THE VITAMIN A (CAROTENE) REQUIREMENTS FOR EGG PRODUCTION AND HATCHABILITY

By

Max Rubin

Thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy

1942

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. H. R. Bird of Maryland University for the direction and assistance which were given to him during the course of study which led to the preparation of this thesis. Also, Dr. J. L. Gvirbely of the National Institute of Health who was most generous in giving his time and advice to the author on vitamin C analysis technic.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

There is a considerable degree of discord in the literature as to what the actual vitamin A requirement is for egg production and hatchability. There seems to be a common agreement that the vitamin A requirement for hatchability is greater than that for egg production. In popular discussions, one will frequently hear that the breeder mash should be supplemented with more vitamin A than the laying mash. Therefore, taking the egg production and hatchability requirement relationship as a fact, it should be possible to produce an egg which is deficient in vitamin A; that is, an egg which can be produced by the hen because the vitamin A requirement for egg production is satisfied though that for hatchability is not. With eggs of this vitamin A content, it should be possible to make a study of how vitamin A enters into the metabolic processes of embryonic formations. Since these eggs would be deficient in vitamin A, some malformations would be likely to develop as a result of this deficiency, and therefore, the embryo should serve as an excellent means of studying how vitamin A actually functions in the cell. This finding would be of importance because very little is known today about how vitamin A is used by the organism.

Therefore, it was believed that if a group of hens were placed on a diet which was deficient in vitamin A, the vitamin A in the body stores of these hens would serve as the source of supply of this vitamin for the carrying on of maintenance and

egg production processes. As these stores became depleted, the point would be reached where eggs would be produced that did not have enough vitamin A in them to enable the embryo to go through to a normal hatching. This point could be determined by incubating all the eggs produced. When the percent hatchability began to drop, it would indicate that the time had been reached where eggs were being produced which did not have enough vitamin A for hatchability. Thus, a more careful study of the early embryonic development of eggs subsequently produced could then be made, knowing that these eggs were deficient in vitamin A for hatchability.

With this hypothesis, a group of hens were placed on a vitamin A deficient diet in order to produce eggs which did not contain enough vitamin A for hatching. This depletion experiment was carried out and then repeated. As a result of these experiments, it was found that the eggs produced, hatched well. In fact, the hens laid eggs until they became vitamin A deficient and died, but their eggs hatched well, even those that were laid last. With this information at hand, it was concluded that there must be some error in the information on which the hypothesis was based, and that the hatchability requirement of vitamin A cannot be greater than the requirement for egg production. A critical review of the data in the work reported on the requirement for hatchability has explained some of the differences of opinion. Also, the experimental work reported in this thesis tends to explain what the real situation is in regard to the relationship between the vitamin A requirement for egg production and hatchability.

LITERATURE REVIEW

Bethke et al (1927) were probably the first workers who showed that there was a relation between the vitamin A content of a hen's egg and the vitamin A content of the hen's diet. However, the stage of vitamin A experimentation had not reached the point where a high degree of quantitativeness was used. Their finding was to the effect that cod liver oil in the diet or blue grass pasture caused eggs to be produced that had a vitamin A potency which was several times that which was produced when the diet contained thirty per cent yellow corn.

Probably the first attempt to put the vitamin A requirement of hens on a quantitative basis is the work reported by Sherwood and Fraps (1932). They fed hens at a level of 270, 120 and zero Sherman - Munsell units of vitamin A daily. They found that the best results in egg production was obtained with the highest level of vitamin A intake. Some data in their paper tends to convey the idea that the same trend is shown for hatchability. However, no conclusion was drawn from this data.

Sherwood and Fraps (1934) reported some additional experiments they had done on the vitamin A requirements of laying hens. In these experiments they used higher levels than in the previously reported experiments. They fed their hens daily 224, 336, and 444 Sherman - Munsell units of vitamin A potency. In these experiments too, they found that there was a relation between the rate of egg production and the level of vitamin A

intake. This was also their finding in the case of hatchability. The best results were obtained on their highest level for both these functions.

In 1935 Sherwood and Fraps repeated the work they had done in the previous year and came to the conclusion that 600 Sherman - Munsell units per day was necessary for laying hens which were laying at the rate of 150 eggs a year. This was the desirable intake if the hens were to lay eggs which had a high vitamin A content. However, if one did not care about the vitamin A content of the eggs produced, 240 units was sufficient for good health and good egg production. There data again showed a trend as far as hatchability was concerned. The higher the intake of vitamin A, the higher was the per cent of hatchability.

Russell et al. (1936) fed hens at a level of 89 U.S.P.XI. units per 100 grams of diet (equivalent to 53 micrograms of carotene) and obtained data which indicated as good egg production and hatchability at this level as at higher levels (6,607 U.S.P. XI. units per 100 grams of diet which is equivalent to 3,964 micrograms of carotene). The only difference they could find between these grades of vitamin A intake was a higher mortality in the chicks hatched from the two lower levels(89 and 154 U.S.P.XI units). Their next higher level was 286 U.S.P.XI. units (equivalent to 172 micrograms of carotene). Hens that died on their lowest level of vitamin A intake contained fair quantities of vitamin A in their livers. None of the birds that died showed vitamin A deficiency symptoms. A possible explanation for the fairly good results they obtained in egg production at the lower levels may be large body stores of vitamin A at the

start of the experiment. The recommended quantity of vitamin A in the diet was 485 U.S.P.X.I. units per 100 grams (equivalent to 291 micrograms of carotene).

Bearse and Miller (1937) found that a diet containing a vitamin A potency of 250 Sherman - Munsell units per 100 grams (equivalent to 210 micrograms) in the form of carotene in alfalfa leaf meal yielded a hatchability of 84.5 per cent. The hatchability improved to 95.8 per cent when the vitamin A potency of the diet was doubled. Any further addition failed to increase hatchability. These authors state, "Although we are not concerned with egg production in the work herein reported, yet it should be mentioned that the production increased as the level of alfalfa was increased in the ration; thus, it appears that a ration containing sufficient vitamin A for maximum hatchability may not contain sufficient for maximum production". The increase in hatchability they obtained with an increase in the quantity of alfalfa leaf meal added to the diet may be due to factors other than carotene contained in the alfalfa.

In some preliminary experiments, Record (1937) found that the best results in egg production and hatchability was obtained when the vitamin A level in the diet was in the range of 350 to 400 U.S.P. units per 100 grams of feed (210 to 240 micrograms of carotene). These workers ran experiments involving the following levels of vitamin A and carotene intake. For vitamin A, they used 160, 240, and 320 U.S.P. units per 100 grams of diet. The carotene was supplemented so as to supply 100, 200, 400, 800, and 1600 micrograms of carotene per 100

grams of feed.

The work of Almquist and Mecchi (1939) showed that egg production and hatchability were satisfactory on all except the lowest carotene level they used. This level, 188 micrograms of carotene, was regarded as satisfactory for egg production but low for hatchability. Their next level was 446 I.U. of vitamin A from shark liver oil per 100 grams of diet (equivalent to 268 micrograms of carotene). However, the egg production they obtained on all levels was not very good. The highest level fed was 663 micrograms of carotene per 100 grams of feed. They recommend a vitamin A potency in the diet of 660 I.U. per 100 grams of diet (equivalent to 396 micrograms of carotene).

Williams et al (1939) have shown that when the hen's daily ration contains 200 micrograms of carotene, fed in the form of alfalfa leaf meal, the diet is adequate to maintain the weight of the hens, keep up a fair egg production, and prevent the incidence of deficiency lesions in the throat. This quantity of carotene was sufficient to obtain good hatchability. When the daily ration of carotene is increased to 500 micrograms, a slightly higher egg production was obtained; however, there was no improvement in any of the other factors. These authors believe that there is no relation between the rate of hatchability and vitamin A in the hen's diet when the level of carotene in the daily ration is sufficient to maintain good health.

With et al (1939) assayed the yolks of eggs for vitamin A. They also incubated eggs from the same source and assayed the whole chicks for vitamin A. The results of these assays showed that there was very little difference between the vitamin A content of the eggs prior to incubation and the vitamin A content of the bodies of the chicks. As a possible explanation, they suggest that in the developing embryo vitamin A is not destroyed in embryonic metabolism. However, after applying a statistical analysis to their data, they state that the maximum utilization of vitamin A during embryonic development is 80 I.U. (48 micrograms).

Baumann et al (1939) reported that the quantity of vitamin A used during embryonic development is quite large. Their egg analyses are reported in micrograms and their chick analyses in blue units of vitamin A. They obtained a conversion value of five blue units equaling one microgram of vitamin A when they used a fish liver oil as their source of vitamin A. On the other hand, they state that for such crude material as chick extracts, the conversion factor may be that one blue unit equals one microgram of vitamin A. Using the former conversion value, the loss during incubation varied on different levels from 91 to 138 micrograms. If the latter conversion value is correct, the loss of vitamin A ranged from 14 to 86 micrograms.

Sherwood and Fraps (1940) have concluded that the vitamin A requirement for birds which are producing eggs not intended for incubation is 450 micrograms of carotene (750 I.U.) per

100 grams of feed. They then added 50 micrograms of carotene to this figure for a safety factor, thus recommending 500 micrograms of carotene (833 I.U.) per 100 grams of feed. They also state that the requirement of vitamin A by hens to be used for breeding purposes is 550 micrograms of carotene (920 I.U.) per 100 grams of feed, and again allowing a safety factor of 50 micrograms of carotene, they recommend 600 micrograms of carotene (1000 I.U.) per 100 grams of diet.

In the first place, these workers did not have any birds on a 450 or a 500 microgram level of carotene intake. They therefore must have interpolated these values from their data which shows that the experimental levels were 225 and 300 micrograms in one series of experiments and 200, 400, and 600 micrograms in another experiment. It is difficult to understand how a single value could be interpolated from as wide a range as 400 to 600 micrograms with any degree of accuracy. Yet, the authors interpolated two values for two different functions, hatchability and egg production. How they decided which required the greater value was not indicated. Secondly, between their 400 and 600 microgram groups there is only four percent difference in hatchability which is not a big enough difference to be considered significant. And furthermore, in their table 14 they show an average of 88.7 per cent hatchability on a 300 microgram level. This value is as high as the value for hatchability which they obtained on the 600 microgram level. Thus, from their data they have no basis for their figure of 550 micrograms of carotene as the requirement of vitamin A for hatchability. Thirdly, they claim that the hatchability requirement is greater than the egg

production requirement. Their data indicates that there may be a significant difference in the egg production on the 400 and 600 microgram levels of intake. All of which would seem to indicate that the work they have done supports the thesis that the egg production requirement is as great or greater than the hatchability requirement.

Polk and Sipe (1940) present data in their paper for a three year experiment with hens on a daily vitamin A intake of 120 Sherman - Munsell units for one group and 1240 Sherman-Munsell units for another group. The data contains the number of eggs produced during the three year period and also the number of eggs set, eggs fertile, and chicks hatched. From this data it is possible to calculate the per cent hatch of fertile eggs for each year. These are presented in tabular form (table 1).and show that the difference in per cent hatch of fertile eggs set is very small; whereas the per cent difference in egg production is quite large. Added significance may be attached to these figures because of the large numbers of eggs involved. Thus,a conclusion which could be drawn from this work is that the hatchability requirement of vitamin A is not greater than the egg production requirement; however, this point was not brought out by the authors.

TABLE I

DATA FROM POLK AND SIPS (1940)

Experimental Year	Hatchability		Per cent difference in egg production
	120 Units per day	1240 Units per day	
1	69.9	72.8	5.5
2	76.3	76.6	39.2
3	72.0	71.2	34.3

This literature review shows that there is a reason why there is so much confusion as to what the true relationship is between the vitamin A requirement for egg production and hatchability

Another phase of the work reported here was undertaken because of Phillip's recent report (1940) that bulls which had a chronic avitaminoses A also had a lowering of the blood plasma ascorbic acid. Other tissues were also depleted of normal quantities of ascorbic acid. Thus, Phillips showed that there is a relationship between vitamins A and C in cattle.

Cattle can synthesize their own vitamin C when they are not vitamin A deficient. The following reports are presented as evidence that the fowl too can synthesize vitamin C. Hart et al (1932) concluded that the quantity of vitamin C present in cereal grains and the skim milk usually present in the fowl's diet was sufficient to supply its needs for this dietary essential. In 1925, Hart et al reported on further work on the ascorbic acid requirements of the fowl. They found that chicks

fed purified food materials devoid of vitamin C did not develop scurvy. The livers of these chicks were assayed for vitamin C by the guinea pig feeding method and were found to contain a good supply of vitamin C. One gram of chicken liver daily cured scurvy sufficiently so that a slight gain in weight was made. Three grams of liver daily in the guinea pig's diet cured scurvy at once. Carrick and Hauge (1925) also found that the livers of chickens which were fed a diet deficient in vitamin C were able to prevent scurvy in guinea pigs. They therefore concluded that it was quiet possible that the fowl could produce vitamin C in its metabolism from substances not available to the guinea pig.

Since the fowl like the cow can synthesize vitamin C, it would be of interest if a similar relationship between the A and C vitamins existed in the fowl. An experiment to determine this point was undertaken.

PROCEDURE

In all of the experimental work which was conducted, the hens were confined to a laying battery. Each hen had its own cage. Thus the eggs were automatically trapnested. The battery was stationed in the hen battery room of the poultry building. This room is subject to some heating from the heating system in other parts of the building. The birds were exposed to artificial light twenty four hours each day. Fertile eggs were produced by means of the artificial insemenation technic. The hens were insemenated twice weekly with a dose of 0.1 c.c. of semen.

Eggs were collected daily except during the hot summer months when they were collected twice daily. All eggs were stored in a refrigerator at approximately 50° F. until they were set in the incubator. The eggs were set in weekly hatches. Thus, no eggs were stored for more than a seven day period prior to being incubated. On the eighteenth day, the eggs were transferred to pedigree baskets and placed in the hatching unit. When the chicks had completed hatching, the number of chicks hatched from the eggs of each hen was recorded. The eggs which did not hatch were broken and a record made of the infertile eggs and the day of death of the dead embryos.

The initial depletion experiment was begun in November, 1940. There were twenty Barred Plymouth Rock hens in the experiment and they were fed a vitamin A deficient diet(table II). Four of this number were selected at random and were used as the control birds. These control hens were dosed twice weekly

with 2.0.c.c. of a cod liver oil containing 1250 -1500 I.U. of vitamin A. per gram. In January, the cod liver oil was changed to a vitamin A oil which had a potency of 5700 I.U. per gram.

TABLE II

THE VITAMIN A DEFICIENT DIET

White corn	46 lbs.
Wheat bran	10 "
Wheat flour middlings	10 "
Oats	15 "
Dried skim milk	10 "
Casein	3 "
Ground oyster shell	3½ "
Yeast	2 "
Bone meal	1½ "
Salt	1 "
Manganous sulfate	12 grams
Delsterol	32 "

The second depletion experiment was begun in January, 1941. In this experiment there were eleven yearling crossbred hens. two of these hens were selected as controls and were dosed with the vitamin A oil as in the previous experiment.

A third depletion experiment was started in July 1941. There were twelve hens in the experiment and no controls. This experiment was run simultaneously with an experiment in which three groups of hens were fed the vitamin A deficient diet supplemented with three levels of carotene respectively. There were twelve birds in each group. The carotene was put in Wesson oil as a carrier and added to the diet so that the three diets contained 350, 550 and 750 micrograms of carotene per 100 grams of diet respectively. Mrall three diets the oil was added so that it composed one per cent of the diet. The carotene was

was added only to small batches of feed at a time and these were held in a refrigerator at approximately 40° F.

In order to obtain a response stimulus which was directly related to the carotene content of the diets, the birds were maintained on the deficient diet for eight weeks before being placed on their supplemented diets. Only a days supply of feed was placed before the hens after they had begun the ninth week of the experiment. In this way, destruction of the carotene was kept down to a minimum.

This hen experiment was continued for thirty-eight weeks. When the experiment was terminated, four of the surviving hens in each group were killed and their livers were assayed for vitamin A. The method of assay is the one used by Rubin et al. (1941).

It was thought desirable to determine what the livability would be of the chicks produced by hens on the different levels of carotene. These chicks were battery brooded for four weeks in a room located in the poultry building. All of the chicks in each hatch were placed in the same deck of the battery; therefore, they were subject to the same environmental conditions. They were fed the Maryland Experiment Station Mash (table III). A record was kept of the day of death of each chick which died up to the end of the fourth week. The mortality data of the first twenty-three hatches was recorded. The first six hatches in all three groups were from eggs produced

while the hens were on the vitamin A deficient diet without any vitamin A supplement. The succeeding hatches came from eggs laid after the diet was supplemented with the three levels of carotene.

TABLE III

MARYLAND STATION MASH

Ground #2 yellow corn	500 lbs.
Wheat bran	300 "
White flour middlings	300 "
Ground heavy oats	300 "
Soybean oil meal	100 "
Alfalfa leaf meal	100 "
Meat scraps (50% protein)	150 "
Fish meal	100 "
Dried skim milk	150 "
Straight cod-liver oil	20 "
Ground oyster shell	10 "
Salt	10 "
Manganous sulfate	½ "

In October 1941, a fourth depletion experiment was started. This experiment consisted of twenty-eight Columbian Rock pullets. Some of these birds were killed when they showed vitamin A deficiency symptoms. Their livers were assayed for both vitamins A and C in order to determine whether or not a similar relationship between these vitamins existed in the hen as was shown to be the case for cattle. At the suggestion of Dr. J.L. Svirbely of the National Institute of Health, the duodenum portion of the intestine was also assayed for vitamin C. The method of assay is the one used by Dr. Svirbely for rat tissues.

The method is as follows, a ten gram sample of liver or intestine is minced with a scissors. The minced tissue is then covered with a twenty per cent solution of trichloracetic acid and allowed to stand for a few minutes in a mortar. This permits the tissue to harden. After hardening, the tissue is then macerated with a pestil. An excess of three per cent of trichloracetic acid is then stirred into the macerated tissue. When the insoluble residue has settled out, the supernatant liquid is then filtered into a volumetric flask. This extraction is repeated twice. The flask is then made up to volume and an aliquote of this solution is titrated with standard sodium 2,6, dichlorobenzenoneindophenol.

RESULTS

Throughout all of the depletion experiments, it was noted that the birds laid eggs until they became vitamin A deficient. These eggs hatched just as well at the latter part of the experiments as they did in the early hatches. Tables IV and V summarize the results of the four depletion experiments. The data in these tables show a definite trend toward a lowering in the per cent of egg production as the depletion of vitamin A progressed. On the other hand, the per cent hatchability, throughout the duration of the experiments, tends towards a constant figure. In all four of the depletion experiments, the experiment was terminated when the number of birds had been reduced by mortality to make succeeding data of very little value or when the number of birds out of production was very great and the death of these birds from vitamin A deficiency was imminent.

The results of the experiment in which hens were supplemented with carotene at the rate of 350, 550 and 750 micrograms per 100 grams of diet has been tabulated in table VI. During the winter period the egg production increased in all groups; however, no change was observed in the hatchability. The rate of egg production between the low and high levels of carotene intake is related to the quantity of carotene in the diet. The hatchability values do not show any relationship to the amount of carotene in the feed.

The vitamin A assays of the livers from the hens which were fed carotene are tabulated in table VII. There were

TABLE IV

DATA OF EXPERIMENTS I AND II

Experiment	Experimental Groups	Month	Number of Eggs Laid	Percent of Eggs Fertilized	Number of Eggs Set	Percent Hatch of Fertile Eggs	Number of Hens Died
1	Parliament	Oct.	138	75.3	96	42.1	0
		Nov.	220	63.6	200	67.5	3
		Dec.	215	51.7	172	60.5	1
		Jan.	96	65.6	68	85.9	4
		Feb.	31	71.0	21	57.9	2
		March	19	57.1	14	64.3	0
	Control	Oct.	2	50.0	2	50.0	0
		Nov.	46	65.7	46	65.0	0
		Dec.	54	51.4	46	76.0	0
		Jan.	54	51.7	29	57.9	0
		Feb.	40	60.4	14	78.6	0
		March	55	78.6	15	100.0	0
2	Parliament	Jan.	36	84.4	29	86.2	0
		Feb.	115	45.6	56	86.2	0
		March	125	49.2	73	82.2	0
		April	25	11.0	5	80.0	4
		May	6	6.7	0	-	1
	Control	Jan.	8	37.1	8	47.0	0
		Feb.	25	41.1	25	92.3	0
		March	43	51.4	37	93.3	0
		April	35	62.5	32	90.9	0
		May	25	51.4	8	100.0	0

TABLE V

DATA OF DEPLETION EXPERIMENTS III AND IV

Experiment Number	Weeks on Depletion	Number of Eggs Laid	Per Cent Egg Production	Per Cent Hatch of Fertile Eggs	Number of Hens Died
3	3 to 7	195	63.4	71.3	0
	7 to 11	162	52.6	74.3	0
	11 to 15	106	37.9	83.8	1
	15 to 19	44	17.5	71.4	1
4	3 to 7	453	64.7	71.3	3
	7 to 11	267	52.5	74.3	5

TABLE VI

EGG PRODUCTION AND HATCHABILITY DATA FOR HENS SUPPLIED CAROTENE IN THE DIET

Period	Per Cent Egg Production			Per Cent Hatch of Fertile Eggs		
	Micrograms of Carotene per 100 Grams of Diet					
	350	550	750	350	550	750
July 21 to Aug. 17	37.5	36.6	36.3	71.1	60.0	71.0
Aug. 18 to Sept.14	44.6	48.2	46.7	82.9	71.3	75.4
Sept.15 to Oct. 12	38.0	45.5	46.1	86.0	72.8	79.8
Oct. 13 to Nov. 9	47.4	51.1	57.1	77.2	74.4	77.2
Nov. 10 to Dec. 7	40.9	47.9	53.3	88.4	78.7	81.0
Dec. 8 to Jan. 4	26.3	32.1	49.4	84.9	60.8	84.6
Jan. 5 to Feb. 1	27.6	24.3	41.7	89.7	56.4	81.9
Feb. 2 to Mar. 1	24.7	21.4	40.0	89.6	62.5	77.0

three cases in the 350 microgram group which had only a trace of vitamin A in their livers. The hens from the 550 and 750 microgram groups contained vitamin A liver stores in relation to the carotene content of their diets.

TABLE VII

THE VITAMIN A ASSAYS OF THE HENS FED CAROTENE

Group	Hen no.	Liver weight, grams	Blue units of vitamin A per gram	Blue units of vitamin A per liver
350	173	58.0	trace	trace
	166	34.5	trace	trace
	204	48.5	trace	trace
	201	29.0	90.0	2610.0
550	233	50.5	142.5	7196.0
	169	48.0	35.0	1680.0
	244	45.5	95.0	4322.5
	187	40.0	140.0	5600.0
750	203	58.5	170.0	9945.0
	207	45.0	85.0	3825.0
	155	43.0	172.5	7417.5
	147	37.0	191.0	7067.0

The weekly mortality of each of the three groups of chicks have been tabulated respectively in tables VIII, IX, and X. During the middle of the experimental period there was a great increase in mortality in all of the groups. This mortality was diagnosed to be due to pneumonia. However, toward the latter part of the experiment, the mortality was reduced somewhat. The total mortality in each group was respectively 29.7, 32.8 and 34.6 per cent. The greatest mortality occurred during the second week of the first and second groups, while the first week had the greatest mortality for the third group.

TABLE VIII

MORTALITY DATA OF CHICKS FROM 350 MICROGRAM GROUP

Hatch No.	No. of Chicks	Died First Week	Died Second Week	Died Third Week	Died Fourth Week
1	6	1	0	0	0
2	19	1	0	0	0
3	20	1	3	2	0
4	18	0	0	0	0
5	19	2	0	0	0
6	26	0	0	1	0
7	16	0	4	1	0
8	27	0	9	7	0
9	21	4	3	1	1
10	18	2	5	7	1
11	14	3	5	1	1
12	20	2	6	3	1
13	23	2	8	3	2
14	14	0	2	2	1
15	20	1	0	0	0
16	20	1	2	0	0
17	15	0	1	0	1
18	12	0	0	1	0
19	14	0	0	1	0
20	15	0	0	0	1
21	18	1	1	2	0
22	5	0	0	0	0
23	14	0	3	1	2
Total Chicks	394	21	52	33	11
Per Cent Mortality	29.70	5.33	13.20	8.38	2.79

TABLE IX

MORTALITY DATA OF CHICKS FROM 500 MICROGRAM GROUP

Hatch No.	No. of Chicks	Died First Week	Died Second Week	Died Third Week	Died Fourth Week
1	9	1	0	0	0
2	17	1	1	0	0
3	9	0	1	0	0
4	12	1	3	1	0
5	21	2	0	0	0
6	31	0	5	2	2
7	17	0	5	6	2
8	23	0	8	3	0
9	17	0	3	3	0
10	20	0	5	4	0
11	23	0	6	5	1
12	25	2	7	6	2
13	23	2	9	5	1
14	19	0	6	3	0
15	21	0	0	0	0
16	20	0	1	0	2
17	12	1	1	0	0
18	13	0	0	1	0
19	14	0	0	1	0
20	13	0	0	1	1
21	8	0	0	0	0
22	8	0	1	0	0
23	2	0	0	0	0
Total Chicks	377	10	62	41	11
Per Cent Mortality	32.89	2.65	16.45	10.88	3.34

TABLE X

MORTALITY DATA OF CHICKS FROM 750 MICROGRAM GROUP

Hatch No.	No. of Chicks	Died First Week	Died Second Week	Died Third Week	Died Fourth Week
1	11	2	0	0	0
2	21	0	2	1	0
3	17	1	2	1	0
4	25	1	5	0	0
5	18	1	2	1	1
6	24	0	0	3	0
7	13	3	8	1	1
8	21	10	4	2	0
9	23	8	3	2	0
10	29	6	4	4	0
11	31	5	3	6	0
12	36	7	8	7	0
13	33	6	6	5	3
14	30	6	7	5	0
15	33	3	0	1	0
16	31	4	0	0	0
17	32	10	0	0	0
18	30	4	3	1	0
19	26	3	2	1	0
20	36	4	1	1	1
21	38	6	2	0	3
22	21	1	1	0	0
23	31	0	1	2	3
Total Chicks	610	91	64	44	12
Per Cent Mortality	34.59	14.92	10.49	7.21	1.97

It was also thought advisable to analyse the data during the portions in which the mortality was not very large. The periods used were the first six and last six hatches. The per cent of mortality in all three groups for these two periods are tabulated in table XI. The mortality for the first six week period for all three groups was respectively 10.2, 20.2, and 19.8 per cent. For the last six week period it was 16.7, 8.6 and 22.0 per cent.

TABLE XI

DATA FROM CHICK MORTALITY EXPERIMENT

Group	Hatch no.	No. of chicks	Per Cent Died				Total per cent died
			First week	Second week	Third week	Fourth week	
Micrograms							
350	1-6	108	4.6	2.8	2.8	0.0	10.2
	18-23	78	1.3	5.1	6.4	3.8	16.7
550	1-6	99	5.1	10.1	3.0	2.0	20.2
	18-23	58	0.0	1.7	5.2	1.7	8.6
750	1-6	115	4.3	9.5	5.2	8.6	19.8
	18-23	182	9.9	5.4	2.7	4.8	22.0

Observations were made in several cases in order to determine the relationship between the microscopic appearance of pustules in a vitamin A deficient hen and its death. Also, in two cases, hens were dosed with a vitamin A oil to determine what would happen to the pustule condition. From the standpoint of borderline vitamin A deficiency, information of this kind is of interest.

There is relatively a considerable length of time between the first microscopic appearance of pustules and the death of

the bird. This time averaged about a month. It was frequently observed that the birds had a fairly good general appearance up until the latter part of this period. Then they lost a considerable portion of their body weight.

The pustules in the esophagus of the treated hens disappeared in less than a week's time even with relatively small doses of the vitamin. However, in a short time the pustules soon returned. The same results were obtained when this therapeutic treatment was applied again. In both cases, eggs were laid between the incidence of pustule formation. Except for two eggs, all the eggs were either broken or infertile. The two which were fertile contained embryos which died on the nineteenth day. The small number of fertile eggs obtained makes this data of limited value.

Table XII is a tabulation of the vitamin A assays. Hens 104 and 87 received the vitamin A deficient diet supplemented with an excess of vitamin A. The W.F. hens were fed the Maryland experiment Station Mash which contains a normal quantity of vitamin A. The remaining hens were on the vitamin A deficient diet and were vitamin A deficient at the time the assay was made. There is no significant difference between the vitamin A content of the birds receiving vitamin A in the diet and those which did not receive any vitamin A. It will be noted that the livers of the hens getting vitamin A in their diet had good stores of vitamin A, while those which showed vitamin A deficiency symptoms did not contain any determinable vitamin A. The quantity of vitamin A found in the duodenum was uniformly great in practically all cases.

TABLE XII

VITAMIN A AND C ASSAYS OF LIVER AND DUODENUM

Hen No.	Mg. c per gm. liver	B.U. per gm. liver	Total C in liver mg.	Mg. c per gm. duodenum	Total c in duodenum mg.
10A	0.0998	190.0	4.0419	0.1718	1.9753
87	0.2116	325.0	11.6380	0.1617	1.2935
S.M.1	0.2574	-	18.1852	0.2567	2.7727
S.M.1	0.1970	-	6.4997	0.2784	2.5080
342*	0.1456	-	7.8629	0.0818	0.6545
332	0.2585	0.0	10.8550	0.2177	1.9592
321	0.2524	trace	7.3190	0.1752	1.0510
323	0.1857	trace	5.1367	0.1532	1.5321
328	0.2085	0.0	17.5140	0.1351	1.8640
337	0.1462	0.0	5.4014	0.1590	1.2720
336	0.1811	trace	5.4330	0.1648	1.6480
350	0.2019	-	7.4703	0.2040	2.3050
343	0.2532	-	9.1152	0.2869	2.8690
340	0.2809	-	13.7641	0.2397	1.5580
351	0.2293	0.0	8.2548	0.2727	2.7270
348	0.2960	0.0	13.3200	0.2270	1.5890
344	0.1736	-	5.3692	0.1526	1.3276
334	0.2124	-	8.4304	0.2296	2.2960
326	0.1954	-	7.0554	0.1843	1.9535
335	0.2154	0.0	5.6004	0.2752	1.3760

* This bird was dead for not more than two hours before the tissues were assayed.

DISCUSSION

The literature which has been cited definitely shows that our knowledge of the relationship between the egg production requirement and hatchability requirement for vitamin A is in a very confused state. Also, the early work done with hens in vitamin A research was probably complicated with riboflavin deficiency. In much of this work, a plant source was used to supply vitamin A potency to the basal diet. There is the possibility that some of the improvement in hatchability obtained in these early reports may have been due to added increments of some other essential needed for hatchability. This would be particularly true in those cases where alfalfa leaf meal was the source of vitamin A potency. In some cases, added confusion was caused by the drawing of two liberal conclusions.

The original purpose of this work was to attempt to determine how chick embryos will react to vitamin A deficiency. By this means, it was believed, more information could be obtained about how vitamin A functions in the organism. Knowledge of this kind is lacking in the literature at the present time. The attempt ended in failure, but as a result of this experiment, a better understanding of the relationship between the egg production and hatchability requirement has been achieved.

When a hen is placed on a vitamin A deficient diet she is dependent on her body stores for this nutritional essential. Payne and Hughes (1933) have stated that a hen on a vitamin A deficient diet behaves normally for quite some time. The affects of the deficiency will cause a sudden decline in the

general health and physiological condition of the fowl. This is quite true; however, there is a relatively considerable length of time between the first macroscopic appearance of pustules and the death of the bird. This time averaged about a month. It was frequently observed that the birds had a fairly good general appearance up until the latter part of this period. Then, they lost a considerable portion of their body weight.

The depletion experiments reported in this thesis have shown that when a group of hens are placed on a deficient diet there is a gradual reduction in egg production. This is because all the hens in the group do not have the same body content of vitamin A. Also, some hens probably use the vitamin A they possess more efficiently than others. Thus, for the individual hen, egg production is maintained at a fairly uniform level. As the bird goes into the vitamin A deficient condition, egg production drops rapidly and then finally ceases.

There is a certain quantity of vitamin A which a hen needs for normal maintenance and production of the maximum number of eggs she is genetically capable of producing. During the production of an egg, a portion of the ingested vitamin A is deposited in the yolk. The quantity deposited is dependent on the amount in the diet. The embryo uses the vitamin A in the egg during incubation. If the quantity present was not large enough, the embryo would die from vitamin A deficiency. Thus, a hen's vitamin A requirement for hatchability would be greater than hen egg production requirement. When the hens in this

experimental work were placed on a deficient diet it was found that there was a decline in egg production but that the per cent hatchability remained fairly constant. Therefore, the eggs which were produced must have been supplied with enough vitamin A from the body stores of the hen for embryonic growth and development. When the hen's supply of vitamin A was depleted she stopped laying eggs. However, the last eggs she laid hatched fairly well. With these results, it is reasonable to believe that the vitamin A requirements of the chick embryo is quite small. Also the vitamin A requirements for egg production is as great or greater than the hatchability requirement when hens are dependent on their body stores of vitamin A.

There was the possibility that a similar relationship did not exist when hens were being supplied with a marginal level of carotene in the diet. The experiment in which hens were fed diets containing 350, 550 and 750 micrograms of carotene per 100 grams of diet was undertaken to determine what the condition was when hens were supplied with a vitamin A source. From the experiment, it appears that there is a relationship between rate of egg production and vitamin A potency of the diet. It is true that the 550 microgram group is somewhat out of order but the difference between the 350 and 750 microgram groups is significant. In addition, since such a situation does exist, it shows that there is a difference between the hen's vitamin A requirement for maintenance and her requirement per egg production. This point has been shown by previous workers.

There was no difference between the hatchability of the 350 and the 750 microgram groups. In this case too, the 550 microgram group was out of order. Thus, hatchability was not related to the vitamin A in the diet of the hen at the levels fed. This experiment checks the depletion experiments. Therefore, the egg production requirement for vitamin A is as great or greater than the egg production requirement when vitamin A is supplied in the diet in the form of crystalline carotene.

A hen is just at the threshold of vitamin A deficiency when she is fed a vitamin A deficient diet which is supplemented with 350 micrograms of carotene per 100 grams of diet under the conditions of the described experiment. The results of the liver assays of the hens which were fed different levels of carotene definitely illustrates this point, since three out of four of the hens in this group had only a trace of vitamin A in a five gram sample. This information adds greater significance to the hatchability results obtained in this hen experiment. It shows that at border-line vitamin A nutrition, egg production is affected but that hatchability is not any different from that of hens with reasonably good stores of liver vitamin A.

The percent of mortality which occurred in the chick livability experiment was excessively high. This was due to the fact that the temperature of the battery room was very irregular. As a result, a large per cent of the chicks died from pneumonia. However, if the dam's diet had any influence on the livability of her chicks as far as the three levels of carotene is con-

cerned, there should have been a mortality trend with the low level having the highest mortality. The high mortality in the experiment does limit its value to some extent, but even during periods of relatively lower mortality there is no trend between the three groups.

Due to the present national emergency there is a conservation program under way at the present time. Any means of conserving vitamin A should be considered of great value to a portion of our national economy. It has been shown that the popular belief that the breeding mash requires more vitamin A is incorrect. Since a very large part of the annual production of vitamin A in this country is consumed by poultry, the thought is introduced that this work may lead to a saving in vitamin A.

The interrelationships between the vitamins is becoming more and more important. It has been shown that cattle do not synthesize vitamin C efficiently when they are chronically vitamin A deficient. Hens which were vitamin A deficient were available for a study of vitamin C storage in avitaminoses A fowls. The vitamin C assays which were made on the liver and duodenum showed that vitamin C storage in these organs is just as high when a hen is vitamin A deficient as when she is adequately supplied with this vitamin. Thus, there is a species difference between cattle and fowls. The deficient hen evidently can synthesize vitamin C as well as the normal hen, whereas cattle do not possess this ability. The duodenum is not generally recognized as a storage organ, yet considerable quantities of this

vitamin were found to be present. This fact is of interest from the standpoint of syntheses in the organism. However, its significance is not known.

CONCLUSIONS

1. The vitamin A requirement for egg production is as reat or greater than the hatchability requirement when the ole source of the vitamin is that which is present in the en'bubody.

2. The vitamin A requirement for egg production is as reat or greater than the hatchability requirement when the en is fed crystalline carotene in its diet at levels high nough to prevent vitamin A deficiency symptoms from appearng.

3. The level of vitamin A in the hen's diet which is ssential for maximum egg production is also adequate for ood livability of chicks produced by these hens.

4. A significant difference was not found between the itamin C contents of the liver and duodenum of hens which ere vitamin A deficient and those which were normal. The itamin A deficient birds possessed no storage of vitamin A n their livers while those which were normal had high storge values.

5. The duodenum of the fowl contains relatively large uantities of vitamin C.

REFERENCES

Almquist, H. J. and E. Mecchi, 1939. Vitamin A requirements of laying hens. Poul. Sci. 18: 129-137.

Baumann, C. A., J. Lamb, C. E. Holmes, and J. G. Halpin, 1939. The determination of vitamin A in the hen's egg. Poul. Sci. 18: 48-53.

Bearse, G. E. and M. W. Miller, 1937. The effect of varying levels of vitamin A in the hen ration on the vitamin A content of the egg yolk, on hatchability and chick livability. Poul. Sci. 16: 39-41.

Bethke, R. M., D. C. Kennard, and H. L. Sassaman, 1937. The fat-soluble vitamin content of hen's egg yolk as affected by the ration and management of the layers. Jour. Biol. Chem. 63: 115-122.

Carrick, C.W. and C. M. Hauge, 1935. Presence of the anti-scorbutic substance in the livers of chickens fed on scorbutic diets. Jour. Biol. Chem. 63:115-122.

Hart, E. B., J. G. Halpin, H. Steenbock, 1922. The nutritional requirements of baby chicks. II Further study of leg weakness in chickens. Jour. Biol. Chem. 52: 379-386.

Hart, E. B., H. Steenbock, and S. Lepkovsky, 1925. The nutritional requirement of the chicken. VI. Does the chicken require vitamin C? Jour. Biol. Chem. 66: 813-818.

Payne L. F. and J. S. Hughes, 1933. The effect of inadequate rations on the production and hatchability of eggs. Kan. Agr. Exp. Sta. Tech. Bul. 34.

Phillips, P. H., 1940. Directions for the ascorbic acid therapy of slow-breeding bulls. Jour. Amer. Vet. Med. Asso. 97: 165-166.

Polk, H. D. and G. R. Sipe, 1940. The effect of vitamin A deficiency on malposition of the chick embryo. 19: 396-400.

Record, Bethke, Wilder, and Chamberlain, 1937. Fifty-fifth annual report. Ohio Agri. Exp. Sta. Bul. 579.

Rubin, M., H. R. Bird, and H. M. DeVolt, 1941. Avitaminosis A in commercial poultry flocks. Poul. Sci. 20: 155-160.

Russel, W. C., C. S. Platt, M. W. Taylor, D. F. Chickester, 1935. The vitamin A requirements of laying pullets. N. J. Agr. Exp. Sta. Cir. 369.

Sherwood, R. M. and G. S. Fraps, 1932. The quantities of vitamin A required by pullets for maintenance and for egg production. Tex. Agr. Exp. Sta. Bul. 468.

Sherwood, R. M. and G. S. Fraps, 1934. The amount of vitamin A required by hens for egg production. Tex. Agr. Exp. Sta. Bul. 493.

Sherwood, R. M. and G. S. Fraps, 1935. The vitamin A requirements of hens for egg production. Tex. Agr. Exp. Sta. Bul. 514.

Sherwood, R. M. and G. S. Fraps, 1940. Requirements of chickens for vitamin A when fed as carotene. Tex. Agr. Sta. Bul. 583

Svirbely, J. L. Personal communication. National Institute of Health. Bethesda, Md.

Williams, J. K., C. E. Tanneman, and D. W. Bolin, 1939. The efficiency of carotene as supplied by alfalfa meal in meeting the vitamin A requirements of laying hens. Poul. Sci. 18: 268-275.

With, T. K. and C. Sanchez, 1939. Investigations into the vitamin A requirements of growing chicks and chick embryos as well as the vitamin A and carotene metabolism of the hen with some remarks about the symptoms of avitaminoses A in the growing chick. Nord. Med. 4: 3425-3429.

ABSTRACT

There are a great many conflicting views in the literature on the relationship between the vitamin A requirements for egg production and hatchability. As the result of experiments intended for an altogether different study, some information was obtained which tends to explain the true condition in the fowl.

Hens were placed on a vitamin A deficient diet. Their sole source of vitamin A was that which was present in their bodies. The eggs produced by these hens were incubated and it was found that the hatchability was relatively good, even the last eggs produced hatched well. However, egg production ceased just previous to the onset of vitamin A deficiency symptoms. It was also found that hens which were fed carotene as the only source of vitamin A at a borderline level produced eggs which hatched just as well as hens fed at higher levels of carotene. The hens which were fed the higher levels of carotene laid eggs at a higher rate of production. It would appear from these experiments that the vitamin A requirement for egg production is as great or greater than the vitamin A requirement for hatchability.

Assays for vitamin C of the liver and duodenum from hens which were vitamin A deficient revealed that these organs possessed as great a store of vitamin C as the organs from hens which were not vitamin A deficient. Thus, the vitamin A and C relationship which was found in cattle does not exist in the fowl.