77 D							13
9165	43	28	100	101	84	72 D						17
9127	45	32	99	107	80	74 D						17
9118	43	36	106	111	97 D							13
9119	43	34	98	89	84	73	60 D					24
9120	42	35	95	88	72	58 D						17
9150	43	33	93	101	84	71	67 D					23
Average	43.2	33.1	97.2	95.0	83.0	69.8	62.0					18.9
Average Live Wt.	43.2	33.1	97.2	95.0	81.7	71.7						

RATS ON VITAMIN A-FREE DIET PLUS 0.50 GRAM YOLK OF EGG
Positive Controls

9194	41	29	101	124	133	150	162	168	168	176	183	56
9215	40	31	101	120	133	147	160	171	182	189	195	56
9189	46	31	98	111	129	158	174	192	210	231	239	56
9200	41	35	102	115	149	170	180	185	200	213	228	56
Average	42.0	31.5	100.5	117.5	136.0	156.2	169.0	179.0	190.0	202.2	211.2	56
Average Live Wt.			100.5	117.5	136.0	156.2	169.0	169.0	190.0	202.2	211.2	

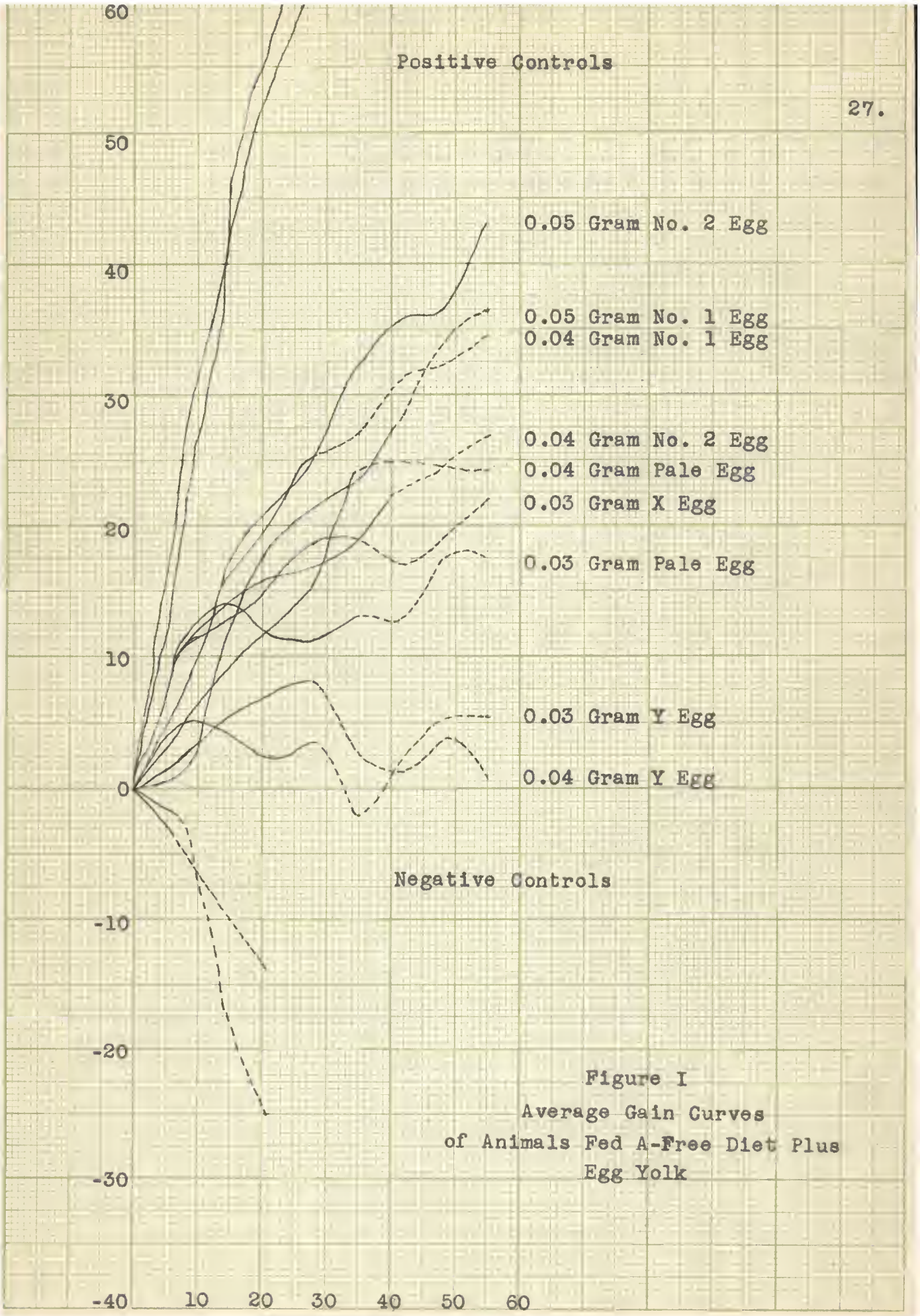

Figure I

Average Gain Curves of Animals Fed A-Free Diet Plus Egg Yolk

DISCUSSION

Tables 2, 3 and 4, and Figure 1 show the growth of rats fed weighed amounts of the X egg (from poor layers) and the Y egg (produced by good layers). Feedings of 0.03 gram daily were used first in each case, since previous experiments (19) with eggs produced by hens on this same ration indicated that this amount would approach closely the Sherman and Munsell (17) standard for 1 unit of vitamin A.

Table 2 shows data on growth of the animal receiving 0.03 gram of the X egg yolk per day. The gains are similar to the standard for 1 unit of vitamin A, the total average gain being 22.8 grams as compared with 24 grams suggested by Sherman and Munsell (17). Some animals died before the end of the 8-week period. Experience indicates that it is difficult to keep an entire group of depleted animals alive for 8 weeks on this low nutritive level. A vitamin A content of nearly 33 units per gram is indicated for the X egg yolk as daily feedings of 0.03 gram contained approximately 1 unit of vitamin A.

Table 3 gives data for the animals fed 0.03 gram portion of the Y egg yolk. These animals made only small gains and few survived the entire 8-week period, some animals dying before the end of the first 4 weeks. As it became evident

that the amount of the Y egg yolk was supplying much less than 1 unit of vitamin A a new group was started receiving 0.04 gram daily. The data for these animals are shown in Table 4. They made distinctly better gains during the first 4 weeks but after this period lost weight and some died so that at the end of the 8 weeks only 54 per cent of the original group survived. These results show that 0.04 gram of the Y egg yolk furnishes much less than 1 unit of vitamin A, so that 1 gram would contain distinctly less than 25 units of the vitamin. It is estimated that the vitamin A content is not over 20 units per gram.

The fall series also included experiments with pale eggs produced on ration devoid of carotene and xanthophyll but containing cod liver oil as a source of vitamin A. Table 5 gives data for the animals fed 0.03 gram of the egg yolk daily to compare with the others in this series. In the early part of the experiment these animals grew much as did those receiving 0.03 gram of the X egg yolk (Table 2). After the first 2 weeks there was some loss of weight, the first death occurring at 30 days and only 33 per cent surviving for the entire 8 weeks. As the amount 0.03 gram of the pale yolk was supplying less than 1 unit of vitamin A, a group receiving 0.04 gram of the pale egg yolk was started. The data in Table 6 show that these animals made small gains

each week to average 24.8 grams for the 8-week period. All animals did not survive the experimental period, a circumstance previously noted. At least 1 unit of vitamin A is furnished by 0.04 gram of the pale yolk or 25 units by 1 gram.

Table 7 gives data for the negative controls of the fall series. These animals lost weight and died surviving an average of 13.9 days. This gives a satisfactory check on the depletion of the animals used for experiment and on the completeness of the removal of vitamin A from the diet. Table 8 shows figures for the positive controls of the series. These animals received A-free diet ad libitum and 0.50 gram of egg yolk per day. Rapid gains were made, averaging 92 grams for the period of 8 weeks. This gain is similar to that reported by King (20) for normal animals of this age. Results of the positive controls show that the depleted animals were free to gain when receiving the A-free diet plus a source of vitamin A.

Eggs for the winter period were laid by young hens in the first 4 months of the pullet year, No. 1 eggs by the poor producers and No. 2 eggs by the good producers. Because the 0.03 gram feedings used during the fall series were not sufficient to produce unit growth in the 8-week period, larger amounts were fed for this series namely 0.04 and 0.05 gram.

Table 9 and Figure 1 show data for the animals

receiving 0.04 gram of the No. 1 egg yolk, which amount provided growth better than that described by Sherman and Munsell (17) for 1 unit of vitamin A. Table 10 gives the data concerning the group of animals fed the larger amount, 0.05 gram, of the same egg yolk. These grew more rapidly, gaining an average of 36.9 grams during the 8 week period. These results are consistent, the first group showing that this egg yolk contained at least 25 units of vitamin A per gram and the second group showing correspondingly better growth.

Data for the animals receiving 0.04 gram of yolk of No. 2 egg are shown in Table 11. This group made small gains each week, with an average curve approaching that of Sherman and Munsell for 1 unit.

Table 12 gives data for the group receiving 0.05 gram of this egg yolk. These animals grew more rapidly, making a weight increase much greater than for unit growth. The No. 2 egg yolk is shown (Figure 1) to be similar to the No. 1 egg yolk in vitamin A content, containing at least 25 units per gram.

Data for satisfactory groups of negative and positive controls are shown in Table 13. These animals were used as controls for the winter series.

Egg production makes pronounced nutritive demands upon the hen. The recognized demand for vitamin A is more

pronounced the greater the number of eggs produced and the longer the laying period. The birds producing eggs X, Y, No. 1 and No. 2 all received the same ration. The pullets (winter series) were in the first 4 months of egg production while the hens (fall period) were in the last part of their first year of production.

The young pullets of both groups, at the end of the first 4 months or production, laid eggs with yolks of similar vitamin A content, at least 25 units per gram. This period of production was apparently too short to have brought about marked differences between the vitamin A content of the yolk of eggs of high producers and low producers. On the other hand, distinct differences were found in the eggs of the older birds, completing the first year of production. The low producers, consuming the desirable ration but laying infrequently, gave yolks of higher vitamin A content, 33 units per gram. The egg yolks of the high producers were less potent in this regard, about 20 units per gram.

The yolk of pale eggs produced on a ration devoid of carotene and xanthophyll but supplying vitamin A in the form of cod liver oil contained 25 units of vitamin A per gram, thus being superior to the Y eggs. This is in agreement with the work of Palmer and Kempster (14) who fed a carotinoid-free ration and obtained fertile eggs from which second generation chickens were eventually produced.

SUMMARY AND CONCLUSIONS

Eggs from the Kansas State College poultry farm were studied for the vitamin A content of the yolk. The investigation included eggs laid by high producers, some laid by low producers, and others that were pigment poor. The biological method of Sherman and Munsell was used for all determinations. The modified vitamin A-free diet contained viosterol to supply vitamin D. Young rats of suitable age and size were fed this vitamin A-free diet during the customary depletion period, after which they were placed on experiment. During the experimental period they received weighed supplementary portions of the yolks of eggs to be tested, at the rate of 0.03, 0.04, and 0.05 gram fed 6 days per week.

Composite tables were prepared from data concerning the various groups of animals. These data were studied and results evaluated according to Sherman and Munsell. Findings are as follows:

1. Young pullets, both high and low producers, at the end of the first 4 months of production, laid eggs with yolks of similar Vitamin A content, at least 25 units per gram. This period of production was apparently too short to have brought about marked differences between the

vitamin A content of the yolk of eggs of the 2 groups.

2. Pullets of high and low production records laid eggs with dissimilar vitamin A content near the close of the first year or egg production. Eggs from low producers at that time showed 33 units of vitamin A per gram of yolk as compared to about 20 units in the high producing group, high producers having decreased and low producers increased the vitamin A content as compared to the content of similar eggs produced at the end of only 4 months of laying.
3. The pale eggs produced on a ration devoid of carotene and xanthophyll but supplying vitamin A in the form of cod liver oil contained 25 units of vitamin A per gram.

LITERATURE CITED

1. McCollum, E. V., and Davis, Marguerite.
The necessity of certain lipins in the diet during growth. J. Biol. Chem. 15: 167-175. June. 1913.

2. Osborne, Thomas B., and Mendel, Lafayette B.
The influence of cod liver oil and some other fats on growth.
J. Biol. Chem. 17: 401-408. March. 1914.

3. Murphy, J. C., and Jones, D. B.
Vitamin A content of fresh eggs.
J. Agr. Res. 29: 253-257. Sept. 1924

4. Jones, D. Breese, Murphy, Joseph D., and Moeller, Otto.
The effect of long continued storage at low temperatures on the vitamin A content of eggs.
Am. J. Physiol. 71: 265-273. Sept. 1925.

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

6. Sherwood, R. M., and Fraps, G. S.
The quantities of vitamin A required by pullets for maintenance and for egg production.
Texas Agr. Expt. Sta. Bul. 468. 19p. Dec. 1932.

7. Fraps, G. S., and Treichler, Ray.
Vitamin A content of foods and feed.
Texas Agr. Expt. Sta. Bul. 477. 24p. July. 1933

8. Ellis, N. R., Miller, David, Titus, Harry W., Byerly, Theodore C.
Effect of diet on egg composition. II. The relation of diet to the vitamin B and the vitamin G content of eggs, together with observations on the vitamin A content.
J. Nutrition. 6: 245-262. May. 1933

9. Palmer, L. S.
Xanthophyll, the principle natural yellow pigment of the egg yolk, body fat, and blood serum of the hen. The physiological relation of the pigment to the xanthophyll of plants.
J. Biol. Chem. 23: 261-280. Sept. 1915.

10. Moore, Thomas.
The distribution of vitamin A and carotene in the body of the rat.
Biochem. J. 25: 275-286. Jan. 1931.

11. Ellis, H. R.
The relation of egg yolk color to some nutritive properties of eggs.
The U. S. Egg and Poultry Magazine. 39: 47-48. Oct. 1933.

12. Thompson, W. C., Philpott, D., and Page, H. C.
The significance of measurements of quantity and quality of egg yield.
New Jersey Agr. Exp. Sta. Bul. 528. 1-40. July. 1931.

13. Jull, Morley A., Poultry husbandry, first ed.
McGraw-Hill Book Co., Inc., New York. 639p. 1930.

14. Palmer, L. S., and Kempster, H. L.,
Relation of plant carotenoids to growth, fecundity and reproduction of fowls.
Jour. Biol. Chem. 39: 299-312. Sept. 1919.

15. Blakeslee, A. F., and Warner, D. E.
Correlation between egg laying and yellow pigment in the domestic fowl. Am. Nat. 49: 360-368. 1915.

16. Sherman, H. C., and Crocker, J.
Growth and reproduction upon simplified food supply. III. The efficiency of growth as influenced by the proportion of milk in the diet.
J. Biol. Chem. 53: 49-52. May. 1922.

17. Sherman, H. C., and Munsell, H. E.
The quantitative determinations of vitamin A.
J. of Am. Chem. Soc. 47: 1639-46. June. 1925.

18. Report of the vitamin A assay committee of the American drug manufacturers association - twentieth annual meeting - May 1931. by Arthur D. Holmes, Chairman.
J. Am. Phar. Soc. 20: 583-594. June. 1931.

19. Condet, Winnie Pearl.
The vitamin A content of the yolk of hen's eggs.
Thesis, Kansas State College of Agriculture and Applied Science. 1933.

20. King, Helen D., Growth and variability in the body weight of the albino rat. Anat. Record. 9; 751-776. Oct. 1915.

ACKNOWLEDGMENT

The writer wishes to express her sincere appreciation to her major instructor, Dr. Martha M. Kramer, for her advice and assistance in all matters pertaining to the study. The writer also wishes to thank Professor L. F. Payne, Head of the Department of Poultry Husbandry, under whose supervision the eggs used for this study were produced.