PARASITE-HOST RELATIONSHIP INFLUENCED BY NUTRITION

PART I. DOES YEAST AFFECT THE INFECTIVITY AND GROWTH OF THE NEMATODE, ASCARIDIA LINEATA (SCHNEIDER), IN CHICKENS

PART II. PARASITISM AFFECTED BY DIFFERENT PROTEIN SUPPLEMENTS

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PART I. DOES YEAST AFFECT THE INFECTIVITY AND GROWTH OF THE NEMATODE, <u>ASCARIDIA</u> <u>LINEATA</u> (SCHNEIDER) IN CHICKENS

INTRODUCTION

Yeast is recognized as being one of the main sources of the vitamin B complex. That this food accessory is required for normal growth in chickens was shown by Eikjman in 1890 according to Sherman and Smith (1931). Eikjman experimentally produced symptoms in fowls similar to those of beri beri by feeding them polished rice. He was able to prevent these symptoms or cure the disease by feeding the fowls extract of the polishings. His first theory was that the disease was caused by toxic substances which were produced by excessive starch and which were not offset by some material in the polishings. However, in 1906, Eikjman stated his second hypothesis. This was to the effect that the disorder was caused by the deficiency of a particular substance present in the rice polishings and he gave the disease the name "nutritional polyneuritis" (Sherman and Smith).

That certain vitamins are essential for the maintenance of normal resistance of chickens to an intestinal parasite has been demonstrated by Ackert and his associates. In 1931, Ackert, McIlvaine and Crawford found more and larger Ascaridia lineata (nematoda) in chickens maintained on rations deficient in Vitamin A than in the controls similarly parasitized and supplied with this vitamin. Ackert and Spindler (1929) found that while the lack of vitamin D did not appear to lower the resistance of the chickens to the growth and development of A. lineata, the supply of this vitamin minimized the effects of the parasites on the chickens.

Ackert and Nolf (1931) in studying the effects of rations deficient of the vitamin B complex, found a larger number of A. lineata in the chickens whose diets lacked this vitamin. This suggested a lowered resistance of the hosts to the parasite. Longer worms, however, were found in the groups of fowls receiving the vitamin B in the form of yeast. This result which suggested the possibility of the growth factor in yeast having a stimulative effect upon the growth of the A. lineata led to the execution of experiments described in Part I of this paper.

ACKNOWLEDGMENTS

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their assistance during the course of the experiments and to Mr. George Montgomery for his advice concerning the statistical methods employed.

MATERIALS AND METHODS

The chickens used in these experiments were obtained as day old, purebred, white leghorn chicks from accredited hatcheries. They were raised in confinement under conditions which were adequate for normal growth (Herrick, Ackert and Danheim, 1923). At the beginning of each experiment the chicks were weighed, banded and separated into groups of equal size and weight. The exceptionally heavy and light birds were not included in any of the experimental groups. Weights were recorded each week for each chicken and the average gain calculated. The results obtained thereby were used in the construction of growth charts and in biometric treatment. From these data it was possible also to study the effects of parasitism.

The worm egg cultures used for parasitizing were made as follows: gravid female A. lineata from recently drawn chicken intestines were placed in a clean petri dish. The anterior ends were cut and the internal organs pressed out through the cut end. The uteri were separated and the proximal ends containing the highest percentage of fertilized eggs macerated in another clean petri dish. Distilled water was used to cover the eggs to which was added a few drops of 2 per cent formalin to prevent the growth of bacteria and mold. The cultures were then placed in an electric incubator kept at a temperature of 30° C. and were allowed to develop to the infective stage. To parasitize each chicken, 50 embryonated eggs (Ackert, Graham, Nolf and Porter, 1931) were counted out on a slide under the low power of the compound microscope. They were then washed off onto a filter paper with distilled water and a pinch of cornmeal added. In parasitizing, the filter paper containing the parasite eggs and the cornmeal was rolled into a pellet and forced down the oesophagus and into the crop of the chicken. In Experiment I the birds were parasitized at the age of six weeks and in Experiment II at the age of nine weeks.

At the end of three weeks of parasitism the chickens were killed and the small intestine removed. The intestine was broken into three or four approximately equal lengths, each length in turn being placed in an Erlenmeyer flask with one end over the nozzle of the flushing apparatus (Ackert and Nolf, 1929), and the contents flushed into the flask by means of warm water under pressure. This material consisting of fecal matter and nematodes (in most cases) was put into glass jars and preserved in formalin. The worms were removed from the fecal material and debris with the aid of a binocular microscope and forceps. They then were placed in vials containing four per cent formalin and the leg band of the chicken from which they were removed for the purpose of identification.

In measuring, the nematodes were placed a few at a time in a petri dish which rested on a glass plate. Under the plate a strong light was used to cast a shadow of the worms vertically through a lens which increased it six diameters on the ground glass of a photographic bellows. This enlarged shadow was traced on onion skin paper. The lengths were then taken by running a calibrated tracing wheel over the tracings which reduced their size six diameters. The use of this method of measuring insured accurate results as the tracing wheel could be made to follow any twist or turn that the worm might have assumed as a result of killing and preserving. Comparisons were made by using as criteria the number of worms per group which supplied the data for the degree of infestation and the length of worms per group which furnished the data for the rate of growth of the worms.

EXPERIMENTAL DATA

Experiment I. One hundred white leghorn chicks of the same hatch reared on an adequate diet for five weeks were weighed, banded and segregated into three groups of thirty each, the total weights of which were the same. The remaining ten were discarded due to their excessive or deficient weights. On the following day one group was put on a ration consisting of the regular diet containing an adequate amount of vitamin B complex plus 6 per cent by weight of Brewer's yeast (Brewer's group). Another group was put on a ration consisting of the regular diet containing an adequate amount of vitamin B complex plus 20 per cent by weight of Baker's yeast which due to the moisture content was equivalent to 6 per cent of dry yeast (Baker's group). The third group was kept on the regular diet containing an adequate amount of vitamin B complex as controls (Control group). One week after the groups were put on their respective diets each chicken was parasitized with 50 embryonated eggs of A. lineata. The chickens were six weeks of age at that time. Three weeks later they were killed and the worms removed. Not one chicken was lost during the experiment.

After being placed on the rations the birds grew rapidly until the second week of parasitism when all three groups not only failed to gain but lost weight (Fig. 1). These results are in accord with those of Ackert and Herrick (1928) who tested effects of these nematodes on chickens throughout various periods of parasitism and found the second week to be the most deleterious to the chicken. This is doubtless due to the young worms burrowing into the intestinal epithelium destroying Brunner's glands during this period (Ackert, 1923). During the third week of parasitism the worms withdraw into the lumen of the intestine and the greatest severity of the parasitism begins to pass. That this condition probably occurred in this experiment is indicated by Fig. 1 which shows that all three groups of chickens gained in weight during the third week. From a study of these gains it was found that the average gain of the Baker's group was 87.6 gm. (Table I) and of the Brewer's 64.6 gm., or a difference of 23 gm. which was significant (6.11 times its probable error). While the reasons for this difference have not been determined, it is thought that the somewhat sour taste of the Brewer's yeast may have resulted in a slightly smaller consumption of the ration by this group of chickens. No significant difference in gains occurred between either of these groups and the control group during this period.

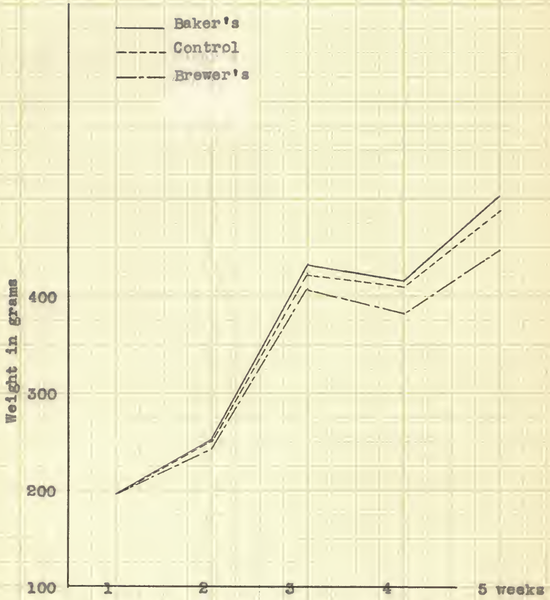

Fig. 1. Showing the growth curves of groups of chickens on Baker's yeast, Brewer's yeast and control diets.

Table 1. Showing comparative gains in weight made by the groups of chickens during the last week of Experiment 1.

Group	Number of Hosts	Means	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio $\frac{D}{E}$
Baker's	30	87.6	17.83	2.19			
					10.64	4.35	2.45
Controls	30	76.96	30.35	3.74			
					12.36	4.83	2.55
Brewer's	30	64.6	24.88	3.06			
					23	3.76	6.11
Baker's	30	87.6	17.83	2.19			

The biometrical treatment of the data on numbers of worms removed from each chicken showed that the Baker's group had an average of 4.43 worms per chicken as compared with an average of 6.9 worms from the controls, or 2.46 worms. As this difference is only 2.79 times its probable error, it is not significant (Table 2). The Brewer's group had an average of 9.06 number of worms per chicken as compared with the 6.9 from the controls, the difference being 2.16 worms which is also within the limits of experimental error. However, the difference between the average number from the Baker's group, 4.43 worms as compared with 9.06 worms from the Brewer's group is 4.63 worms. This is 4.6 times the probable error and may be considered significant.

The data obtained from measuring the worms showed that the average length of those from the Baker's group was 19.41 mm. as compared with 19.83 mm. from the controls. This difference of 0.41 mm. is not significant (Table 2). The worms from the Brewer's group had a mean length of 20.92 mm. as compared with 19.83 mm. for the controls. The difference of 1.09 mm. likewise is not significant. The average length of 19.41 mm. for the Baker's group as compared with 20.92 mm. for the Brewer's group showed a difference of 1.5 mm. which is 2.15 times its probable error and therefore not significant.

The results of this experiment seemed to indicate that yeast fed in the presence of an adequate diet does not alter the resistance of chickens to the nematode Ascaridia lineata nor does it, under the same conditions, affect the growth of this nematode. Although there were significantly more A. lineata found in the chickens of the Brewer's group than in those from the Baker's group it was not considered an index of infectivity because the difference existed between two experimental groups receiving different types of yeast. It appeared also that the presence of Baker's yeast in the ration increased the rate of growth of the chickens whereas the presence of the Brewer's yeast somewhat retarded their growth. It is possible that this difference might be due to the dissimilarity of the palatability of the rations.

Table 2. Showing numbers and lengths of *Ascaridia lineata* from chickens in Experiment I.

Group	Number of Hosts	Means	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio D/E
			Numbers of Worms				
Baker's	30	4.43	3.16	0.39	2.46	0.88	2.79
Control	30	6.90	6.41	0.79	2.16	1.21	1.77
Brewer's	30	9.06	7.54	0.92	4.63	1.0	4.63
Baker's	30	4.43	3.16	0.39			
			Average lengths of worms (mm.)				
Baker's	30	19.41	10.89	0.63	0.41	0.71	0.58
Control	30	19.83	6.86	0.32			
Brewer's	30	20.92	7.48	0.3	1.09	0.44	2.46
Baker's	30	19.41	10.89	0.63	1.5	0.7	2.15

Experiment 2. The second experiment followed closely the procedure of Experiment 1 except that the parasitizing was done four weeks after the chickens had been put on their respective diets. One hundred fifty purebred, white leghorn day old chicks of the same hatch were raised in confinement on the same adequate diet as those in Experiment 1 for five weeks. At the end of that time they were banded, weighed and segregated into four groups of 43 each, the remainder being discarded due to their excessive or deficient weights. The groups of chickens were put on their respective diets, viz., a Baker's group receiving the regular diet containing an adequate amoung of vitamin B in addition to 20 per cent by weight of Baker's yeast, a Brewer's group receiving the regular diet containing an adequate amount of vitamin B plus 6 per cent of Brewer's yeast and an additional group receiving the regular diet containing an adequate amount of vitamin B complex plus 6 per cent by weight of dry Baker's yeast (dry Baker's group). Besides the aforementioned groups, there was a control group receiving the regular ration containing an adequate amount of vitamin B complex.

After the chickens had been on their respective diets four weeks they were parasitized, each with 50 embryonated eggs of A. lineata from a culture which had been incubated three weeks. After three weeks of parasitism the chickens were killed, the intestines removed and the parasites isolated. During the experiment several of the birds died, eight of them were from the control group, two from the moist Baker's group, two from the Brewer's group, and none from the dry Baker's group. The fact that such a large percentage of them were from the control group would seem to indicate that the yeast helped to keep the birds in a more healthful condition.

Upon examination of the growth curves (Fig. 2) it will be seen that those in the (dry) Baker's and the moist Baker's groups made better gains than those of the control group and the Brewer's groups, while the Brewer's group made less gain than the controls. However, there were no significant gains made by any one group over another.

Comparing the numbers of worms it was found that the Baker's group had an average of 3.24 worms per bird and the controls, 2.8 worms. The difference was 0.44 worms which is not significant (Table 3). The Brewer's group had an average of 3.41 worms and the controls 2.8 worms or a difference of 0.61 worms which likewise was not significant. The average of 2.28 worms of the dry Baker's group as compared with the 2.8 worms of the controls made a difference of 0.5 worm which is within the range of experimental error. The consideration of the differences between the groups in

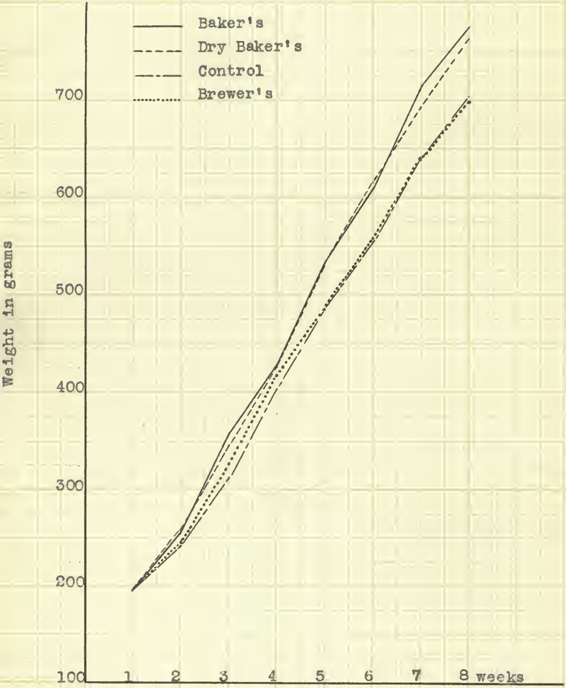

Fig. 2. Showing the growth curves of groups of chickens on (moist) Baker's dry Baker's yeast, Brewer's yeast and control diets.

Table 3. Showing numbers and lengths of <u>Ascaridia lineata</u> from chickens on different yeast rations in Experiment II

Group	Number of Hosts	Means	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio $\frac{D}{E}$
Numbers of Worms							
Baker's	41	3.24	4.5	0.47			
					0.44	0.59	0.74
Control	35	2.8	3.13	0.35			
					0.61	0.63	0.96
Brewer's	41	3.41	5.0	0.52			
					1.12	0.61	1.82
Dry Baker's	42	2.28	3.13	0.32			
					0.51	0.48	1.06
Control	35	2.8	3.13	0.35			
Brewer's	41	3.41	5.0	0.52			
					0.17	0.7	0.24
Baker's	41	3.24	4.5	0.47			
					0.95	0.57	1.66
Dry Baker's	42	2.28	3.13	0.32			
Average lengths of worms (mm.)							
Baker's	41	12.56	5.68	0.33			
					1.12	0.47	2.39
Control	35	11.43	5.12	0.35			
					2.8	1.0	2.8
Brewer's	41	14.24	16.16	0.93			
					2.0	1.45	1.37
Dry Baker's	42	12.23	15.89	1.11			
					0.88	1.16	0.76
Control	35	11.43	5.12	0.35			
Brewer's	41	14.24	16.16	0.93			
					1.68	0.99	1.69
Baker's	41	12.56	5.68	0.33			
					0.32	1.54	0.21
Dry Baker's	42	12.23	15.89	1.11			

which yeast was used showed that the difference in the averages of the Baker's group and the Brewer's group was 0.17 worm which was not significant. Between the Baker's and Dry Baker's groups the difference was only 0.95 worm and likewise not significant. The Dry Baker's and the Brewer's difference amounted to 1.12 worms which was 1.82 times its probable error and therefore within the range of experimental error.

The average length of worms found in the Baker's group was 12.56 mm. as compared with 11.43 mm. in the controls. The difference was 1.12 mm. which is 2.39 times its probable error and not significant. The worms in the Brewer's group had an average length of 14.24 mm. and the controls 11.43 which made a difference of 2.8 mm. which was not significant. The 12.23 mm. average from the Dry Baker's group as compared with 11.43 for the controls made a difference of 0.88 mm. which was within the range of experimental error. The consideration of the groups in which yeast was used showed that the difference in the averages between the Baker's and Brewer's was 1.68 mm. which was not significant. Between the Baker's and the Dry Baker's groups the difference was 0.32 mm. which was not significant. The Dry Baker's and the Brewer's difference amounted to 2.0 mm. which likewise was not significant.

It was suggested from the results of this experiment that yeast, when fed in the presence of an adequate diet, does not alter the infectivity or affect the growth of *A. lineata* in chickens. However, the presence of Baker's yeast in the ration, whether dry or moist, seemed to increase the rate of growth of the chickens.

SUMMARY (Part I)

1. Two controlled experiments involving 262 chickens were conducted to ascertain if yeast is a factor in the infectivity and growth of *Ascaridia lineata* when the host chickens were fed different forms of yeast in addition to an adequate diet.

2. After being on the regular ration for five weeks, the chickens were banded, weighed and segregated into groups of equal size and weight. The first group received, in addition, 20 per cent (by weight) of Baker's yeast; and the second group 6 per cent of Brewer's yeast. In Experiment 2, an additional group received 6 per cent (by weight) of dry Baker's yeast. A control group on the regular ration was maintained in each experiment.

3. Each chicken was parasitized with 50 embryonated eggs of *A. lineata* after being on the special rations (one week Experiment 1; four weeks, Experiment 2). At the end of three weeks the experiments were terminated and the

worms isolated.

4. Throughout Experiment 1 all groups gained in weight except during the second week of parasitism when they lost weight. In the final week of the experiment, the Baker's group made a marked (significant) gain over that of the Brewer's group, but not over the gain of the control group. It is thought that the difference between the two yeast groups may have been due to decreased palatability of the Brewer's ration. Likewise in Experiment 2 the chickens receiving the Brewer's yeast made the poorest gains.

5. The criterion used for ascertaining the degree of infectivity was the number of worms removed at autopsy; that for judging the growth of worms was their length at autopsy.

6. In Experiment 1 there were no significant differences between the control group and the experimental groups for numbers of worms. There was a significant difference in numbers of worms between the Baker's group and the Brewer's group. Since this was a difference between two forms of yeast and not between the control and experimental groups, it was not considered as an index of infectivity.

7. The results of Experiment 2 showed no significant differences between any of the groups on either number of worms or on lengths of worms.

8. It has been concluded from these experiments that yeast does not contain a special growth factor for Ascaridia lineata when used in the presence of an adequate diet; nor does it, under the same conditions, constantly alter the infectivity (degree of infestation) of this nematode.

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*Ascaridia perspicillum refers to A. lineata (Schneider).

PART II. PARASITISM AFFECTED BY DIFFERENT PROTEIN SUPPLEMENTS

INTRODUCTION

While the studies on vitamin A (Ackert, Fisher and Crawford, 1931) and vitamin B complex (Ackert and Nolf, 1931) as factors in the resistance of chickens to parasitism were in progress, two experiments were carried out in 1927 to ascertain if the resistance of chickens to the nematode, <u>Ascaridia lineata</u> (Schneider), might be influenced by different protein supplements in diets. The data from these experiments which were turned over to the writer have been examined, the preserved <u>A. lineata</u> from the groups of chickens under comparison have been counted and measured, and the descriptions of the experiments prepared as a portion of Part II of this paper. The general method of procedure was similar to that described for the problem in Part I.

ACKNOWLEDGMENTS

The writer wishes to acknowledge his indebtedness to Dr. James E. Ackert for his planning and supervising of the work set forth in this paper and to Dr. J. S. Hughes for his help and suggestions in the planning and mixing of the

rations used. Thanks are also due Mr. Dale A. Porter and Mrs. W. W. Crawford for their assistance during the course of the experiments and Dr. W. H. Andrews for his advice concerning the statistical methods employed.

EXPERIMENTAL DATA

Experiment 1. The materials used and the methods employed were the same as those in Part I of this paper. Seventy-five pure bred white leghorn day old chicks were received from the Smith Hatcheries of Boonville, Missouri. They were separated into three groups of 25 each at four days of age and put on their respective diets. Except for the first five and one-half weeks the basal rations consisted of the following:

	gm.	protein gm.	fat gm.	carbohydrates gm.
Cornmeal	60	5.52	1.14	45.24
Wheat	20	2.22	0.34	15.10
Oats	10	1.6	0.77	6.7
Salt Mixture	5			
Cod liver oil	2			
	2			
	99	9.34	2.25	67.04

In addition to this basal ration, Group 1 (animal protein plus milk) received 54.3 per cent of its protein in the form of meat meal which amounted to 11.22 per cent by weight of the ration, 0.7 gm. of crisco and 3.2 gm. of corn starch. The crisco and corn starch were added to equalize the fat and carbohydrate content of the other groups. Twice daily green alfalfa was fed and sweet skim milk was given _ad libitum._ Group 2 (animal protein) was given a diet identical with that of Group 1 except the skim milk, which was omitted. Group 3 (plant protein) was reared on a diet entirely lacking in animal protein. In the place of the protein furnished by the meat meal in Groups 1 and 2, peanut meal was used as a supplement. This furnished 54.3 per cent of the protein in the ration, which constituted 11.1 per cent by weight of the ration. The balance of the protein was supplied by the wheat, oats and corn in the basal ration. During the first part of the experiment smaller amounts of protein supplement were used in the rations. Groups 1 and 2 received 27.8 per cent of protein supplement and Group 3, 28.3 per cent of protein supplement. These amounts proved to be insufficient and were, therefore, increased to the aforementioned amounts.

Each chick was parasitized at the age of seven weeks by being fed approximately 500 embryonated eggs of _A. lineata._ Upon examination of Fig. 1 it is seen that the group receiving the animal protein plus milk supplement made noticeably greater gains in weight than did the other two groups. The animal protein group made better gains than the plant protein group but markedly less than the animal protein plus milk groups. The chicks remained on

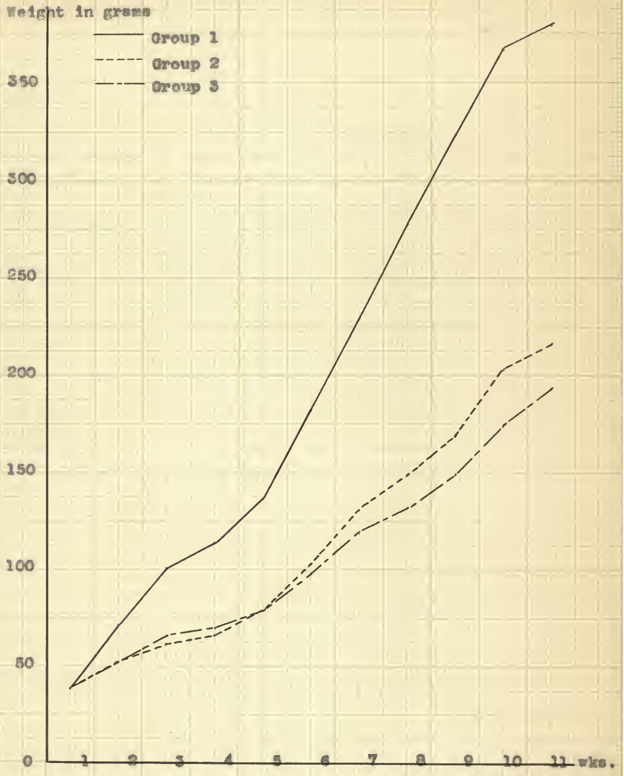

Fig. 1. Showing the growth curves of groups of chickens on animal protein plus milk (Group 1), animal protein (Group 2) and plant protein (Group 3) supplements.

their rations for three weeks after parasitizing at which time they were killed, the nematodes isolated from their intestines and later counted and measured.

The biometrical treatment of the data on numbers of worms removed from each chicken showed that the chickens in Group 1 (animal protein plus milk) had an average of 8.54 worms per chicken as compared with 49.6 worms in Group 2 (animal protein), the difference amounting to 41.06 worms. This is 4.29 times its probable error, and is considered to be significant. Group 3 (plant protein group) had an average of 38.7 worms per chicken; comparing this with the 8.54 worms from Group 1, gave a difference of 30.1 worms. This difference is 4.14 times its probable error and is likewise significant. The difference between the average numbers of worms from Groups 2 and 3 was 10.9 worms, but due to the large probable error this difference was within the range of experimental error (Table 1).

The analysis of the data obtained from measuring the worms showed that Group 1 had an average worm length of 13.02 mm. and Group 2, 22.6 mm. This difference was 9.58 mm. which is 17.1 times its probable error and obviously significant. The average of the length of worms of Group 3 was 23.87 mm. This average length compared with 13.02 mm. of Group 1 gave a difference of 10.85 mm. which was significant (22.6 times its probable error). Comparison of the average length of Group 2 and that of Group 3 gave a difference of only 1.27 mm. which fell within the range of experimental error.

The results of this experiment indicate that the presence of milk in the diet significantly increased the resistance of the chickens to A. lineata. Not only did the milk appear to decrease the size of infestations found at autopsy, but judging from their length it seemed to have an inhibitory effect upon the growth of the worms present. As to the effects of the animal and plant protein supplements on resistance, there apparently was no difference. Although there were many more worms found in the plant protein group, the high probable error precluded the possibility of a significant difference. The presence of milk in the diet seemed to induce rapid growth of the chickens. The presence of animal protein without milk appeared to lack something essential for the normal growth of the chickens as also seemed to be the case to a greater degree of those receiving plant protein. There seemed to be a retarding of the growth of the worms in the animal protein group when compared to the lengths of those from the plant protein group. The plant protein supplement appeared not to have everything required for normal growth, as was likewise the case of the chickens receiving animal protein alone, but to a less noticeable extent than the plant protein group.

Table 1. Showing numbers and lengths of <u>Ascaridia lineata</u> from chickens on diets including the different protein supplements in Experiment 1

Group	Number of Hosts	Means	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio D/E
		Average numbers of worms					
(1) Animal Protein plus skim milk	24	8.54	8.43	1.16	41.06	9.56	4.29
(2) Animal Protein	23	49.6	67.42	9.49	10.9	11.9	0.91
(3) Plant Protein	24	38.7	52.07	7.18	30.1	7.27	4.14
(1) Animal Protein plus skim milk	24	8.54	8.43	1.16			
		Average lengths of worms (mm.)					
(1) Animal Protein plus skim milk	24	13.02	8.04	0.42	9.58	0.56	17.1
(2) Animal Protein	23	22.6	9.08	0.39	1.27	0.46	2.76
(3) Plant Protein	24	23.87	6.63	0.26	10.85	0.48	22.6
(1) Animal Protein plus skim milk	24	13.02	8.04	0.42			

Experiment 2. The procedure in this experiment closely followed that of the previous one. Seventy-five pure bred, day old, white leghorn chickens were segregated into three groups of 25 each. At four days of age they were put on their respective diets as follows: Same basal ration as in Experiment 1. Group 1, in addition to the basal ration, received 51 per cent of its protein in the form of meat meal which constituted 17.6 per cent by weight of the ration; also 0.7 gm. of crisco and 3.2 gm. of starch to equalize the fat and carbohydrate content of the other groups. Green alfalfa was fed twice a day and fresh skim milk was given ad libitum. The ration for Group 2 (animal protein) was identical with that of Group 1 with the exception of the absence of the skim milk. Group 3 (plant protein) received no animal protein in any form. Instead, peanut meal was fed as a supplement, which amounted to 51.1 per cent of the protein, the balance being present in the cornmeal, oats and wheat. The peanut meal constituted 20.1 per cent of the ration.

At the age of seven weeks, each chicken was parasitized with approximately 500 embryonated eggs of A. lineata. The effects of the rations on the growths of the chicken is illustrated in Figure 2. The animal protein and milk group, as before, made decidedly better gains than the animal protein group and the plant protein groups. Also, as before

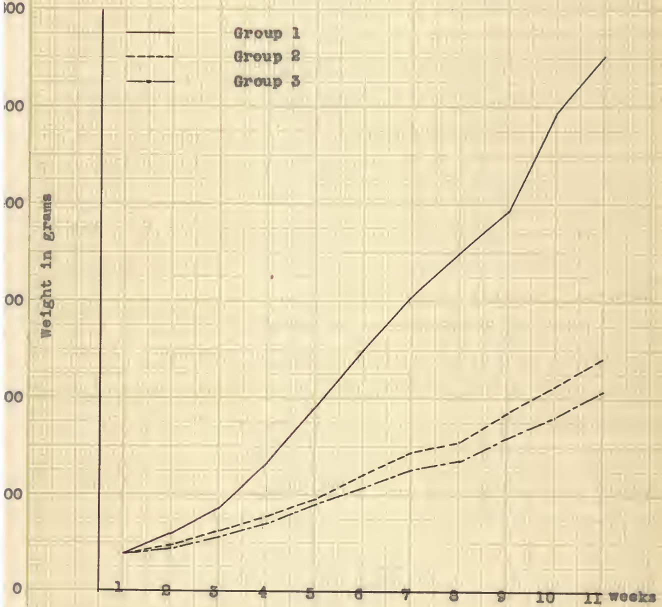

Fig. 2. Showing the growth curves of groups of chickens on animal protein plus milk (Group 1), animal protein (Group 2) and plant protein (Group 3) supplements.

the animal protein group made faster gains than the group on plant protein. The chickens were kept on their respective diets for a period of three weeks of parasitism at the end of which time they were killed, the intestines removed, and the worms isolated, counted and measured.

The analysis of these results showed that the number of worms from the chickens in Group 1 (animal protein plus milk) averaged 3.15 as compared with 23.1 worms in Group 2 (animal protein). This difference was 19.95 worms which was 6.39 times its probable error. Group 3 (plant protein) had an average of 32.52 worms which when compared with the 3.15 worms from Group 1, gave a difference of 29.37 worms. This difference was significant (9.30 times its probable error). The difference between the average number from Group 2, 23.1 worms, as compared with 32.52 worms from Group 3 was 9.42 worms, but due to the high probable error was not significant (Table II). The results from measuring showed that the average length of the worms of Group 1 was 9.48 mm. as compared with 13.35 mm. of Group 2. This difference of 3.87 mm. was 7.58 times its probable error. The average length of the worms of Group 3 was 15.52 mm. This average length compared with 9.48 mm. of those from Group 1 gave a difference of 6.04 mm. This was 11.61 times its probable error and likewise considered significant. The difference between the average of Group 2, 13.35 mm., and the average of group 3, 15.52 mm., amounted to 2.17 mm. which due to the very small probable error was 7.23 times its error (Table 2).

These results suggest that the addition of milk to the ration increases the resistance of the chickens to A. lineata. Not only were there fewer worms present in the groups receiving the milk, but those found were shorter, indicating the possibility of an inhibitory effect against the growth of the worms. Although there existed a considerable difference in the numbers of worms found in the animal and plant protein groups, the plant protein group having the greater number, the high probable error prevented a significant difference.

Experiment 3. One hundred white leghorn chickens used in this experiment were raised for the first month on a diet adequate for chickens reared in confinement. At the end of that time they were separated into four groups of equal size and weight and put on their respective diets as follows:

Table 2. Showing numbers and lengths of *Ascaridia lineata* from chickens on different protein rations

Group	Number of Hosts	Means	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio $\frac{D}{E}$
Average numbers of worms							
(1) Animal Protein plus skim milk	20	3.15	3.73	0.56			
					19.95	3.7	5.39
(2) Animal Protein	19	23.1	23.9	3.7			
					9.42	4.81	1.95
(3) Plant Protein	19	32.52	19.99	3.09			
					29.37	3.13	9.38
(1) Animal Protein plus skim milk	20	3.15	3.73	0.56			
Average lengths of worms (mm.)							
(1) Animal Protein plus skim milk	20	9.48	5.11	0.47			
					3.87	0.51	7.58
(2) Animal Protein	19	13.35	4.53	0.2			
					2.17	0.3	7.23
(3) Plant Protein	19	15.52	7.3	0.24			
					6.04	0.52	11.61
(1) Animal Protein plus skim milk	20	9.48	5.11	0.47			

Control Group (1)

	gm.	protein gm.	ash gm.
Yellow cornmeal	40	4.03	.6
Cracked wheat	15	1.36	.28
Oatmeal	12	1.92	.24
Meat meal	10.4	6.28	1.58
Milk powder	6.4	2.44	.51
Alfalfa meal	4	.59	.34
Cod liver oil	1.6		
	89.4	17.12	3.55

Ani-al Protein Group (Group 2)

	gm.	protein gm.	ash gm.
Yellow cornmeal	48	4.84	.72
Cracked wheat	20	2.48	.38
Oatmeal	15	2.4	.30
Meat meal	14	8.46	2.14
Alfalfa meal	7.5	1.11	.65
Cod liver oil	2		
	106.5	19.29	4.19

Plant Protein Group (Group 3)

	gm.	protein gm.	ash gm.
Yellow cornmeal	48	4.84	.72
Cracked wheat	20	2.48	.38
Oatmeal	15	2.4	.30
Peanut meal	22	6.71	.51
Alfalfa meal	7.5	1.11	.65
Salt (NaCl)			
Cod liver oil	2.0		
	114.5	17.54	2.56

Protein Deficient Group (Group 4)

	gm.	protein gm.	ash gm.
Yellow cornmeal	48	4.84	.72
Cracked wheat	20	2.48	.38
Oatmeal	15	2.4	.30
Meat meal	4	2.42	.61
Corn starch	10	1.11	.65
Alfalfa meal	7.5		
Salt (NaCl)	0.3		
Crisco	1.7		
Cod liver oil	2.0		
	108.5	13.25	2.66

The chickens were kept on their respective diets for a period of six weeks, at the end of which time the experiment was terminated. As will be seen by the accompanying chart the growth rate of Group 1 (animal protein) was considerably less than that of the control group. A great deal of this was no doubt due to the presence of a large number of males in the control group. However, a part of this weight increase of the control group was undoubtedly brought about by the presence of the powdered milk in the diet as would be expected from the results of Experiments 1 and 2. Group 2, on a plant protein diet, as would also be expected from the previous results, made considerably less gains than the control and animal protein groups. Group 3 (protein deficient group) made extremely poor gains due to the lack of sufficient protein in the ration (Figure 3).

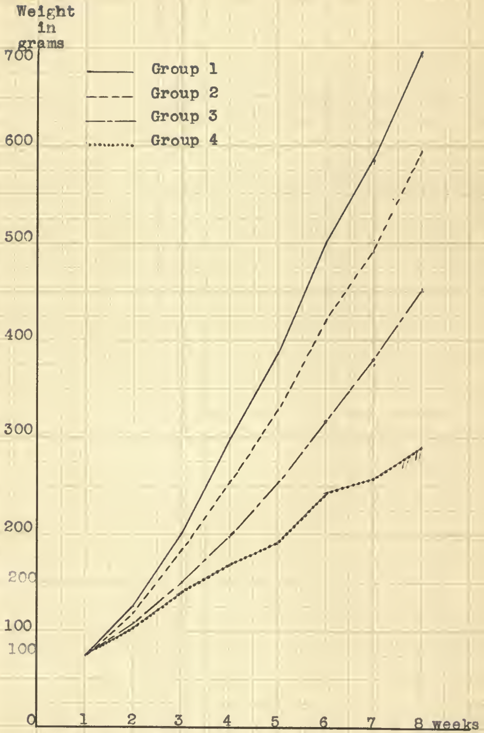

Fig. 3. Showing the growth curves of chickens on control (Group 1), animal protein (Group 2), plant protein (Group 3), and protein deficient (Group 4) supplements.

Table 3 shows the results of the biometric treatment of gains of all experimental groups for both the first and last weeks during which the groups were on their respective diets.

From a study of the results of the experiment it appeared that the powdered milk in addition to the animal protein present in the ration of the control group, produced more rapid gains in the chickens. That something was lacking in the plant protein ration, was evidenced by the fact that the chickens receiving this ration were unable to maintain a weight increase, equivalent to the animal protein group and considerably less than the animal protein plus milk group. The lack of sufficient protein in the diet apparently inhibited the growth of the chickens, thus making a small, irregular gain. Likewise, the chickens remained in a poor state of health as was evidenced by their ruffled feathers and droopy wings.

<u>Experiment IV.</u> In this experiment, slight variations were made in the amounts of protein supplement and the chickens used were much younger when started on their respective rations. Otherwise, the procedure closely followed the previous experiment. One hundred chickens were raised for the first ten days on an adequate diet. At the end of that time they were weighed, banded, and separated

Table 3. Showing gains made by groups of chickens on animal protein, plant protein, protein deficient and control supplements for the first and last weeks on rations

Group	Number of Indiv.	Ave. Gain	Stand. Dev.	P. E. of Mean	Act. Diff. of Means	P. E. of Diff.	Ratio D/E
		First Week Gains (gm.)					
Controls	22	52.04	8.85	1.27	7.04	2.3	3.06
Animal Protein	25	45.0	14.33	1.93	12.09	2.83	4.27
Plant Protein	24	32.91	8.91	2.08	2.17	2.31	0.93
Protein Deficient	25	30.16	8.92	1.02	14.84	2.18	6.8
Animal Protein	25	45.0	14.33	1.93			
Plant Protein	24	32.91	8.91	2.08	19.13	2.43	7.87
Controls	22	52.04	8.85	1.27			
		Last Week Gains (gm.)					
Controls	22	106.5	19.48	2.8	5.02	3.08	1.62
Animal Protein	25	101.48	9.71	1.3	31.32	3.34	9.37
Plant Protein	24	70.16	21.48	3.08	36.12	3.86	9.35
Protein Deficient	24	34.04	16.96	2.33	67.44	3.08	21.18
Animal Protein	25	101.48	9.71	1.3			
Plant Protein	24	70.16	21.48	3.08	36.34	4.16	8.73
Controls	22	106.5	19.48	2.8	72.46	3.64	19.9
Protein Deficient	24	34.04	19.96	2.33			

into three groups of equal weight and size, each group containing 30 chickens. On the same day they were put on their respective diets, consisting of three distinct protein supplements as follows:

Basal Ration:

	gm.	Protein gm.	Fat gm.	Fiber gm.	Ash gm.
Yellow cornmeal	65	5.64	2.8	1.12	.84
Cracked wheat	20	2.43	.42	.44	.[illegible]
Otona	15	2.4	.98	.22	.30
Alfalfa leaf meal	5	.74	1.16	1.41	.43
Salt (NaCl)	1				
Cod liver oil	1				
Steamed bone meal	2				1.8
	100	11.26	5.36	3.19	3.75

Group A, in addition to the basal ration, contained 17 per cent (by weight) of protein supplement consisting of:

	gm.	Protein gm.	Ash gm.
Meat meal	9.15	6.4	.76
Milk	4.57	1.6	.36
Starch (filler)	7.03		
	20.75	8.0	1.12

Group B, in addition to the basal ration, contained 17 per cent (by weight) of protein supplement consisting of:

	gm.	Protein gm.	Ash gm.
Meat meal	11.43	8.0	.95
Bone meal	.31		.27
Starch (filler)	9.00		
	20.74	8.0	1.22

Group C, in addition to the basal ration, contained 17 per cent. (by weight) of protein supplement consisting of:

	gm.	Protein gm.	Ash gm.
Peanut meal	20.46	8.0	.87
Bone meal	.30		.27
	20.76	8.0	1.14

The chickens remained on these rations for a period of six weeks, at which time the experiment was terminated. Figure 4 shows that the results of this experiment very closely follows the results obtained in the previous experiments. The group receiving the milk in addition to animal protein in the supplement made better gains throughout than both the other groups, and the group on animal protein supplement made better gains than the group receiving the plant protein supplement. It might be mentioned at this time that very little difference could be detected in the amounts of feed consumed by the animal protein plus milk group and the plant protein group thus precluding the possibility of any great increase in gains of the one group over the others due to the differences in palatability.

Even though the percentage of milk was quite low in this experiment, there was an indication that it produced more rapid gains. There seemed to be a growth requirement not present in the plant protein as the chickens were unable to maintain a normal growth throughout the experiment.

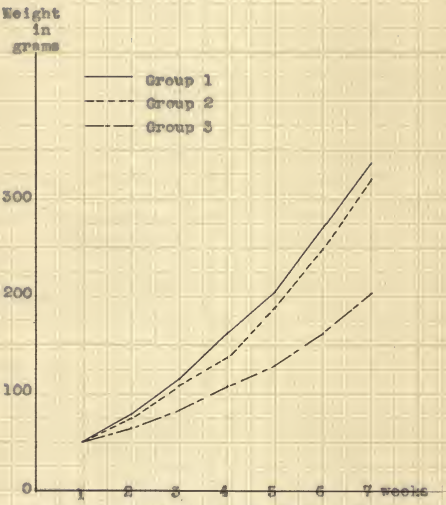

Fig. 4. Showing the growth curves of groups of chickens on animal protein plus milk (Group 1), animal protein (Group 2), and plant protein (Group 3) supplements.

DISCUSSION

It is generally understood that a reduction in quantity of food up to a certain point is not harmful and may, in some instances, be beneficial. However, the quality of the food, or more strictly speaking, the proper amount of the necessary constituents of a food or ration may have a significant relationship to growth and resistance to parasitism. The necessity for the presence of vitamin A (Ackert, McIlvaine and Crawford, 1931) and vitamin B (Ackert and Nolf, 1931) in rations fed to chickens in order to maintain normal growth of chickens and high resistance to nematode infestation was pointed out in Part I of this paper. This point is supported in part by the work of Foster and Cort (1931) who showed that dogs maintained on a deficient diet were much more susceptible to hookworm (Ancylostoma caninum) infestation than were those on an adequate diet. That the deficient ration lacked vitamins and minerals was pointed out by the authors, and, although it is not mentioned, there apparently was a lack of animal protein as well.

Besides an adequate amoung of necessary constituents, the addition of milk in a diet seems to play an important part in growth and host-parasite relationship. Circumstantial evidence on this point was furnished by Smillie

(1922). He cited an example of eight peasants who, in addition to an apparently adequate diet, were able to consume all the milk they wanted. Although the infestations of hookworm were large in this group, the peasants appeared to be in good health and with a haemoglobin index of 66*, although each individual harbored an average of 350 worms. This group he compared with 16 field workers who, apparently were receiving adequate rations, but to them the milk was not available. Although they harbored fewer hookworms, 224 as compared with 350, their haemoglobin index was low, an average of 57, and they did not have the strong, active and ruddy appearance of those who were receiving milk.

In addition to these groups, Smillie observed three male mountaineers who evidently were ill-fed and whose diet was deficient in many respects. Their average number of hookworms was 518 and their haemoglobin index, 33. They had a yellow, waxy skin, oedema of the face and feet, and were greatly depressed in spirit. These observations suggested a relationship between nutrition, especially milk, and the effects of parasitism. Differences in effects of parasitism were noted by Ackert and Spindler (1927) who found that chickens supplied with vitamin D suffered less from parasitism than those fowls from which it was withheld. Likewise,

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*Smillie suggested that the haemoglobin per cent was an index to the effect of parasitism.

the groups which received milk in addition to animal protein (Experiments 1 and 2) evidenced very little, if any, effect of parasitism. Of course, the numbers of worms were fewer in the groups receiving milk, therefore less damage from their presence would be expected. That the milk in the rations was the chief factor in the greater resistance to parasitism in the present experiments is quite evident. Also, from the results of these experiments it has been concluded that an adequate diet is necessary for a high degree of resistance of chickens to A. lineata and for normal growth.

SUMMARY

1. Four experiments, involving a total of 340 chicks, reared under controlled conditions, were conducted to study the effects of various protein supplements on the resistance of the chickens to the nematode Ascaridia lineata (Schneider).

2. The chickens were banded, weighed and segregated into equal groups, Group 1 receiving a protein supplement containing animal protein and milk, Group 2 receiving a protein supplement containing animal protein but no milk. Group 3 was fed no animal protein, but received its protein supplement in the form of peanut meal. An additional group in Experiment 3 received a ration containing only 28 per cent of the required amount of protein. By means of weekly

weights and graphs, the growths of the various groups were compared.

3. The chickens in Experiments 1 and 2 were each parasitized with 50 embryonated eggs which were allowed to grow for three weeks, at which time the experiment was terminated and the worms isolated. In Experiments 3 and 4, studies were made on the effects of the different diets on the growth of the chickens.

4. The criteria for judging whether or not the resistance of the chickens was affected by the various rations were the numbers and lengths of worms removed from the various groups at autopsy.

5. In Experiment 1, the results showed significantly more and longer worms in the group receiving an animal protein supplement and also in the group receiving a plant protein supplement than in the group receiving animal protein plus milk supplement. No significant difference existed between the animal and plant protein groups.

6. The results of Experiment 2 likewise show significantly more and longer worms from the group receiving the animal protein supplement and the group receiving the plant protein supplement than in the group receiving animal protein plus milk supplement. No significant difference existed between these two groups' numbers of worms. However, there was a significance between lengths of worms of

the animal protein group and the plant protein group.

7. It was quite evident from the appearance of the chickens as well as their records that those receiving both animal protein and milk were in much better health and were growing more normally than the animal protein group and the plant protein group. The plant protein group chickens failed worse than either of the other two groups.

8. From the results obtained there was strong evidence that the milk present in the diet caused the chickens to develop normally and to maintain a high degree of resistance to the parasites.

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