A STUDY OF GENETIC RESISTANCE IN CHICKS TO A DEFICIENCY OF VITAMIN A IN THE DIET

by

JAMES ROBERT DE DELL

B. S., Kansas State College
of Agriculture and Applied Science, 1949

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Poultry Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

TABLE OF CONTENTS

INTRODUCTION

Studies have been made for the past several years on the fowl's genetic resistance to disease, genetic resistance to parasites and genetic variations in nutritional requirements. Results from the studies have shown that some breeds and strains of fowl are relatively resistant to avian diphtheria, pullorum, fowl typhoid, and lymphomatosis. Other studies have shown some breeds to be more resistant to certain parasitic infections and nutritional deficiencies than others.

Much work has been done on vitamin A at the Kansas Agricultural Experiment Station during recent years. The Diary, Animal, and Poultry Husbandry departments have cooperated with the Chemistry Department to study the nutritional problems arising in dairy, swine, sheep, and poultry. Practically all of the work on vitamin A in each department was from a nutrition standpoint. It was noted very early in the vitamin A studies with poultry that hens differed from each other in the ability to transfer vitamin A to the egg yolk. It was also noted that the amount of vitamin A transferred from a particular hen to her yolk was fairly constant although there was some degree of variability among hens when the hens were fed the same ration.

The foregoing points of interest provided impetus for investigation of the genetic differences among White Leghorns in the utilization of vitamin A as compared to the differences in the transmission of vitamin A from the hen to the egg yolk in two differentiated strains. If there were differences of utili-

zation within the breed of White Leghorns there should be a difference in longevity of chicks on a basal ration deficient or low in vitamin A. An experiment was designed to test the authenticity of such reasoning.

REVIEW OF LITERATURE

Frateur (1924), as described by Hutt (1949), showed that fowls differed gentically in their capacity to withstand infection by the bacterium causing avian diphtheria, Corynebacterium diphtheriae. Hutt and Scholes (1941) showed that White Leghorn chicks were more highly resistant to the bacterium, Salmonella pullorum, than were Rhode Island Red, White Wyandotte, and Barred Plymouth Rock chicks. Roberts and Card (1935), Lambert (1932), and Hutt, Cole, and Bruckner (1945) showed differences in resistance to pullorum, fowl typhoid, and lymphomatosis, respectively, in various strains of White Leghorns.

Ackert et al. (1935) found that the White Leghorn was more susceptible to the nematode, Ascaridia lineata, than other breeds of poultry. Lamoreaux and Hutt (1939a) gave evidence that White Leghorn chicks had a lower requirement for thiamine than Rhode Island Red and Barred Plymouth Rock chicks. From their data, it was shown that White Leghorns have the ability to tolerate diets low in thiamine and that this is a definite breed characteristic. Evidence in support of this was given by crossing Rhode Island Red females to White Leghorn males. Chicks of the F_1 generation were intermediate between the two parental breeds in thiamine requirements.

Lamoreaux and Hutt (1948) supported the evidence of Davis et al. (1938) that there were genetic differences among White Leghorns in the utilization of riboflavin.

Dairy cattle, as reported by Baumann, Steenbock, Beeson, and Rupel (1934) showed breed differences in their ability to transmit vitamin A to their milk.

There have been conflicting opinions among the many investigators regarding vitamin A utilization in the various species of animals. Baumann and Semb (1939) observed that when chicks were placed on a diet low in vitamin A the periods of survival were roughly proportional to the amount of vitamin A present in the newly-hatched chick or to the amount of vitamin A in the original yolk. Their data were based on a relatively small number of chicks, varying from 12 to 34 for different groups. They pointed out that the survival time of chicks on a low vitamin A diet was 13.2, 25.0 and 31.6 days for three groups of chicks which averaged 96, 146, and 170 micrograms of vitamin A, respectively, in the yolk.

Bearse and Miller (1937) noted that eggs from hens which had received different amounts of vitamin A in their rations contained different quantities of vitamin A in the egg yolks in proportion to the amount of vitamin A in the ration. Chicks hatched from such eggs and reared on a diet deficient in vitamin A lived and grew in proportion to the amount of this vitamin in the breeding ration. Table 1 gives a resume of their results.

Table 1. The periods of survival of chicks from hens receiving various levels of vitamin A in the ration. (Bearse and Miller, 1937.)

I.U.* of vitamin A in hen ration	Number chicks	Average survival time (days)
125	4	7.3
250	19	8.3
500	68	19.2
1000	92	44.7

*I.U. = International Units.

Bolin; Lampman, and Berg (1943) observed that there was a definite loss of vitamin A from livers of chicks receiving 100 micrograms or less of carotene per 100 grams of ration regardless of the initial amount present or the carotene intake of the dam. Chicks fed a diet deficient in vitamin A potency lost almost all their liver-stored vitamin A by the 19th day, regardless of the initial amount present. They noted the greatest loss occurred in the chicks which had the highest initial liver storage of vitamin A.

The Committee on Animal Nutrition of the National Research Council (1950) reported that pathological lesions, on autopsy, were confined largely to the mucous membranes of the mouth, pharynx, esophagus, and respiratory and urinary systems. Creamy white postules were found on the roof of the mouth and along the esophagus extending to the crop. Ureates accumulated in the ureters and in the kidney tubules, causing these organs to become

enlarged and creamy white in color. A cheesy exudate from the eyes was observed as well as a sticky discharge from the nostrils.

Dann (1932) observed no correlation in rats between the amount of vitamin A present in the liver at weaning and the survival time when subsequently placed upon a diet deficient in vitamin A.

Davies and Moore (1935) showed that when adult rats already possessing high liver reserves of vitamin A were given massive doses of vitamin A concentrate, the liver reserves reached levels having a mean value of about 18,000 B. U. (blue units) per g., representing a supply sufficient to satisfy the theoretical requirement of the rat for about a century if used up at a rate corresponding to the minimum physiological requirements. When the rats were subsequently restricted to a diet low in vitamin A a rapid elimination of vitamin A from the liver took place, the mean vitamin A reserve falling to 400 B. U. per g. after 12 weeks. At this level a condition of stable storage adequate for maintenance of the vitamin A level appeared to have been attained.

Brenner, Brookes, and Roberts (1941) observed a decrease in the average vitamin A content of the livers, blood, and eyes of hypervitaminotic rats on a diet deficient in vitamin A. This decrease of vitamin A content in the 3 organs amounted to 64 per cent in males and 60 per cent in females within two weeks. A decrease of 91 per cent in males and 79 per cent in females was noted at the end of six weeks; and a total decrease of 98 per cent in males and 91 per cent in females was evident at the

end of 13 weeks.

According to Leong (1941) the time required for depletion of vitamin A in the blood of dogs was not related to the original amount of vitamin A present at the beginning of the depletion period.

Booth (1950), Brenner et al. (1941), Moore and Sharman (1950), Davies and Moore (1925), and Popper et al. (1941) noted the ability of female rats to store more vitamin A than male rats when they both had been placed previously on a diet deficient in vitamin A. They also observed that the males eliminated their liver stores of vitamin A much faster than the females.

Moore and Sharman (1950) noted in rats on a diet deficient in vitamin A that there was more vitamin A in the kidneys of male rats than in the kidneys of the females. Popper and Brenner (1941) pointed out by their method of florescent microscopy, under conditions of large vitamin supply and advanced depletion, that there was a greater excess of vitamin A in the Kupffer cells than in the liver cells of rats. That these Kupffer cells in hypervitaminosis store the excess of vitamin A and apparently destroy it was shown to explain the uneconomic utilization of vitamin A under conditions of large supply.

MATERIALS AND METHODS

History of the Stock

Previous to this study, two lines of Single Comb White Leghorns had been differentiated during a 3 year period in the flocks

at the Kansas State College Poultry Farm. The females of one line were selected for the relatively high amounts of vitamin A they transferred to the egg yolks, while the females of the other line were selected for the relatively low amounts of vitamin A they transferred. The selection in each line was toward the extremes from the original means. The line having the ability to transfer relatively high amounts of vitamin A from the hen to the yolk will be hereafter referred to as Line H, and the line transferring low amounts of vitamin A to the egg will be referred to as Line L.

The matings were composed of both hens and pullets each generation. Analysis of the vitamin A content of yolks was made by the Chemistry Department of Kansas State College during the months from March to May. The vitamin A analysis was by the modified Carr-Price method for total vitamin A, described by Neff et al. (1949).

Selection of Breeders

For the present study, the females in both lines which were selected for the 1951 mating season had three years of previous selection of vitamin A transmission from hen to yolk. The hens from Line H were selected on the basis of having transferred considerably more vitamin A to the egg yolks than the females from Line L.

The male used in Line H was from a dam whose vitamin A classification was next to the highest for the 19 hens classified

in 1950. Of the 3 one-year old males used in Line L, two were from a yearling hen whose vitamin A classification was the lowest among the hens classified in 1950. The other male's dam was classified as intermediate among the low vitamin A transmitters in 1950.

Both lines had similar environments during the mating season. They were housed in adjacent pens in the same building. The equipment was similar in each pen. Both lines were housed together the remainder of the year.

The mating pens for this study were made up early in March, 1951. The hens were trapnested and the eggs were pedigree hatched.

Rations

Breeding Stock. The diet of both the females and males for each year was adequate in all the food nutrients. Throughout the year, the recommended 3300 International units (I.U.) of vitamin A per pound of ration were fed the breeders. The all-mash breeder ration consisted of the following feedstuffs:

Selected white corn, ground	360 pounds
Wheat shorts	60
Ground oats	60
Wheat bran	30
Meat scraps	21
Soybean oil meal	27
Fish meal	9
Brewers yeast	9
Dried skim milk	9
Calcium carbonate	6
Salt (NaCl)	3

Delsterol (vitamin D)	120 grams
Riboflavin	9
Manganese	45
Choline chloride	216
Vitamin B_{12}, Merck Sp 626	150
Prot-A (vitamin A)	100

Chicks. The basal ration for the chicks in this study consisted of the following feedstuffs:

Selected white corn, ground	60.5 pounds
Wheat bran	4.0
Soybean oil meal, 44% protein	27.0
Dried brewers yeast	3.0
Dried skimmilk	2.0
Steamed bonemeal	1.0
Calcium carbonate	2.0
Sodium chloride	0.5
Manganese sulphate	25 grams
Delsterol	40
Riboflavin	5
Choline chloride	9
Calcium pantothenate	1
Niacin	5
Vitamin B_{12}, Merck Sp 626	23
Prot-A	0

Management

The feed was prepared in small quantities at bi-weekly intervals to avoid unnecessary loss of vitamin A potency. The customary practice of feeding chicks on egg flats for the first three days was followed. Thereafter, the regular metal hoppers accompanying the brooder were used.

The chicks were individually wingbanded and weighed at the beginning of the experiment and at weekly intervals thereafter. The sex of each chick was determined after death by a post-mortem examination.

EXPERIMENTAL PROCEDURE

Experiment One (Preliminary)

On April 5, 1951, a test was begun with 140 chicks to determine what levels of vitamin A to use in the experiments to follow. The 140 chicks were divided equally into 5 groups and placed in the 5 decks of an electrically heated battery brooder. The chicks in this test were from White Leghorns of both Lines H and L. They were randomly assorted when placed in the 5 groups numbered 1, 2, 3, 4, and 5. Varying levels of vitamin A were added to the basal diet deficient in this vitamin.

The five levels of vitamin A added to the basal ration in the form of Prot-A were 0, 250, 500, 1000, and 2000 I.U. per pound of ration for Groups 1, 2, 3, 4, and 5, respectively.

Weights in this experiment were taken semi-weekly. An accurate account of mortality was maintained. The results after a 30-day trial revealed 100 per cent mortality in Group 1, 14.27 per cent mortality in Group 2, attributed to A-avitaminosis, and no mortality attributable to lack of vitamin A in Groups 3, 4, and 5.

To test the genetic tolerance to a deficiency of vitamin A, it was felt that a diet containing small amounts of the vitamin might provide information of a different nature than the results obtained from rearing chicks on a diet containing very little, if any, vitamin A. The survival time of the chicks fed a diet containing no vitamin A might be due only to the amount of the

vitamin present in the livers and yolks of the chicks at time of hatching and might not actually test the tolerance of chicks to survive on a diet low in vitamin A. The chicks having the greatest tolerance to a deficiency of vitamin A should live longer than those with less tolerance when all are fed a diet which contained a low amount of the vitamin.

From the results of this preliminary experiment, it was decided to use a level of 100 I.U. of vitamin A per pound of basal feed for Experiment 2 in order to obtain at least 50 per cent mortality for a 30-day trial.

Experiment Two

To determine whether there were genetic differences between the two lines of White Leghorns in the tolerance to a diet low in vitamin A, 180 chicks were hatched May 10, 1951. Of these, 65 chicks were from Line H and the remaining 115 were from Line L. To alleviate all possible differences in the environment during the experiment, both lines of chicks were reared together. Half of the chicks from each line were placed in one deck of a battery brooder. The remaining 90 chicks were placed in the deck immediately below. The chicks were fed the basal diet plus 100 I.U. of vitamin A per pound of ration.

Experiment Three

On May 18, 1951, 58 chicks from the same stock were started. There were 17 chicks from Line H and 41 from Line L. Thirty-one

crossbred and inbred White Rock chicks were added to this lot of 58 since they were available. The 89 chicks were brooded together in the same battery brooder as chicks in Experiment 2. They were fed the basal ration.

Experiment Four

Because of the results obtained in Experiments 2 and 3 (Figs. 4 and 5), and, too, because 125 White Rock-New Hampshire crossbred chicks were available at that time, Experiment 4 was designed to give further light on the differences in mortality between males and females when subjected to a diet low in vitamin A potency. These chicks were started the first week in June, 1951. Their diet consisted of the basal ration plus 100 I.U. of vitamin A per pound. Table 2 shows how the 125 chicks were divided into groups and the treatment with sex hormone given each group.

Table 2. Treatment with male and female hormone given chicks in Experiment 4 from day old to time of death.

Group	Number of chicks	Treatment
6	49	Control
7	41	Testosterone, 0.03 ml of 25 mg/ml solution
8	45	Stibesterol, 0.05 ml of 15 mg/ml solution

Injections of the sex hormones were made on alternate days. Since sex could be determined by color pattern at the beginning

of the experiment in chicks from certain hens, the females were placed in Group 7 and the males placed in Group 8. However, the sex could not be determined in all the chicks. Group 6 consisted of unsexed chicks. Group 7 and Group 8 consisted of chicks of both sexes, but Group 7 had a majority of females and Group 8 a majority of males.

RESULTS

Experiment One (Preliminary)

The results from the preliminary feeding experiment provided information along three lines. It gave a clue as to the level of vitamin A to use for testing the hypothesis on vitamin A utilization in order to have the desired 50 per cent mortality reached within close range of a 30-day feeding trial. It provided a standard to measure future rates of mortality on a diet deficient in vitamin A, and it revealed comparative growth rates on the various levels of vitamin A used (Fig. 1). It is of interest to note that with an increasing amount of vitamin A in the five different diets, there was a proportionate increase in the average growth rate of chicks in the five different groups.

Mortality in Group 1 began at 6 days of age and 100 per cent of the chicks had died by the 28th day. Only 14.28 per cent of the chicks in Group 2 had succumbed by the same date. Pathological lesions of the mouth, eye, pharynx and urinary system on autopsy were noted in the chicks in both groups. There were no deaths in Groups 3, 4 and 5 attributable to a deficiency of

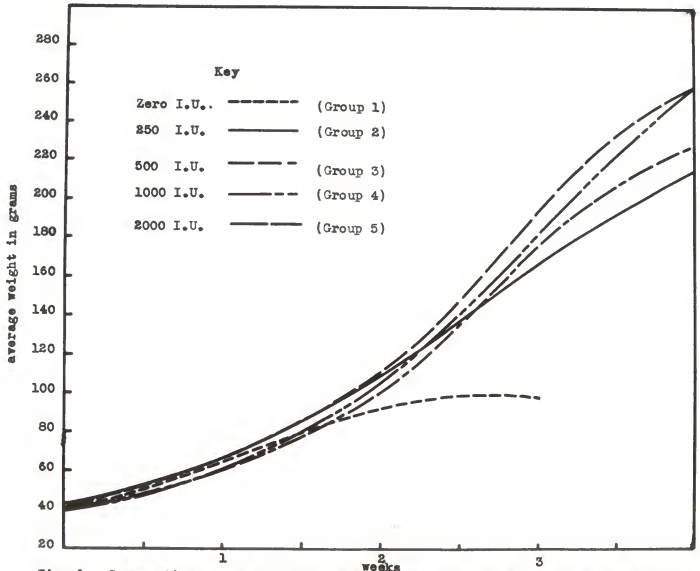

Fig. 1. Comparative rates of growth in White Leghorn chicks reared on diets containing varying amounts of vitamin A.

vitamin A, although 4 chicks in Group 3, 2 chicks in Group 4, and 4 chicks in Group 5 died during the first 2 days of the preliminary trial. Due to the fact that only 14.28 per cent of the chicks died on a diet containing 250 I.U. of vitamin A within a 28-day period (and these occurred between the 23rd and 28th days) it was decided that a diet containing 100 I.U. should be used in one of the future experiments to test the genetic tolerance to a deficiency of vitamin A.

The basal feed was analyzed for vitamin A-active carotenoids by a modification of the Association of Official Agricultural Chemists' procedure (1951) for carotene in dried plants. Since it contained no animal products, its content of preformed vitamin A was zero. No measurable quantities of vitamin A-active carotenoids were found.

The diet containing 100 I.U. of vitamin A was analyzed for vitamin A by the method of Neff et al. (1949) for vitamin A in egg yolks. The concentration was found to be somewhat less than 100 I.U. per pound of feed.

Experiment Two

It was assumed that the tolerance in the line of chicks having the higher amount of vitamin A in the yolk at time of hatching might be greater than the line of chicks having small amounts of the vitamin in the yolk. Contrary to this opinion the results of the second experiment showed that the chicks from hens selected for high amounts of vitamin A in the egg yolk had the

least tolerance when fed a diet containing 100 I.U. of vitamin A per pound of ration. Mortality began earlier in Line H and continued to be higher throughout the experiment than in Line L as shown in Figure 2. At 3 weeks and 2 days of age when 50 per cent mortality had been reached in the 180 chicks, the mortality of chicks in Line H was 72.30 per cent and in Line L it was 37.39 per cent. The average longevity of chicks in Line H was 16.75 days and in Line L, it was 21.45 days. Table 3 shows the trend of the mortality in Experiment 2. The difference in average longevity of $4.700 \pm 1.081^{**}$[1] days was statistically significant at the 1 per cent level. The average longevity for chicks in Experiment 2 was 19.75 days.

Table 3. Accumulative per cent mortality in the 180 chicks of Experiment 2.

Week	Line H 65 chicks	Line L 115 chicks	Total 180 chicks
1	12.31	6.08	8.33
2	38.31	16.52	24.44
3	67.54	31.30	44.44
4	98.31	96.52	97.22
5	100.00	100.00	100.00

[1] Throughout this thesis, statistical significance is denoted as follows: *means statistically significant at the 5 per cent level. **means highly statistically significant at the 1 per cent level.

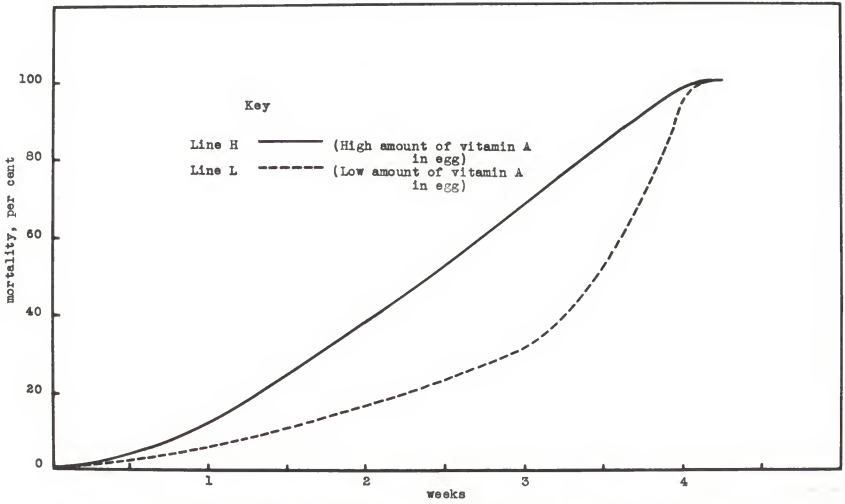

Fig. 2. Accumulative mortality in chicks between two lines of White Leghorns selected for high and low amounts of vitamin A in the egg yolk.

The mortality in Line H was more than twice that in Line L during any of the first 3 weeks of the test period, as shown in Table 3. It was during the fourth and fifth weeks that mortality in Line L caught up with that in Line H, as shown in Figure 3.

It was noted early in the experiment that early mortality was greater in the males than in the females. That the greater mortality in males occurred earlier in the test is shown in Table 4.

Table 4. Sex differences in weekly mortality of the 180 chicks in Experiment 2.

Week	Number died		Accumulative per cent mortality	
	Males	Females	Males	Females
1	11	4	11.95	4.54
2	20	9	33.69	14.77
3	17	19	52.17	36.36
4	41	54	96.74	97.72
5	3	2	100.00	100.00
Total	92	88		

Figure 4 reveals differences in the accumulative mortality between the males and females throughout the test period. The difference of 2.740 ± 1.153* days in average longevity of males and females in the 180 chicks was statistically significant. Table 5 shows the average survival time of chicks in days.

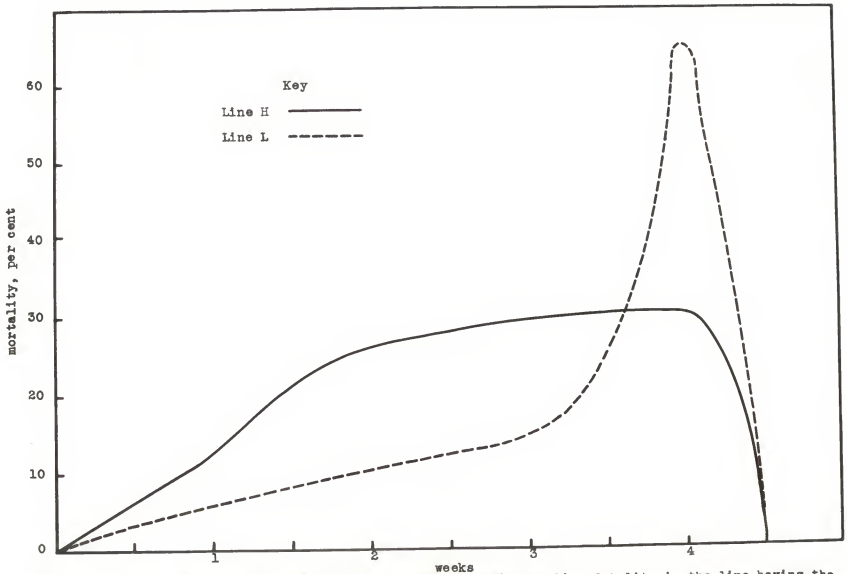

Fig. 3. Chick mortality in 2 lines of White Leghorns, showing earlier fatality in the line having the greater amount of vitamin A at time of hatching.

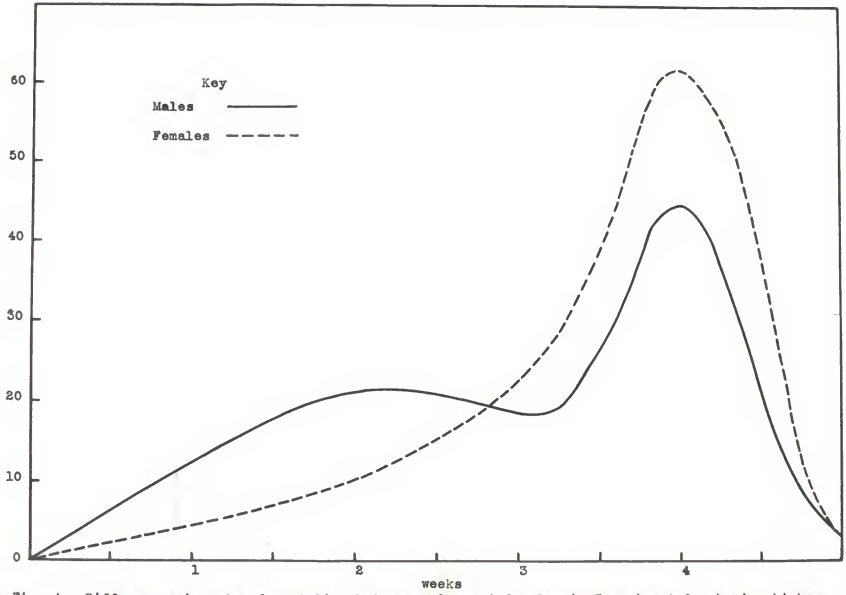

Fig. 4. Differences in rate of mortality between males and females in Experiment 2, showing higher early mortality among the males.

Table 5. Comparison of survival time of 180 chicks in Lines H and L, whose diet consisted of the basal ration plus 100 I.U. of vitamin A.

	Line H		Line L	
	Number of chicks	Average longevity, days	Number of chicks	Average longevity, days
Males	38	15.47	54	20.48
Females	27	18.55	61	22.47
Total	65	16.75	115	21.45

When 50 per cent mortality had occurred in the 180 chicks, 54 males and 36 females had succumbed. Of the 90 chicks that had died, 60 per cent were males and 40 per cent were females.

When the males and females in Lines H and L were treated separately, the mortality to 2 weeks was significantly higher in the males. Figure 5 shows the differences in mortality by sex in Lines H and L. At the time when 50 per cent mortality of the 180 chicks had been reached (3 weeks and 2 days of age), 81.57 per cent of the males and 59.25 per cent of the females in Line H had succumbed compared to 42.59 per cent of the males and 35.0 per cent of the females in Line L.

Experiment Three

As in Experiment 2, the chicks in Line H of this experiment showed the lesser tolerance of the two lines of White Leghorns to a diet more deficient in vitamin A when fed the basal ration. The chicks in Line L lived significantly longer than the chicks

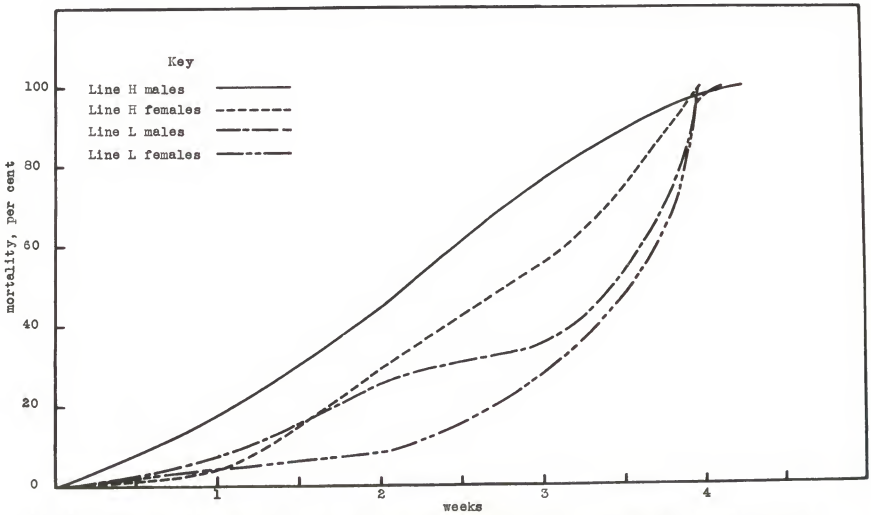

Fig. 5. Accumulative mortality in male and female chicks in two lines of White Leghorns, showing earlier mortality in males than in females in both lines.

in Line H. The difference in the average survival time of Line H and Line L of the 58 chicks was 3.03 ± 1.324* days. The average survival time in days for each line is shown in Table 6.

Table 6. Comparison of survival time of 58 chicks in Lines H and L when fed the basal ration containing no vitamin A.

	Line H		Line L	
	Number of chicks	Average longevity, days	Number of chicks	Average longevity, days
Males	3	11.00	26	11.80
Females	14	8.65	15	15.93
Total	17	10.58	41	13.61

The per cent of accumulated weekly mortality of the chicks is revealed in Table 7. The chicks in Line H succumbed at a rate of 2.41 times as much the first week and 1.43 times as much during the second week as did the chicks in Line L. The average longevity of chicks in Experiment 3 was 13.70 days. Figure 6 shows the trend of mortality in Experiment 3.

Table 7. Weekly accumulated per cent mortality of chicks receiving the basal diet containing no vitamin A.

Week	Line H	Line L
1	29.41	12.19
2	76.46	53.65
3	100.00	97.65
4		100.00

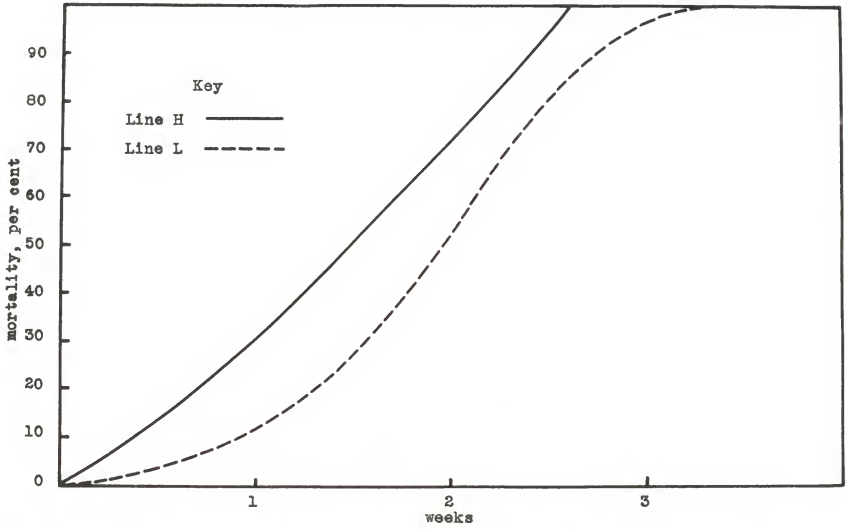

Fig. 6. Accumulative mortality in chicks in 2 lines of White Leghorns, showing earlier mortality in stock having higher amounts of vitamin A in the egg yolk at time of hatching.

There was a disproportionate number of male and female chicks in Line H, so a comparison of differences in mortality among sexes would be of little value. There were 3 males and 14 females in the 17 chicks. Of the 41 chicks in Line L, the mortality to 2 weeks was significantly higher in the males. There was a difference in the average survival of 3.97 ± 1.700* days between the males and females in Line L. The weekly rates of mortality according to the sex of the 41 chicks in Line L is shown in Table 8.

Table 8. Percentage accumulative mortality by weeks among 26 males and 15 females in Line L. (Diet consisted of the basal ration without vitamin A.)

Week	Accumulative mortality, per cent	
	Males	Females
1	15.38	6.66
2	69.23	20.66
3	100.00	93.32
4	-	100.00

The heavy breed chicks in this experiment when fed the basal diet showed an average survival time of 15.35 days. This is an increase of 2.25 days above the average survival time for the White Leghorn chicks. The average survival time of the heavy breed male chicks was 14.58 days; and for the females, it was 16.21 days. The mortality was higher in the males but was not significantly higher than in the females. At 2 weeks of age the males outnumbered the females in mortality by 11.30 per cent.

Table 9 shows the percentage mortality by weeks in the 31 inbred and crossbred chicks.

Table 9. Accumulative percentage mortality of the 31 heavy breed chicks.

Weeks	Males		Females	
	Chicks, number	Mortality, per cent	Chicks, number	Mortality, per cent
1	1	5.88	1	7.14
2	7	47.00	4	35.71
3	17	100.00	13	92.85
4	-	-	14	100.00

Experiment Four

After a 45-day trial, when 100 per cent mortality had been reached, the average longevity of the female chicks that were given the male hormone in Group 7 was 21.28 days. The average longevity of the male chicks that were given the female hormone was 24.00 days. Table 10 shows the comparative longevity of male and female chicks which were treated with the sex hormones.

Table 10. Comparison of longevity of male and female chicks when injected with male and female sex hormone.

Sex of chicks	Average longevity, days injected with	
	Male hormone	Female hormone
Males	17.50 (2 males)	24.00 (33 males)
Females	21.18 (39 females)	25.83 (12 females)

The male chicks receiving the female sex hormone survived longer than females who received the male sex hormone. The difference in survival time was not significant, however. Evidence that the addition of the female sex hormone to the female chicks increased the ability to utilize minute amounts of vitamin A is demonstrated by the longevity greater by 4.65 days than in the female chicks which received the male sex hormone.

Analyses of Average Longevity of Individual Families in Lines H and L

Individual families within each line were examined for longevity, and no correlation could be found in regard to the amount of vitamin A each dam transferred to the yolk and the longevity of her chicks. There were 10 dams of known vitamin A classification in each line. Coefficients of correlation were computed comparing the average survival in days for the chicks with the ability of the dam to transfer vitamin A to the egg. The coefficient of correlation in Line H was +0.5787. To be significant statistically at the 1 and 5 per cent levels with this number of families, a value of 0.765 and 0.632, respectively, was necessary. The coefficient of correlation in Line L was +0.127. This figure was far from the 0.632 necessary for significance at the 5 per cent level. The data in Table 11 compare the longevity of chicks with the level of vitamin A transferred to the egg yolk by the dam.

Table 11. Comparison of vitamin A in the yolk of eggs laid by dams with longevity in the chicks reared on 100 I.U. of vitamin A in the diet.

Line H			Line L		
Classification of dam with respect to vitamin A in the egg, mg/gm yolk		Average longevity of chicks, days	Classification of dam with respect to vitamin A in the egg, mg/gm yolk		Average longevity of chicks, days
5 highest dams	Mg.		5 highest dams	Mg.	
9424	5.00	15.57	4481	4.81	14.36
3975	4.76	22.00	4478	3.81	22.25
4487	4.40	25.00	4466	3.75	12.90
4473	4.27	20.30	4472	3.60	16.37
1847	4.20	16.00	1802	3.45	24.00
5 lowest dams			5 lowest dams		
4453	4.17	12.00	4490	3.40	20.50
4463	4.03	23.25	1820	3.30	13.61
4448	3.86	8.25	4439	2.67	13.00
1810	3.70	10.42	1832	2.20	12.25
1848	3.50	8.12	1805	1.81	13.66

Upper 5

$r = -0.064$

Lower 5

$r = -0.3061$

Upper 5

$r = -0.285$

Lower 5

$r = +0.8000$

DISCUSSION

The level of 100 I.U. of vitamin A for a diet low in vitamin A, chosen by the interpolation of the results of the preliminary test (Experiment 1) was apparently satisfactory for measuring tolerance of chicks to such deficient diets. Chicks in Experiment 2, whose diet included 100 I.U. of vitamin A, had an average longevity of 19.75 days; whereas, the average longevity of chicks was 13.70 days in Experiment 3 where the diet contained no vitamin A. The distribution of mortality by 2 day periods in the two experiments are compared in Fig. 7.

In this study, genetic tolerance was measured by the average survival time in 2 lines of chicks, differentiated by the amount of vitamin A which the dams transferred to the egg yolk. One might expect that the tolerance to deficient diets for the line of chicks from stock transferring the higher amount of vitamin A to the yolk to be greater than that in the line of chicks from stock which transferred small amounts of the vitamin. The results of both experiments did not show this to be true. The period of survival of chicks from Line H, from stock transferring the greater amounts of vitamin A to the yolk, was significantly shorter than that of chicks in the other line. The average longevity of chicks in Line H for Experiments 2 and 3 was 16.75 and 10.58 days and in Line L for the same experiments was 21.45 and 13.61 days, respectively. The overall average longevity of chicks in Experiments 2 and 3 combined in Line H was 15.47 days and in Line L it was 19.29 days. There were 82 chicks in Line H and 156 chicks in

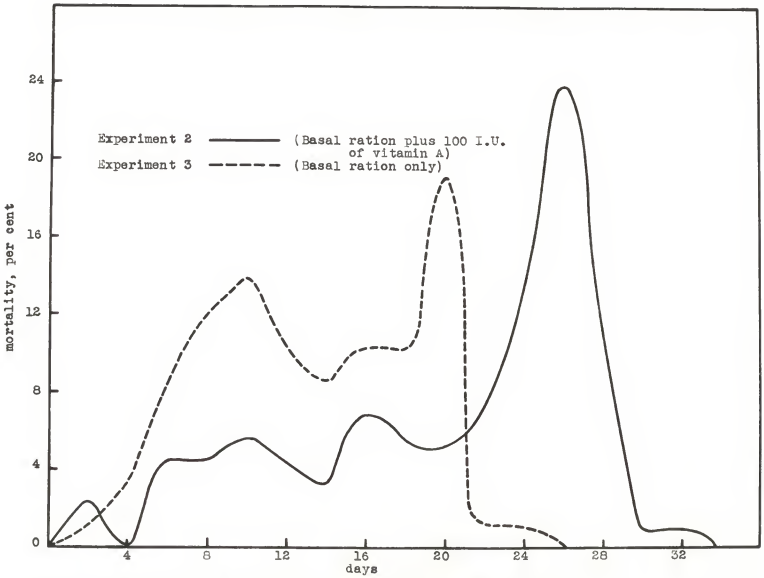

Fig. 7. Distribution of mortality in chicks for Experiments 2 and 3.

Line L in the combined experiments.

This finding is in agreement with Bolin et al. (1943), who also found that the greatest loss occurred in the 3 lots of chicks having the highest initial liver storage of vitamin A at time of hatching. This does not conform to the results of Baumann and coworkers (1939) and Bearse and Miller (1937). Their results possibly may be due to the small number of chicks used in certain groups.

The results obtained in this test with the domestic fowl agree, in general, with the work done with other species of animals. It is in agreement with Dann (1932) who could find no relationship between the survival time and the amount of vitamin A present in the livers of rats at weaning when subsequently fed a diet deficient in vitamin A.

The rapid depletion of vitamin A in chicks agrees with the results of Leong (1941) who showed that the depletion time of vitamin A in dogs was comparatively the same regardless of the original amount present at the beginning of the depletion period. The fact that high amounts of vitamin A in rats at the start of a depletion experiment is not an advantage was also noted by Davies and Moore (1925) and Brenner et al. (1941). Davies and Moore (1925), Brenner et al. (1941) and Horton et al. (1941) observed no advantage of excessive amounts of liver-stored vitamin A in rats in that a rapid elimination of the excess occurred. The period of depletion of excessive amounts of liver-stored vitamin A did not depend entirely upon the original amount present according to Dann (1932) and Horton et al. (1941).

A possible reason for the rapid loss of the excess vitamin A in chicks may be due to the phagocytic action of the Kupffer cells which comprise a part of the reticulo-endothelial system. This was shown to have occurred in rats, as reported by Popper and Brenner (1941). This may account for the excessive amounts of vitamin A found in the rat kidney when placed on a diet depleted of vitamin A as reported by Moore and Sharman (1950).

Another possibility for the rapid loss of vitamin A would be a greater utilization of it when found in abundance in the chicks.

It was seen by comparing results for Lines H and L that longevity in chicks on a diet deficient in vitamin A could not be determined by the amount of the vitamin the chick had at the time of hatching. Neither could longevity be determined by comparing individual high and low families. The trend in longevity between Lines H and L, favoring a longer survival in chicks from Line L, was the same between high and low families within lines. That is, a dam whose chicks had the shortest survival period usually transferred a relatively larger amount of vitamin A to the yolk.

Since the females in the fowl were shown to live significantly longer than the males, this scope of knowledge can be added to the reports of others who had found differences between males and females of other species with regard to the ability to store vitamin A in the liver and differences in the rates of depletion of liver-stored vitamin A.

The greater longevity of the female fowl is probably due to the increased vitamin A storage in the liver and to their being able to retain this vitamin A store longer than males. This would agree with Popper and Brenner (1941), Brenner et al. (1941), Booth (1950), and Moore and Sharman (1950) who have shown that female rats store considerably more vitamin A in the livers and retain their vitamin A stores longer than males. Booth (1950) concluded that the difference between liver storage of vitamin A in favor of female rats was not due to differences in rates of growth between males and females.

The greater longevity of the female fowl may be due to the action of the female sex hormone, estrogen. It was shown in Experiment 4 that male chicks which were injected with the female sex hormone lived longer than did the female chicks which were treated with the male hormone. A greater significance was seen when female chicks lived longer which received additional amounts of the female sex hormone than those that received the male sex hormone. The presence of the female sex hormone may assist in increasing and maintaining the storage of vitamin A in the liver allowing a longer period of survival of the female chicks.

There was more significance in the results of Experiment 2 than in Experiment 3 with respect to both the greater longevity of chicks in Line L and the greater longevity of the female chicks. This was attributed to the greater numbers of chicks in Experiment 2.

It is difficult to understand why a higher amount of vitamin

A should be a disadvantage to a chick, even though survival on a deficient diet depended entirely upon the ability to utilize minute amounts of vitamin A in the daily diet. Data reported for chicks, rats and dogs are in general agreement that high amounts of the vitamin present at the beginning of a depletion diet actually are comparatively detrimental to relative survival.

That the survival of the chick depended upon its ability to utilize the minute amounts of the vitamin was seen when the longevity was reduced by 46 per cent when given a diet which contained no added vitamin A. Since the survival time was increased by 7 days (from 12.70 days in Experiment 3 to 19.75 days in Experiment 2) by adding 100 I.U. of vitamin A to the basal ration, this was evidence that the survival to 12 days was due in part to the minute amounts of the vitamin in the basal diet. The greater survival of the chicks in the line having small amounts of vitamin A at the time of hatching undoubtedly was due to their lower requirements of the vitamin.

The evidence submitted also suggests that the female sex hormone increased the ability to utilize the minute amounts of vitamin A in the daily diet.

SUMMARY AND CONCLUSIONS

Experiments were conducted with White Leghorn chicks to test for tolerance to a diet deficient in vitamin A. The results, based on the average survival time in 2 lines of White Leghorn chicks, showed (1) the least tolerance in the line of chicks

from stock that transferred greater amounts of the vitamin to the yolk and (2) that the females have a greater tolerance than males.

An experiment was conducted with mixed, heavy breed chicks to determine the effect of sex hormones on longevity of chicks on diets deficient in vitamin A. The results based on the average survival time of male and female chicks when fed a diet containing 100 I.U. of vitamin A showed that the chicks that were treated with the female sex hormone lived longer than those that were treated with the male sex hormone. Results were as follows:

1. The growth and weight of chicks to 5 weeks in the preliminary experiment was in direct relationship to the amount of vitamin A in the feed.

2. Less tolerance to a diet deficient in vitamin A was noted in the line of chicks from stock having transferred greater amounts of vitamin A to the yolk.

3. Females had a greater tolerance to a diet depleted of vitamin A than did males.

4. The increased tolerance of females to a diet deficient in vitamin A was apparently due to the presence of the female sex hormone, estrogen.

ACKNOWLEDGMENT

The author is deeply indebted to Dr. Clyde D. Mueller, major instructor, for his advice, constructive criticism, and aid through the course of this investigation.

Sincere gratitude is extended the Chemistry Department for the valuable service rendered in connection with the analyzing of eggs and the formulation of the diets.

Words of appreciation are offered to John D. Harvey for his valuable time in helping with this course of study.

LITERATURE CITED

Ackert, J. E., L. L. Eisenbrandt, J. H. Wilmoth, B. Glading, I. Pratt. Comparative resistance of five breeds of chickens to the nematode Ascaridia lineata (Schneider). U.S.D.A. Jour. Agr. Research 50:607-622. 1935.

Association of Official Agricultural Chemists. Vitamin A in mixed feeds. Jour. Assoc. Official Agr. Chemists. 34:97-98. 1951.

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

Baumann, C. A., H. Steenbock, W. M. Beeson, and I. W. Rupel. The influence of breed and diet of cows on the carotene and vitamin A content of butter. Jour. Biol. Chem. 105:167-176. 1934.

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

Bolin, D. W., C. E. Lampman, and L. R. Berg. The influence of carotene intake as supplied by dehydrated alfalfa on the storage of vitamin A and pigments in the livers of the young chick. Poul. Sci. 22:348-353. 1943.

Booth, V. H. The influence of sex on the storage of vitamin A. Proc. of the Biochemical Society. 47:4, p. xliii. 1950.

Brenner, S., M. C. Brookes, and L. J. Roberts. The relation of liver stores to the occurence of early signs of vitamin A deficiency in the white rat. Jour. Nutr. 23: 459-471. 1941.

Dann, W. J. The transmission of vitamin A from parents to young in mammals. Biochem. Jour. 36:1072-1076.

Davis, H. J., L. C. Norris, and G. F. Heuser. Further evidence of the amount of vitamin G required for reproduction in poultry. Poul. Sci. 17:87-92. 1938.

Davies, W. A., and T. Moore. Elimination of vitamin A from the livers of rats previously given massive doses of vitamin A concentrate. Biochem. Jour. 29:147-150. 1925.

Horton, F. B., W. A. Murrill, and A. C. Curtis.
Vitamin A in the liver and blood of rats. Jour. Clin. Invest. 20:387-393. 1941.

Hutt, F. B.
Genetics of The Fowl. New York: McGraw-Hill, pp. 413-418. 1949.

Hutt, F. B., H. K. Cole and J. H. Bruckner.
A test of fowls bred for resistance to lymphomatosis. Poul. Sci. 24:564-571. 1945.

Hutt, F. B., and J. C. Scholes.
Breed differences in susceptibility to Salmonella pullorum. Poul. Sci. 20:342-352. 1941.

Lambert, W. V.
The comparative resistance of different breeds. Jour. Immunol. 23:253-259. 1932.

Lambert, W. V.
Bacteriological studies upon surviving birds of the resistant stock in relation to progeny resistance. Jour. Immun. 23:241-251. 1932.

Lambert, W. V.
The effect of selective breeding on natural resistance to fowl typhoid. Jour. Immun. 23:229-240. 1932.

Lamoreaux, W. F. and F. B. Hutt.
Breed differences in resistance to a deficiency of vitamin B_1 in the fowl. Jour. Agr. Res. 58:307-316. 1939a.

Lamoreaux, W. F. and F. B. Hutt.
Genetic resistance to deficiency of riboflavin in the chick. Poul. Sci. 27:334-341. 1948.

Leong, P. C.
Vitamin A in blood and its relation to body reserves. Biochem. Jour. 35:806-812. 1941.

Moore, T., and I. M. Sharman.
Vitamin A in the kidneys of male and female rats. Proc. of Biochem. Soc. 47:4, pp. xliii, xliv. 1950.

National Research Council.
Recommended nutrient allowances for poultry. A report of the Committee on Animal Nutrition, p. 7. 1950.

Neff, A. W., D. B. Parrish, J. S. Hughes and L. F. Payne.
The state of vitamin A in eggs. Archives of Biochem. 21:315-320. 1949.

Popper, H., and S. Brenner.
The fate of excess vitamin A stores during depletion. Jour. Nutr. 23:431-441. 1941.

Roberts, E. and L. E. Card.
Inheritance of resistance to bacterial infection in animals. A genetic study of pullorum disease. Ill. Agr. Expt. Sta. Bul. 419. 1935.

APPENDIX

Table A. Average weekly weight of White Leghorn chicks reared on diets varying in vitamin A level.

Group	I.U. vitamin A added to basal diet	Weight in grams: Day old	One week	Two weeks	Three weeks	Four weeks
I	0	40.40	63.85	92.78	99.9	-
II	250	41.50	64.18	107.88	167.38	215.66
III	500	40.25	58.29	99.70	178.60	229.47
IV	1000	41.32	61.50	109.00	197.77	258.96
V	2000	39.00	57.28	104.00	183.40	258.68

Table B. A comparison of mortality in Lines H and L when 50 per cent of all chicks had died. Diet included 100 I.U. of vitamin A.*

Line	Number of chicks: Dead	Alive	Total
H	47	18	65
L	43	72	115
Total	90	90	180

*The Chi-square value for this distribution of chicks is 20.25, indicating a highly significant difference between Lines H and L.

Table C. Test of significance in difference of longevity of the chicks in Lines H and L.

Statistical item	Line H	Line L
Number of chicks	65.000	115.000
Mean longevity, days	16.753	21.452
Standard deviation	7.141	6.1537
Standard error	0.89365	0.06109
Difference and standard error of difference	4.70 ± 1.081**	

**Denotes highly significant difference (P = <.01)

Table D. Test of significance in difference of longevity of the chicks in Lines H and L.*

Statistical item	Line H	Line L
Number of chicks	17.00	41.00
Mean longevity, days	10.58	13.61
Standard deviation	4.300	5.248
Standard error	1.042	0.8190
Difference and standard error of difference	3.03 ± 1.324	

*P = <.05

Table E. Comparison of mortality in males and females of the 180 chicks in Experiment 2 when 50 per cent of the chicks had died. The chi-square value for this distribution of chicks is 5.56, showing a significant difference between males and females.

Sex of chicks	Number of chicks		
	Dead	Alive	Total
Males	54	38	92
Females	36	52	88
Total	90	90	180

Table F. Differences in mortality between males and females in the 65 chicks in Line H when reared on diet containing 100 I.U. vitamin A. The chi-square value for this distribution of chicks is 16.08, indicating a highly significant difference between males and females.

Sex of chicks	Number of chicks		
	Dead	Alive	Total
Males	31	7	38
Females	16	11	27
Total	47	18	65

Table G. Differences in mortality between males and females in the 115 chicks in Line L. The chi-square value is 7.10 for the distribution of chicks.

Sex of chicks	Number of chicks		
	Dead	Alive	Total
Males	33	21	54
Females	21	40	61
Total	44	71	115

Table H. Test of significance in difference of longevity of male and female chicks.*

Statistical item	Sex of chicks: Males	Females
Number of chicks	92.00	88.000
Mean longevity, days	18.41	21.159
Standard deviation	7.771	6.649
Standard error	0.81023	0.70889
Difference and standard error of difference	= 2.748 ± 1.073 days	

*P = <.05

Table I. Test of significance in difference of longevity of male and female chicks.*

Statistical item	Sex of chicks: Males	Females
Number of chicks	26.00	15.00
Mean longevity, days	12.23	16.00
Standard deviation	4.829	6.458
Standard error	0.9470	1.667
Difference and standard error of difference	= 3.97 ± 1.700	

*P = <.05

Table J. Coefficient of correlation for period of survival of chicks in Lines H and L with known vitamin A in yolk at time of hatching.

Line H		Line L	
X*	Y**	X*	Y**
10.42	3.70	24.00	3.45
16.00	4.20	15.66	1.81
8.12	3.50	13.61	3.30
22.00	4.76	12.25	2.20
8.25	3.86	15.00	2.67
12.00	4.17	12.90	3.75
23.25	4.03	16.37	3.60
20.30	4.27	22.25	3.81
25.00	4.40	14.36	4.81
15.57	5.00	20.50	3.40
Average 16.09	4.18	16.29	3.28
r = +0.5787		r = +0.127	

*X = Average longevity of chicks.

**Y = Classification of dam.

A STUDY OF GENETIC RESISTANCE IN CHICKS TO A DEFICIENCY OF VITAMIN A IN THE DIET

by

JAMES ROBERT BE DELL

B. S., Kansas State College
of Agriculture and Applied Science, 1949

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Poultry Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

A preliminary experiment was conducted with 140 White Leghorn chicks to determine what levels of vitamin A to use to test for tolerance to a diet deficient in the vitamin. Two experiments following the preliminary one were conducted to test for the tolerance to a diet free of vitamin A with two differentiated lines of White Leghorn chicks. The chicks were from stock that had been selected for 3 consecutive years for the amount of vitamin A that was transferred from the hen to her egg yolk. The chicks in one line were from stock transferring the high amounts of vitamin A to the yolk, (Line H), and chicks in the other line were from stock having transferred low amounts of the vitamin to the yolk (Line L).

The results, based on the average survival time in 2 lines of White Leghorn chicks, showed the least tolerance in the line of chicks from stock that transferred greater amounts of the vitamin to the yolk. One might expect that the tolerance to deficient diets for the line of chicks from stock transferring the higher amount of vitamin A to the yolk to be greater than that in the line of chicks from stock which transferred small amounts of the vitamin. The results of two experiments did not show this to be true. The overall average longevity in the 2 experiments was 15.47 days for chicks from stock transferring large amounts of vitamin A to the yolk, and the average longevity was 19.29 days for chicks from stock transferring to the yolk small amounts of the vitamin.

The White Leghorn female chicks outlived the male chicks in both experiments. The difference of 2.74 $\pm$ 1.153 days in average

longevity of males and females in the 180 chicks in Experiment 2 was statistically significant. Also there was a difference in the average survival of 3.97 ± 1.700 days between males and females in Experiment 3.

Experiment 4 was conducted with mixed, heavy breed chicks to determine the effect of sex hormones on longevity of chicks on diets deficient in vitamin A. The results based on the average survival time of male and female chicks when fed a diet containing low amounts of vitamin A showed that the male chicks that were treated with the female sex hormone lived longer than female chicks that were treated with the male sex hormone.

Summary

1. The growth and weight of chicks to 5 weeks in the preliminary trial was in direct relationship to the amount of vitamin A in the feed.

2. Less tolerance to a diet deficient in vitamin A was noted in the line of chicks from stock having transferred greater amounts of vitamin A to the yolk.

3. Female chicks had greater tolerance to a diet depleted of vitamin A then did males.

4. The increased tolerance of females to a diet deficient in vitamin A was apparently due to the presence of the female sex hormones.