THE RESISTANCE OF CHICKENS TO ASCARIDIA GALLI (SCHRANK) AS AFFECTED BY AN AUREOMYCIN-VITAMIN B_{12} DIETARY SUPPLEMENT

by

MARK GILBERT NORRIS, JR.

B. S., New Mexico College of Agriculture
and Mechanical Arts, 1950

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

Experimental evidence presented in the past thirty years has indicated that the resistance of a vertebrate host to a parasitic infection is influenced by several factors. Ackert (1942), in a review of the literature on natural resistance to helminthic infections, considered the factors involved to be mainly in the diet, age and genetic constitution of the host. The present study has been largely devoted to the resistance of chickens to an ascarid infection as influenced by diet; therefore, only the literature directly related to this phase of the subject will be reviewed.

Apparently for the first time, it was reported by Zimmerman, Vincent and Ackert (1926) that the resistance of young chickens to Ascaridia galli (syn. A. perspicillum and A. lineata) was lowered when the chickens were placed on vitamin B deficient diets. Ackert, Fisher and Zimmerman (1927) reported that a deficient vitamin A diet for chickens likewise lowered their resistance to A. galli. These observations were supported by further investigations of Ackert, McIlvaine and Crawford (1931). That A. galli does not require vitamins A and B for normal growth was shown by Ackert (1930).

Ackert and Nolf (1931) found the resistance of chickens to A. galli to be significantly lowered when the chickens were given diets lacking vitamin B complex. This research also suggested that yeast, which was used as the source of vitamin B complex,

contained a growth factor for the nematode. Later Beach and Ackert (1932) reported that such a factor was not present in yeast.

A study made by Ackert and Spindler (1929) failed to show any significant relationship between vitamin D deficient diets and the growth of A. galli in chickens. However it was noted that the general effects of the parasite on the chickens were reduced when the chickens were well supplied with the vitamin.

A recent paper by Sadun et al. (1950) indicated that pteroylglutamic acid deficiencies reduced the resistance of chickens to A. galli. These workers also observed that chickens on diets supplemented with liver extract harbored significantly longer worms than did the chickens on a non-supplemented diet and suggested that vitamin B, present in the extract, may have been responsible for the growth stimulus to the nematodes.

Further investigations to determine the effects of other dietary deficiencies and supplements on the resistance of chickens to an ascarid infection were conducted by Ackert and Beach (1933). These workers found that chickens reared on diets of entirely vegetable sources were considerably less resistant than chickens given cereal rations supplemented with skim milk and meat meal. Ackert (1947) and Riedel and Ackert (1950) reported that soybean oil meal could be substituted for skim milk or meat scraps to aid the chickens in maintaining their resistance to ascariasis. Riedel and Ackert (1950) also found a soybean oil meal and skim milk supplemented diet

to be superior to either soybean oil meal or meat scrap supplements for maintaining the resistance of the chickens to A. galli.

During the past two years considerable progress has been made in the field of animal nutrition with regard to growth responses in young animals. Stokstad et al. (1949) reported significant weight gains in young chickens on an all vegetable protein diet supplemented with aureomycin fermentation mash and vitamin B_{12}. Further investigations by Stokstad and Jukes (1951) have shown that diets supplemented with small amounts of aureomycin resulted in greater weight gains in chickens than did diets supplemented with vitamin B_{12}. Research conducted by Cunha et al. (1949) and Carpenter (1950) has shown that low level aureomycin and vitamin B_{12} supplemented diets produced significant weight gains in young pigs. Stokstad and Jukes (1950) reported significant weight gains in turkey poults when placed on diets containing small amounts of aureomycin.

At present it is a common practice to include low level aureomycin and vitamin B_{12} supplements in many commercial feedstuffs. This is usually added in the form of Aurofac, a fermentation product manufactured in conjunction with aureomycin production. The manufacturer, American Cyanimid Company, Lederle Laboratories Division, have recommended the use of Aurofac in concentrations of 0.1 to 0.5 per cent in the basal ration for optimal growth responses in young animals.

With the foregoing information in mind, the present research was conducted to determine the resistance of two to eight week old chickens to an experimental infection of A. galli as affected by an all vegetable protein diet supplemented with low level aureomycin and vitamin B_{12}. The criteria used for judging the resistance of the chickens to ascarid infections were the numbers and lengths of the worms recovered, weight gains made by the chickens, percentage mortality and percentage infection as determined by the number of chickens harboring the nematodes at the termination of the experiments.

MATERIALS AND METHODS

The chickens used in this study were received from the Kansas State College Poultry Farm on the day of hatching or the succeeding day. All were mixed lots of White Plymouth Rocks and White Leghorn crosses.

Upon receipt the chickens were weighed, banded and immunized against Newcastle Disease with intranasal doses of Newcastle Disease Live Virus supplied by the American Cyanimid Company, Lederle Laboratories Division. The chickens were divided into four groups (designated as Groups I, II, III and IV) of approximately equal weights and placed into an electric brooder battery. They were then given their experimental diets and supplied with water at all times. The weekly weights of the chickens were recorded.

An all vegetable protein basal ration, complete with the necessary vitamins and minerals, was obtained from the Kansas State College Poultry Farm for use in the experiments (Table 1). Groups I and II of each experiment received the basal ration, whereas, Groups III and IV received the basal ration supplemented with 1/2 lb. of Aurofac (supplied by the Lederle Laboratories) per 100 lb. of feed. The Aurofac, containing 1.9 gm of aureomycin and 1.9 mg of vitamin B_{12} per pound, thus supplied approximately 0.9 gm of aureomycin and 0.9 mg of vitamin B_{12} per 100 lb. of feed. The supplemented ration was mixed in 25-lb. batches by placing small portions of a 25-lb. quantity of basal ration into a 30-gal. galvanized can. As each portion was added it was sprinkled with small amounts of Aurofac until 1/8 lb. (56.7 gm) of Aurofac had been added. The entire batch was then agitated by holding the can at a 30° angle to the floor and rotating it from left to right approximately 100 times.

A method described by Riedel (1947) was used to prepare egg cultures from living, gravid female A. galli. The anterior ends of the nematodes were excised and the body contents were pressed out. The uteri were isolated, placed into Petri dishes containing a few millimeters of tap water, and cut into small pieces by means of teasing needles. These cultures were then incubated at 28° C. until used.

The chickens in Groups I and IV of Experiments 1 and 2 were given, at the age of two weeks, 100 ± 10 embryonated ova of A. galli. In Experiments 3 and 4, which utilized Groups II and III (controls) of the first two experiments, the

Table 1. Formula for the all vegetable protein basal diet used in the experiments.

Ingredient	Quantity
Ground yellow corn	63.8 lbs.
Wheat bran	4.0 "
17 per cent dehydrated alfalfa meal	1.0 "
44 per cent solvent extracted soybean oil meal	30.0 "
$CaCO_3$	1.0 "
Steamed bone meal	1.0 "
NaCl	0.5 "
$MnSO_4$	25.0 gm
Prot. A (2400 U. S. P. units per gm)	100.0 "
Delsterol (200 A. O. A. C. units per gm)	40.0 "
Riboflavin (Merck Mix no. 54=1 gm of riboflavin per ounce of mix)	5.0 "
Choline Chloride (Crystalline)	9.0 "
Calcium Panthothenate	1.0 "
Niacin	5.0 "
Total	100.5 lbs.

chickens were given, at the age of five weeks, 100±10 embryonated ova.

Administration of the embryonated ova to the experimental chickens was done by a modification of the method described by Riedel (1947). A quantity of egg culture of known age was transferred to a vial containing a small amount of clean sand; the contents then were agitated gently by inverting the vial. Egg counts of one drop of the suspension, as delivered by a calibrated pipette, were determined by use of a microslide and a compound microscope. The egg concentration of the vial contents was adjusted by adding either water or egg culture, depending upon sample counts, until the desired count was obtained.

The experimental chickens were given the desired quantity of egg culture per os by means of the calibrated pipette. The vial contents were agitated between administrations to insure an even distribution of eggs throughout the suspension. In order to distribute error in egg concentration evenly between the two groups, experimental chickens from each group were exposed alternately. Following the administration of the egg culture, all groups of chickens were transferred to a growing battery.

Three weeks following administration of the A. galli ova, the chickens from the experimental groups were killed and the body cavity was opened. The region of the intestine extending from the duodenal loop to the yolk sac remnant was removed and placed in a pint jar along with the corresponding wing band.

After the intestine had been flushed by the hydraulic method of Ackert and Nolf (1929) it was opened and the mucosa scraped to remove any embedded worms. These scrapings were then added to the intestinal flushings. The sex of each chicken was recorded following a macroscopic examination of the gonads.

The intestinal contents were allowed to stand for a period of six to eight hours to permit relaxation of the worms. Formalin was added to the jars to give a concentration of approximately 10 per cent. Small quantities of the jar contents were poured into a flat bottomed glass bowl, eight inches in diameter, to facilitate location and recovery of the nematodes. All nematodes and wing band of the chicken from which they were collected were recovered by means of a teasing needle and stored in 12 ml specimen vials containing 10 per cent formalin.

The worms were measured by placing several between two glass plates to prevent coiling and to retain the entire lengths of the worms in one plane. The worm images were projected through a lens and bellows arrangement which produced a magnification of 6X on a ground glass plate. These magnified images were drawn on tracing paper. The sex of each worm was determined by use of a compound dissecting scope. Individual worm lengths were then determined by traversing the image tracing with a Dietzgen Map Measure and dividing the reading thus obtained by six.

In assembling the data in all tables, male and female worms were all whole specimens, whereas, the total number of worms includes a few severed specimens.

EXPERIMENTAL RESULTS

Experiment 1

The chickens used in this and the subsequent three experiments were White Plymouth Rocks and White Leghorn crosses in the approximate ratio of one to three. The chickens were weighed, banded and distributed into four groups (I, II, III and IV) of approximately equal weights. All four groups received the all vegetable protein basal ration containing the necessary vitamins and minerals, however, Groups III and IV were given the basal ration supplemented with 0.5 per cent concentration of Aurofac.

Experiment 1, which utilized 177 chickens, was begun February 20, 1951 and was terminated March 27, 1951. When two weeks of age, the chickens of Groups I and IV were given 100±10 embryonated A. galli ova from a 21-day old culture. Three weeks later, the chickens of Groups I and IV were killed and examined for worms.

The results of this experiment, recorded in Table 2, showed that Group I, consisting of 37 chickens, yielded 246 worms or an average of 6.65 worms per chicken. The 42 survivors of Group IV were found to harbor 107 worms, an average of 2.55 worms per chicken.

Of the worms collected and measured from Group I, 104 were males with an average length of 18.82 mm. The 135 female

Table 2. Results of Experiment 1 showing total number of worms recovered, maximum, minimum and average worm numbers per chicken, worm lengths, percentage mortality, percentage infection and average weights of the chickens at the termination of the experiments.

	Chickens						Worms										
Gp.	No. surviving	Av. weight (gm)	No. infected.	% infected.	No. begin.	% mortal	Total	Av. per chick.	Min. No.	Max. No.	Males No.	Males Length (mm) Av.	Min.	Max.	Females No.	Females Length (mm) Av.	Min.	Max.
I	37	246.00	24	64.86	45	17.78	246	6.65	1	48	104	18.82	3.33	40.00	135	21.36	2.50	41.66
II	41	254.63			43	4.64												
III	44	380.91			45	2.22												
IV	42	359.92	24	57.14	44	4.55	107	2.55	1	21	39	20.92	3.66	36.66	64	22.47	2.50	43.33

worms recovered from this group of chickens had an average length of 21.36 mm. The chickens of Group IV yielded 39 male worms which had an average length of 20.92 mm and 64 females which had an average length of 22.47 mm.

The infection rates of Groups I and IV were 64.86 and 57.14 per cent, respectively, or there were 7.22 per cent fewer infections in Group IV. The percentage mortality of the four groups was: Group I, 17.78; Group II, 4.65; Group III, 2.22 and Group IV, 4.55. Though the mortality of the parasitized chickens of Groups I and IV was higher than that of the chickens in their respective controls (Groups II and III), there was a difference in mortality of 2.33 per cent between the chickens of Groups III and IV.

The average weight of the chickens in Groups I and IV was 246.00 gm and 359.92 gm respectively, a difference of 113.92 gm. The chickens of Groups II and III weighed an average of 254.63 gm and 380.91 gm, respectively, a difference of 126.28 gm. A comparison of the average weights of parasitized versus non-parasitized chickens on the same diets showed that the chickens of Group I were 8.63 gm lighter than their control chickens (Group II). However, the chickens of Groups IV averaged 20.99 gm lighter than their control chickens (Group III).

The results of this experiment suggest that the resistance of young chickens to A. galli was influenced by the aureomycin and vitamin B_{12} dietary supplement. The increased resistance was reflected in the reduction of worm numbers per chicken as

well as by the decreased mortality and rate of infection in those chickens which received the supplement. It has also been shown that those chickens given the supplement made larger weight gains than did those chickens given the non-supplemented diet.

Experiment 2

This experiment which was conducted from April 4, 1951 to May 12, 1951 included 197 chickens of the same breeds as used in Experiment 1.

When the chickens were 14 days old, Groups I and IV were given 100±10 embryonated ova from 21-day old cultures. At the termination of the experiments, 21 days subsequent to feeding the embryonated ova, the chickens of Groups I and IV were killed and examined for worms.

The results of this experiment, compiled in Table 3, show fewer worms in Group IV than in Group I. The 48 chickens comprising Group I were found to harbor 792 worms or an average of 16.50 worms per chicken, whereas, the 49 chickens of Group IV harbored 590 worms or an average of 12.04 worms per chicken.

The average length of the 395 male worms recovered from Group I was 21.54 mm, and the average length of the 390 female worms was 26.45 mm. Group IV yielded 296 male worms which had an average length of 21.13 mm and 289 females with an average length of 25.01 mm.

Table 3. Results of Experiment 2 showing total number of worms recovered, maximum, minimum and average worm numbers per chicken, worm lengths, percentage mortality, percentage infection and average weights of the chickens at the termination of the experiments.

Gp.	No. surviving	Av. weight (gm)	No. infect.	% infect.	No. begin.	% mortal	Worms									
							Total	Av. per chick.	Min. No.	Max. No.	Males				Females			
											No.	Length (mm)			No.	Length (mm)		
												Av.	Min.	Max.		Av.	Min.	Max.
I	48	314.70	47	97.92	49	2.04	792	16.50	1	93	395	21.54	3.33	43.33	390	26.45	3.33	47.33
II	48	328.52			50	4.00												
III	49	379.85			49	0.00												
IV	49	364.59	43	87.76	49	0.00	590	12.04	1	47	296	21.13	2.00	38.33	289	25.01	2.00	48.33

The rates of infection of the chickens in Groups I and IV were 97.92 and 87.76 per cent, respectively. Percentage mortality of the chickens in Groups I and II was 2.04 and 4.00, respectively. There were no chickens lost in Groups III and IV.

The average weights of the chickens of the four groups at the termination of the experiment were: Group I, 314.70 gm; Group II, 328.52 gm; Group III, 379.85 gm and Group IV, 364.59 gm. The chickens of Groups I and IV (parasitized groups) were 13.82 gm and 15.26 gm lighter than the chickens of their respective controls (Groups II and III).

The comparative reduction in numbers of worms per chicken, infection rates, and mortality of young chickens given the dietary supplement in this experiment suggests further that the resistance of fowls to A. galli was increased. Again, as in Experiment 1, the chickens given the dietary supplement made greater weight gains than did those in the groups given the non-supplemented diet.

Experiment 3

This experiment which utilized the 85 control chickens (Groups II and III) of Experiment 2, was conducted to determine the resistance of chickens at a different age level to an ascarid infection as influenced by an aureomycin and vitamin B_{12} supplemented diet. These chickens were five weeks old when they were given 100±10 embryonated A. galli ova from a 45-day old

culture. The chickens were continued on their former diets. When eight weeks of age, 21 days after they were given the A. galli ova, the chickens were killed and examined for worms.

As shown in Table 4, the 37 surviving chickens of Group II harbored 299 worms or an average of 8.08 worms per chicken. Group III, consisting of 38 chickens, yielded 125 worms or an average of 3.28 worms per chicken.

The 107 male nematodes recovered from the chickens of Group II averaged 13.68 mm in length and the 190 females averaged 15.01 mm in length. The chickens of Group III harbored 48 male worms which averaged 10.34 mm in length and 73 females with an average length of 12.34 mm.

The infection rates of Groups II and III were 81.08 and 76.32 per cent, respectively. There was a mortality of 9.76 per cent among the chickens in Group II as compared with a mortality of 13.64 per cent among the chickens of Group III.

At the termination of the experiment, the average weights of the chickens in Groups II and III were 595.32 gm and 808.10 gm, respectively.

The results of this experiment showed a greater reduction in numbers of worms per chicken as well as a lower percentage infection in those chickens given the dietary supplement as compared with the chickens given the non-supplemented diet. Also there were considerable differences in the average weights of the chickens in the two groups. The mortality of the chickens given the non-supplemented diet was somewhat higher than that

Table 4. Results of Experiments 3 and 4 showing total number of worms recovered, maximum, minimum and average worm numbers per chicken, worm lengths, percentage mortality, percentage infection and average weights of the chickens at the termination of the experiments.

Gp.	Chickens: No. surviving	Av. weight (gm)	No. infected	% infected	No. begin.	% mortal	Worms: Total	Av. per chick.	Min. No.	Max. No.	Males: No.	Males: Length (mm) Av.	Min.	Max.	Females: No.	Females: Length (mm) Av.	Min.	Max.
Experiment 3																		
II	37	595.32	30	81.08	41	9.76	299	8.08	1	32	107	13.68	2.33	26.66	190	15.01	2.33	32.50
III	38	808.10	29	76.32	44	13.64	125	3.28	1	18	48	10.34	3.33	19.66	73	12.34	3.00	27.00
Experiment 4																		
II	45	703.13	32	71.11	48	6.25	118	2.62	1	15	48	17.56	2.50	43.00	70	19.70	3.00	41.33
III	48	791.6	29	60.42	49	2.04	119	2.48	1	30	51	18.02	3.33	30.86	65	22.39	2.5	36.50

of the chickens which received the non-supplemented diet. These data suggest that the resistance of chickens to A. galli is increased by the dietary supplement.

Experiment 4

The 97 control chickens (Groups II and III) of Experiment 2 were used in this experiment. At the termination of Experiment 2 these chickens were five weeks of age at which time they were given 100±10 embryonated A. galli ova from a 45-day old culture. The chickens were supplied with their former diets until they were eight weeks old. They were then killed and examined for worms.

The results of this experiment, recorded in Table 4, show that the 45 surviving chickens of Group II harbored 118 worms, or an average of 2.62 worms per chicken, while the 48 chickens of Group III yielded 119 worms, or an average of 2.48 worms per chicken.

Of the 118 worms recovered from Group II, 48 were males averaging 17.56 mm in length and 70 were females averaging 19.70 mm in length. The average length of the 51 male worms harbored by the chickens in Group III was 18.02 mm as compared with an average length of 22.39 mm for the 65 female worms recovered from Group III.

The percentage infection of the chickens in Group II was 71.11 as contrasted to the 60.42 per cent infection of those chickens in Group III. The mortality of the chickens in Groups

II and III was 6.25 and 2.04 per cent, respectively.

At the termination of the experiment, the average weight of the chickens in Group II was 703.13 gm and that of the chickens in Group III was 791.60 gm.

Though the results of this experiment did not indicate a marked reduction in the number of worms in those chickens receiving the dietary supplement as compared with the chickens receiving the non-supplemented diet, there was some indication that the dietary supplement increased the resistance of chickens to A. galli as reflected in the lowered rates of infection and percentage mortality among those chickens on the supplemented diet.

COMBINED RESULTS AND DISCUSSION

This study has demonstrated that the aureomycin-vitamin B_{12} dietary supplement augmented the resistance of chickens to an experimental A. galli infection. Evidence of this resistance is shown by (1) the numbers of worms harbored by the chickens, (2) the percentage of chickens becoming infected after being given 100±10 embryonated ova of A. galli and (3) the percentage mortality of chickens used in the experiments.

The combined results of Experiments 1 and 2 (Table 5) show that the chickens which received the non-supplemented diet (Group I) harbored the sum of 1,038 worms, or an average of 12.21 worms per chicken; whereas, those chickens which

Table 5. Combined results of Experiments 1 and 2 showing total number of worms recovered, maximum, minimum and average worm numbers per chicken, worm lengths, percentage mortality, percentage infection and average weight gains made by the chickens.

Gp.	Chickens						Worms									
	No. surviving	Av. weight (gm)	No. infected	% infect.	No. begin.	% mortal	Total	Av. per chick.	Min. No.	Max. No.	Males No.	Males Length (mm) Av.	Min.	Max.	Females No.	Females Length (mm) Av.	Min.	Max.
I	85	248.46	71	83.53	94	9.57	1038	12.21	1	93	499	20.98	3.33	43.33	525	25.14	2.50	47.33
II	89	257.93			93	4.30												
III	93	343.76			94	1.06												
IV	91	326.22	67	73.63	93	2.15	697	7.66	1	47	335	21.11	2.00	38.33	353	24.55	2.00	48.33

received the dietary supplement (Group IV) harbored a total of 697 worms, or an average of 7.66 worms per chicken. An analysis of variance of the numbers of worms recovered from the chickens in these groups yielded an F value of 4.42 which was significant at the one per cent level. This suggests that a greater degree of resistance to A. galli was developed by the chickens which received the supplemented ration.

The mortality and infection rates of the chickens which received the supplemented diet were considerably lower than those of the chickens given the basal ration. The infection rates of Groups I (non-supplemented diet) and IV (supplemented diet) were 83.53 and 73.63, respectively, or a difference of 9.9 per cent (Table 5). The mortality of the chickens in Groups I and IV was 9.57 and 2.15 per cent, respectively, a difference of 7.42 per cent. The mortality of the chickens in the control Groups II and III was lower than that of the chickens in either of the parasitized Groups I and IV, the extreme low being 1.06 per cent in Group III.

A comparison of the average lengths of the worms recovered from the chickens of Groups I and IV gives no indication of an increased resistance of Group IV to A. galli (Table 5). The average lengths of the male worms recovered from Groups I and IV were 20.98 mm and 21.11 mm, respectively, a difference of 0.13 mm. The average lengths of the female worms differed by 0.59 mm between the two groups. In both instances these differences fall within the range of experimental error.

The male to female worm ratios of the worms recovered from the chickens were 1:1.05 in each group.

The combined results of Experiments 3 and 4 supply further evidence of an increased resistance of chickens to A. galli induced by the Aurofac supplement. Table 6 shows the average number of worms recovered from the 82 survivors of the combined Group II to be 5.08. However, the average number of worms per chicken in the combined Group III was only 2.84. An analysis of variance of worm numbers showed that the difference was significant beyond the one per cent level (F value, 82.29).

The mortality of the chickens in Groups II and III was 7.87 and 7.53 per cent, respectively. However, the infection rates of the combined Groups II and III were 75.61 and 66.28 per cent, respectively, a difference of 8.33 per cent.

A comparison of the average lengths of the male worms from Groups II and III showed a difference of 0.60 mm; while the difference between the average lengths of the female worms in the two groups was 0.80 mm. In both instances these differences proved to be within the range of experimental error. The male to female worm ratios of the worms recovered from Groups II and III were 1:1.68 and 1:1.39, respectively.

It is of interest to compare the sex ratios of the worms recovered from the chickens used in Experiments 1 and 2 with those of the worms recovered in Experiments 3 and 4. Though the male to female ratios of the worms from the first two experiments were in both cases 1:1.05, these ratios of the worms recovered from the last two experiments were 1:1.68 for

Table 6. Combined results of Experiments 3 and 4 showing total number of worms recovered, maximum, minimum and average worm numbers per chicken, worm lengths, percentage mortality, percentage infection and average weight gains made by the chickens.

Gp.	Chickens						Worms									
	No. surviving	Av. weight (gm)	No. infect.	% infect.	No. begin.	% mortal	Total	Av. per chick.	Min. No.	Max. No.	Males No.	Males Length (mm) Av.	Min.	Max.	Females No.	Females Length (mm) Av.	Min.	Max.
II	82	612	62	75.61	89	7.87	417	5.08	1	32	155	14.89	2.33	43.00	260	16.27	2.33	41.33
III	86	763	58	66.28	93	7.53	244	2.84	1	30	99	14.29	2.50	30.86	138	17.07	2.50	36.50

Group II and 1:1.39 for Group III. In both instances there was a slight increase in numbers of female worms as compared with the numbers of female worms recovered from the first two experiments. Whether this difference can be attributed to the aging of the chickens is questionable. However, these sex ratios are somewhat similar to those reported by Todd (1948) who recovered 354 adult males and 488 adult female A. galli (sex ratio 1:1.38) from 1,014 chickens of varying ages and breeds.

The data of Tables 5 and 6 indicate that the chickens of Experiments 1 and 2 were considerably less resistant than those in Experiments 3 and 4. Since the ages of the chickens used in the two series of experiments differed by three weeks, the increased resistance of those chickens in Experiments 3 and 4 may possibly be attributed to an age factor. Ackert and Herrick (1928) found four-month old chickens to be considerably more resistant to A. galli than chickens one month of age. Other investigations by Herrick (1926) have shown this age resistance to increase up to 103 days. Ackert, Porter and Beach (1935) found the resistance of 45 day old chickens to increase significantly up to 93 days. In evaluation the data of Tables 5 and 6, it must be recalled that 45-day old A. galli ova cultures were given to the chickens of Experiments 3 and 4, whereas, the experimental chickens of Experiments 1 and 2 were given A. galli ova from a 21-day old culture. This factor of age difference between the ova cultures may have

been partially responsible for the decreased rate of infection as well as shorter worms in the chickens of Experiments 3 and 4. Ackert, Cooper and Dewhirst (1947) have shown A. galli ova from 120-day old cultures to be significantly less viable than ova from 36-day old cultures. Todd et al. (1950) reported that A. galli ova cultured 14-21 days had the greatest viability.

The results of the present study showed, in addition, that those chickens which were given the aureomycin-vitamin B_{12} dietary supplement made significantly greater weight gains than did those chickens given the non-supplemented diet (Fig. 1). It was also observed that the chickens given the dietary supplement not only feathered out earlier and matured earlier, as shown by their greater comb development, but they also consumed more feed and water, and voided larger amounts of droppings than did those chickens given only the basal ration. This empirical observation on feed and water consumption was confirmed in a pilot experiment in which data were collected by accurately measuring for five days the feed and water consumption, as well as fecal output, of 100 White Leghorn cockrels on the same diets as were the chickens used in Experiments 1 through 4. However, the results of this pilot experiment did not indicate an increased feed efficiency of the chickens.

The significant weight gains made by those chickens given the dietary supplement (Fig. 1) are comparable to the weight gains of chickens used in experimental studies made by Couch

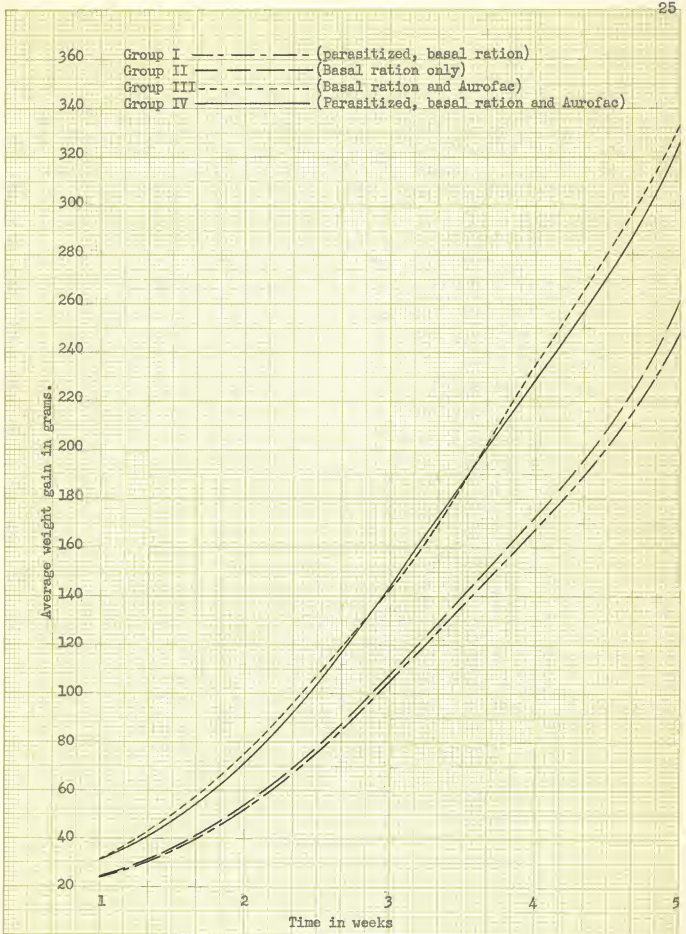

Fig. 1. Combined growth curves of the chickens used in Experiments 1 and 2.

(1950) and Stokstad and Jukes (1950). These workers found small concentrations (0.25 to 2.0 per cent) of the Aurofac supplement to be effective in producing significant weight gains in chickens. These and other studies point out the correlation of the significant weight gains of animals receiving the dietary supplement with the increased efficiency (feed consumed per pound of weight gained) of those animals.

A comparison of the combined average weight gains made by the parasitized (Group I) versus non-parasitized (Group II) chickens on the basal ration in Experiments 1 and 2 shows a difference of 9.47 gm at the termination of the experiments (Table 5). An analysis of variance of the individual weight gains of the chickens showed this difference was not significant. In contrast to the difference in weight gains made by these chickens, those given the dietary supplement in Group III (non-parasitized) and Group IV (parasitized) showed a difference of 17.54 gm between their weight gains. Statistical treatment of the individual weight gains of these chickens yielded an F value of 5.16 which was significant at the one per cent level. Furthermore, as previously shown, the chickens given the dietary supplement (Group IV) harbored significantly fewer worms than did those chickens receiving the non-supplemented diet (Table 5).

These facts suggest that the worms harbored by the chickens of Group IV (supplemented diet), though occurring in lesser numbers, were significantly detrimental to the chickens as shown by the comparative weight gains of the chickens in Groups

III and IV. However, the chickens in Group I were not seriously affected by the nematodes even though a greater number were present.

The foregoing observation may be explained by a comparison of the efficiency (feed consumed per pound of weight gained) of those chickens given the aureomycin-vitamin B_{12} supplemented diet to that of chickens given the basal ration only. Those chickens which received the dietary supplement were apparently functioning at such a high degree of efficiency that this efficiency was significantly offset by the effects of a few worms, whereas, the relatively low efficiency of chickens receiving only the basal ration was not significantly disturbed by a comparatively heavier ascarid infection.

That young chickens on an adequate diet can tolerate moderate infections of A. galli for one month was shown by Ackert and Wisseman (1946) who failed to find any constant differences between the control and parasitized groups of chickens in weight gains, blood sugar content or hemoglobin percentages.

The experimental results of this study and their statistical treatment indicate a new and important aspect concerning the economic importance of ascarid parasitism in young chickens. With the development of the aureomycin-vitamin B_{12} dietary supplement to produce rapid growth in chickens and consequent increased marketable weights at an earlier age, A. galli may assume a role of greater economic importance than was formerly accorded the parasite.

The effectiveness of the dietary supplement used in these experiments to increase the resistance of chickens to A. galli is reflected in the reduction of worm numbers as well as in the comparatively lower mortality and infection rates of chickens receiving the supplement. That this influence on the resistance to the nematode can be attributed to the antibiotic properties of aureomycin contained in the supplement was not demonstrable in this study.

SUMMARY

A series of four experiments, utilizing 374 chickens, was conducted to determine the effect of an aureomycin-vitamin B_{12} dietary supplement on the resistance of chickens to an experimental A. galli infection. Chickens used in Experiments 1 and 2 were five weeks of age at the termination of the experiments, whereas, the chickens used in Experiments 3 and 4 (controls of Experiments 1 and 2) were eight weeks of age when the experiments were terminated. All groups (I, II, III and IV) of chickens were given an all vegetable protein basal ration, complete with the necessary vitamins and minerals for normal growth of chickens; however, the chickens in Groups III and IV, in addition to the basal ration, received a supplement of 0.9 gm of aureomycin and 0.9 mg of vitamin B_{12} per 100 lbs of basal ration. Certain of the above mentioned groups of chickens, specifically designated in the following

summary, were fed 100±10 embryonated ova of A. galli. The results of this study were as follows:

1. The parasitized chickens of Groups I and IV harbored 1,038 and 697 worms, respectively, or an average of 12.21 worms per chicken in Group I and 7.66 worms per chicken in Group IV. Statistical treatment of these data yielded an F value of 4.42 which was significant at the one per cent level.

2. The infection rates of the chickens in Groups I and IV were 83.53 and 73.63 per cent, respectively. The percentage mortality of the four groups was: Group I, 9.57; Group II, 4.30; Group III, 1.06 and Group IV, 2.15.

3. The average length of the 499 male worms recovered from the chickens of Group I was 20.98 mm and that of the 525 female worms was 25.14 mm. Group IV harbored 335 male worms which had an average length of 24.55 mm. The ratio of male to female worms recovered from the chickens of the two groups was 1:1.05 in both groups.

4. The chickens of Groups II and III (controls of Experiments 1 and 2) were parasitized when five weeks of age. These groups were found to harbor averages of 5.08 and 2.84 worms per chicken, respectively. Statistical treatment of these data yielded an F value of 82.29 which was significant beyond the one per cent level.

5. The percentage mortality of Groups II and III was 7.87 and 7.53 respectively. The percentage infection of Group II was 75.61 and that of Group III was 66.28.

6. There was a difference of 0.60 mm between the average lengths of the male worms recovered from Groups II and III and there was a difference of 0.80 mm between the average lengths of the female worms in these two groups. In both cases, these differences were within the range of experimental error.

7. The older chickens used in Experiments 3 and 4 were apparently more resistant than the younger chickens used in the first two experiments. The increased resistance may have been due to an age factor, however, it is pointed out that a 45 day old A. galli ova culture was given to the older chickens and this may have been partially responsible for a decreased number of worms as well as shorter worms in the older chickens.

8. The chickens given the dietary supplement made greater weight gains than did those given the nonsupplemented diet.

9. Statistical treatment of average weight gains of parasitized versus non-parasitized chickens given only the basal ration showed that the difference of 9.47 gm was not significant, whereas, statistical treatment of the average weight gains of parasitized versus nonparasitized chickens (difference of 17.54 gm) given the dietary supplement yielded an F value of 5.16 which was significant at the one per cent level.

10. The chickens given the dietary supplement harbored significantly fewer worms than did those given the non-supplemented diet. Those chickens given the dietary supplement were apparently functioning at such a high degree of efficiency that

this was significantly offset by the affects of a few worms, whereas, the relatively low efficiency of chickens given only the basal ration was not significantly disturbed by a comparatively heavier ascarid infection.

11. This study has indicated that A. galli may assume a role of greater economic importance than formerly accorded the parasite.

12. This study did not demonstrate that influence on the resistance of chickens to A. galli could be attributed to the antibiotic properties of the aureomycin contained in the supplement.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. M. F. Hansen, major instructor, for suggesting the problem, for his counsel throughout the course of the study and for aid in statistical treatment of the data; to Dr. James E. Ackert for suggestions on the manuscript; and to Dr. Paul B. Sanford, Poultry Nutritionist, for his counsel.

LITERATURE CITED

Ackert, J. E.
Fowl resistance to parasitism affected by vitamins A and B. Archive Zoologico Italiano. 16:1369-1379. 1930.

Ackert, J. E.
Natural resistance to a helminthic infection. Jour. Parasitol. 28:1-24. 1942.

Ackert, J. E.
Soybean oil meal supplement effective in maintaining host resistance to Ascarids. Kuba (Havana, Cuba). 3:92-94. 1947.

Ackert, J. E., and T. D. Beach.
Resistance of chickens to the nematode Ascaridia lineata affected by dietary supplements. Amer. Micros. Soc. Trans. 52:51-58. 1933.

Ackert, J. E., R. M. Cooper and L. W. Dewhirst.
Viability of Ascaridia eggs under varying conditions of age and administration. Amer. Micro. Soc. Trans. 66:383-389. 1947.

Ackert, J. E., Marion L. Fisher and Naomi B. Zimmerman.
Resistance to parasitism affected by fat soluble vitamin A. Jour. Parasitol. 13:219-220. 1927.

Ackert, J. E., Marion Fisher McIlvaine and Naomi Zimmerman Crawford. Resistance of chickens to parasitism affected by vitamin A. Amer. Jour. Hyg. 13:320-336. 1931.

Ackert, J. E., and C. A. Herrick.
Effects of the nematode Ascaridia lineata (Schneider) on growing chickens. Jour. Parasitol. 15:1-13. 1928.

Ackert, J. E., D. A. Porter and T. D. Beach.
Age resistance of chickens to the nematode Ascaridia lineata (Schneider). Jour. Parasitol. 11:205-213. 1935.

Ackert, J. E., and L. O. Nolf.
New technique for collecting intestinal roundworms. Science. 70:310-311. 1929.

Ackert, J. E., and L. O. Nolf.
Resistance of chickens to parasitism affected by vitamin B. Amer. Jour. Hyg. 13:337-344. 1931.

Ackert, J. E., and L. A. Spindler.
Vitamin D and the resistance of chickens to parasitism. Amer. Jour. Hyg. 9:292-307. 1929.

Ackert, J. E., and Charles L. Wisseman, Jr.
Tolerance of fowls for moderate infections of intestinal helminths. Amer. Jour. Trop. Med. 26:721-729. 1946.

Beach, T. D., and J. E. Ackert.
Does yeast affect the growth and infectivity of the nematode, Ascaridia lineata, (Schneider), in chickens. Jour. Parasitol. 19:121-129. 1932.

Carpenter, L. A.
Effect of aureomycin on the growth of weaned pigs. Arch. Biochem. 27:469-471. 1950.

Couch, J. R.
From unpublished data presented in a paper given by E. L. R. Jukes at the Feed and Poultry Conference. Atlanta, Ga. 1950.

Cunha, T. J., H. H. Hopper, J. E. Burnside, A. M. Pearson, R. S. Glasscock and A. L. Shealy. Effects of vitamin B_{12} APF supplement on methionine needs of the pig. Arch Biochem. 23:510-512. 1949.

Herrick, C. A.
Studies on the resistance of the chicken to the nematode Ascaridia perspicillum (Rud.). Amer. Jour. Hyg. 6:153-172. 1926.

Riedel, Bernard B.
New technique on culturing and feeding Ascarid eggs. Amer. Micros. Soc. Trans. 66:396-397. 1947.

Riedel, Bernard B., and J. E. Ackert.
The resistance of chickens to ascarids as affected by protein supplements of soybean oil meal and skim milk. Poultry Sci. 29:611-612. 1950.

Sadun, E. H., C. K. Keith, M. J. Pankey and J. R. Totter.
The influence of dietary pteroyglutamic acid and A.P.A. liver extract on survival and growth of the nematode Ascaridia galli in chickens fed purified and natural diets. Jour. Hyg. 51:274-291. 1950.

Stokstad, E. L. R., and T. H. Jukes.
Growth promoting effect of aureomycin on turkey poults. Poultry Sci. 29:611-612. 1950.

Stokstad, E. L. R., and T. H. Jukes.
Effect of various levels of vitamin B_{12} upon growth responses produced by aureomycin in chickens. (18391). Proc. Soc. Exp. Biol. and Med. 76:73-76. 1951.

Stokstad, E. L. R., J. Pierce, A. C. Page, Jr., and A. L. Franklin. The multiple nature of the animal protein factor. Jour. Biol. Chem. 180:647. 1949.

Todd, A. C.
Worm parasites of Tennessee chickens. Tennessee Agr. Exp. Sta. Bul. 205. 1948.

Todd, A. C., M. F. Hansen, G. W. Kelley and Z. N. Wyant. Age differences in virulence of Ascaridia galli eggs. Amer. Micros. Soc. Trans. 69:394-397. 1950.

Zimmerman, Naomi B., Lola B. Vincent, and J. E. Ackert. Vitamin B, a factor in the resistance of chickens to Ascaridia perspicillum (Rud.). Jour. Parasitol. 12:164 (abs.). 1926.

THE RESISTANCE OF CHICKENS TO ASCARIDIA GALLI (SCHRANK) AS AFFECTED BY AN AUREOMYCIN-VITAMIN B_{12} DIETARY SUPPLEMENT

by

MARK GILBERT NORRIS JR.

B. S., New Mexico College of Agriculture
and Mechanical Arts, 1950

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1951

This research was conducted to determine the effects of an aureomycin and vitamin B_{12} dietary supplement on the resistance of two to eight week old chickens to Ascaridia galli. Criteria used for judging resistance were the numbers and lengths of worms recovered, weight gains made by the chickens, percentage mortality and percentage infection as determined by the number of chickens harboring the nematodes at the termination of the experiments.

A series of four experiments were conducted utilizing 374 chickens of mixed lots of White Plymouth Rock and White Leghorn crosses. The chickens for each experiment were received within two days of hatching and given an intranasal dose of Lederle Newcastle Vaccine Live Virus. Following vaccination, the chicks were banded, weighed and distributed into four groups (I, II, III and IV) of approximately equal weights. Weekly weights of the chickens were recorded throughout the experiments.

All four groups were given an all vegetable protein basal ration containing the necessary vitamins and minerals for normal growth of chickens, however, Groups III and IV were given the basal ration supplemented with 0.9 gm of aureomycin and 0.9 mg of vitamin B_{12} per 100 lb. of basal ration. The two supplements were added in the form of Aurofac, a fermentation product manufactured in conjunction with aureomycin production by the American Cyanimid Company, Lederle Laboratories Division.

The chickens in Groups I and IV of Experiments 1 and 2,

when two weeks of age, were given 100±10 embryonated A. galli ova from 21 day old water cultures. The chickens used in Experiments 3 and 4, control Groups II and III of Experiments 1 and 2, were five weeks of age when given 100±10 embryonated A. galli ova from 45 day old water cultures. All parasitized chickens were killed 21 days after the administration of the ova and the worms were recovered from the intestines. The worms were fixed in 10 per cent formalin, measured and sexed.

The results of each experiment showed that the resistance of two to eight week old chickens was increased by the Aureomycin-vitamin B_{12} dietary supplement. Evidence of an increased resistance was shown by (1) the numbers of worms harbored by the chickens, (2) the percentage of chcikens becoming infected after being given 100±10 embryonated ova and (3) the percentage mortality among the chickens.

The combined results of Experiments 1 and 2 showed that 85 chickens, comprising Group I, harbored 1,038 worms or an average of 12.21 worms per chicken. The 91 chickens in Group IV harbored 697 worms or an average of 7.66 worms per chicken. Statistical treatment of the worm numbers gave an F value of 4.42 which was significant at the one per cent level.

There were 83.53 per cent infections in the chickens of Group I, whereas, there were 73.63 per cent infections in Group IV. Percentage mortality of the four Groups was: Group I, 9.57; Group II, 4.30; Group III, 1.06; and Group IV, 2.15.

The average worm length of the 499 male worms recovered from the chickens of Group I was 20.98 mm, and the average lengths of 525 female worms was 25.14 mm. Group IV harbored 335 male worms with an average length of 21.11 mm and 353 female worms with an average length of 24.55 mm. The differences of 0.13 mm and 0.59 mm in average lengths of male and female worms, respectively, recovered from Groups I and IV were within the range of experimental error. The male to female worm ratios of the worms in Groups I and IV were 1:1.05 in each group.

Further evidence of an increased resistance of chickens to A. galli as induced by the dietary supplement was shown in the results of combined Experiments 3 and 4. Group II, consisting of 82 chickens, harbored 417 worms or an average of 5.04 worms per chicken, whereas, Group III, comprising 86 chickens, harbored 244 worms or an average of 2.84 worms per chicken. Statistical treatment of these data gave an F value of 82.29 which was significant beyond the one per cent level.

The mortality of the chickens in Groups II and III was 7.87 and 7.53 per cent, respectively. However, the infection rates of the Groups II and III were 76.6 and 66.28 per cent, respectively.

The average length of the 155 male worms recovered from the chickens of Group II was 14.89 mm, and the average length of the 260 female worms from this Group was 16.27 mm. Group III harbored 99 male worms with an average length of 14.29 mm and 138 female worms with an average length of 17.07 mm. The

differences of 0.60 mm and 0.80 mm between the average lengths of male and female worms, respectively, recovered from the two groups were not statistically significant. The male to female worm ratios of Groups II and III were 1:1.68 and 1:1.39 respectively.

The comparative results of the combined Experiments 1 and 2 and Experiments 3 and 4 suggested that the older chickens were more resistant to A. galli than the younger chickens used in Experiments 1 and 2. However, the fact that the chickens in Experiments 3 and 4 were given A. galli ova from a 45 day old ova culture may have accounted for decreased worm numbers, shorter worms, lower mortality and infection rates in the older chickens.

All chickens given the dietary supplement made greater weight gains than did those given only the basal ration. The average weight gains of the four groups used in Experiments 1 and 2 were: Group I (parasitized), 248.46 gm; Group II (controls), 257.92 gm; Group III (controls), 343.76 gm; Group IV (parasitized), 326.22 gm.

Statistical treatment of average weight gains of parasitized (Group I) versus non-parasitized (Group II) chickens given only the basal ration showed that the difference of average weight gains (9.47 gm) between the two groups was not significant. Statistical treatment of the difference in weight gains (17.54 gm) between the chickens in Groups III (non-parasitized) and IV (parasitized) given the dietary supplement yielded an F value of 5.16 which was significant at the one per cent level.

The parasitized chickens given the dietary supplement (Group IV) harbored significantly fewer worms than did the parasitized chickens given only the basal ration (Group I). These data suggest that the chickens given the dietary supplement were functioning at such a high degree of efficiency, as reflected in weight gains, that the effects of a few worms significantly offset this efficiency. However, the lower efficiency of parasitized chickens given the non-supplemented ration was not significantly disturbed by a comparatively heavier ascarid infection.

This study has indicated that, along with the development and use of the aureomycin-vitamin B_{12} dietary supplement to produce rapid growth in chickens and consequent increased marketable weights at an earlier age, A. galli may assume a role of greater economic importance than was formerly accorded the parasite.

The effectiveness of the dietary supplement used in these experiments in increasing the resistance of chickens to A. galli is reflected in the reduction of worm numbers as well as in the comparatively lower mortality and infection rates of parasitized chickens given the supplement. This study did not demonstrate that the influence on the resistance of chickens to A. galli could be attributed to the antibiotic properties of the aureomycin contained in the supplement.