# STUDIES ON THE EFFECT OF HOST DIETARY FACTORS ON THE HOST-PARASITE RELATIONSHIP BETWEEN *HETERAKIS GALLINARUM* (NEMATODA: HETERAKIDAE) AND THE CHICKEN

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# Chapter I

## INTRODUCTION

The influence of nutrition on experimental infections in general, and on host-helminth relationships in particular, has been discussed by several groups of workers. Lapage (1962) lists seventy-four species of helminths that may be found in the domestic fowl, but of these, only the nematodes, Ascaridia galli, Heterakis gallinarum and Capillaria sp. are of economic importance and probably only A. galli and C. obsignata are of pathogenic significance. Occasionally cestodes, such as Raillietina and Davainea sp., are found and trematodes very rarely. Any appraisal of the problem must take into account the following aspects: (1) the virulence of the parasite, (2) the size of the infective dose and route of entry, (3) the host that becomes infected, and in this respect genetics, age and sex are important, (4) the nutritional status of the host, and (5) the nature of the resistance produced. Consequently, the host-parasite interrelationship is never static but complex and dynamic with either organism capable of producing constant changes in the other. Thus, any particular combination of the host and parasite can produce effects which range from no observable interaction to a serious disease which may result in the death of the host. Between these two extremes lies

a whole range of interactions which can be influenced by nutrition (Hunter, 1953).

Heterakis gallinarum (Schrank, 1788) Madsen, 1949, the poultry caecal worm, has been reported from more than 30 species of gallinaceous birds and is one of the most commonly encountered nematodes of domestic poultry reared on the soil. Graybill and Smith (1920) associated this nematode with transmission of the protozoan, Histomonas meleagridis (Smith, 1895) Tyzzer, 1920, responsible for blackhead, or histomoniasis. Prevalence rates from 60-100% among birds are commonly reported, with arithmetic mean worm burdens up to 175 worms per bird (Norton, 1964). There is little information in the literature relevant to the effect of host's diet on the growth and development of this parasite. Previous work provided some important information concerning the effect of dietary levels of proteins and amino acids, vitamins, and different minerals on the development of H. gallinarum and on the resistance of exposed hosts. No information is available concerning the effect of various levels of dietary sodium chloride, energy source, and other dietary factors such as inert bulk (e.g., polyethylene) on this parasite during a course of experimental infection. Therefore, this study is intended to describe the development of H. gallinarum in Leghorn chicks fed prescribed diets and the performance of exposed chicks during the course of experimental infection.

# Chapter II

# LITERATURE REVIEW

## Nutrition of Chicken

### Salt Addition to Chick Diets

It is well known that excessive intake of sodium chloride is toxic to poultry. Most of the earlier investigations concerning the salt tolerance of chickens were conducted with mature birds. The minimum lethal single dose of salt for birds weighing from 3 to 5 pounds was found to be close to 4 grams per kilogram of body weight (Mitchell et al., 1926).

A deficiency of dietary sodium chloride causes growth retardation accompanied by a reduction in feed conversion in chickens (Burns et al., 1953; Nott and Combs, 1969; Ross, 1977). In practical rations the average ingredients will usually meet the sodium chloride requirements. The addition of a small amount of salt to the average chick ration usually improves growth. The National Research Council (1946) recommended the addition of 0.5 percent NaCl to all poultry rations. Further minimal additions have no effect but growth is finally depressed when large amounts (5% and above) are included. With rations containing 0.25, 0.5, 1.0, 2.0, and 3.0 percent salt, Bearse and Berg (1946) reported greatest growth in birds fed the 0.5 percent salt ration from

3 to 8 weeks of age. Differences in mortality were not significant until the 3.0 percent of salt level was reached. Barlow et al. (1948) concluded that 1.0 percent of added salt was the optimum, although 2.0 percent and 3.0 percent added salt was neither better nor worse than the 1.0 percent level. Levels of 5.0 percent or more added salt depressed growth somewhat. Feed consumption did not appear to be appreciably influenced by salt levels in amounts from 0 to 10 percent. Quigley and Waite (1932) fed baby chicks rations containing from 1 to 15 percent salt. Levels of 8 percent or higher resulted in reduced growth; mortality was not excessive until the level of salt in the ration was greater than 5% by weight.

Excessive amounts of salt are toxic and will increase mortality. Three percent is usually considered the lower level to show an effect in this respect (Barlow et al., 1948; Bearse and Berg, 1946). The signs of salt intoxication, as reported by Scott et al. (1978), were inability to stand, intense thirst, pronounced muscular weakness, and convulsive movements preceding death. Necropsy has revealed lesions in many organs, particularly hemorrhages and severe congestion in the gastro-intestinal tract, muscles, liver, and lungs. In chronic cases a straw colored, gelatinous exudate is found beneath the skin in the region of the crop, and frequently in other portions of the body (Quigley and Waite, 1932). Bird (1943) and Dam (1944) reported that increasing the soluble salts in chick diets, particularly sodium chloride or other inorganic salts which tend to accumulate in the extracellular fluid, enhanced the occurrence of exudative diathesis when chicks were fed a diet low in vitamin E.

It has been frequently noted that high salt diets increase the water consumption which has an effect on the consistency of the droppings. Kare and Biely (1948) found that the water intake per gram of feed consumed increased progressively with the salt percentage of the mash diet. They also observed that chicks exhibited individual differences in tolerance for sodium chloride. For some chicks 5.18 percent salt in the mash was fatal; for others it was apparently harmless. Salt in many instances has proved to be very laxative. In fact, many broiler raisers have been known to regulate the droppings of the birds they are feeding, particularly in batteries, by the amount of salt in the ration (Halpin et al., 1936).

Slinger et al. (1950) reported that evidence indicates that the salt requirement of chickens expressed as a percentage of the feed is influenced by several factors. Using low-fiber, high-energy diets maximum growth was obtained with 0.25 percent or less of added salt. As the low-fiber, high-energy grains were replaced by high-fiber, low-energy grains, wheat by-products and cellulose, the salt requirement increased progressively to at least 2.0 percent. Halpin et al. (1936) found that 0.5 percent of added salt to the diet will undoubtedly meet the requirements of either the growing chick or the laying hen, judged by growth, mortality and egg production. In fact, when poultry rations contain the usual amounts of meat scraps, fish meal, or dried skim milk, as little as 0.5 percent of salt is sometimes probably excessive.

Burns et al. (1950) showed an interrelationship between sodium and potassium. When birds were fed semipurified diets containing 0.74 percent potassium, about 0.08 percent sodium was sufficient to produce

optimum growth, but if potassium intake dropped to 0.30 percent, about 0.15 percent sodium was required to produce the same growth. For best all-around results it appears that 0.5 percent of salt is about right although higher amounts may not be harmful in certain instances.

## The Use of Molasses in Chick Diets

Cane blackstrap molasses is a product of the sugar industry. Many feed manufactures make it a practice to include from 2.0 to as high as 5.0 percent of cane molasses in their poultry feeds the year round. The soluble carbohydrates in molasses are chiefly sugar and are of first importance in determining its feeding value. Chemical analysis of molasses has revealed that the total sugar content in samples analyzed ranged from 59.9% to 61.6%, with an average of 60.5%. These sugars were shown to be almost entirely sucrose, glucose and fructose, which were present at levels of 34.05%, 14.05% and 12.10% of the molasses, respectively. In all the samples a small amount (0.3%) of an unidentified sugar was also present. The mineral content of molasses was mainly potassium, magnesium and sodium, which were present at levels of 3.45%, 0.45% and 0.41% of the molasses, respectively (Cuervo et al., 1972).

Experiments have shown that diets containing as high as 10 percent of molasses could be fed at times. However, the higher percentages usually were too laxative for birds. Ott and Boucher (1942) replaced ground yellow corn by 0.0, 2.0, 4.0, and 6.0 percent feeding-cane molasses. The investigation was designed to show whether or not the addition of feeding-cane molasses would improve an already high-grade

ration. Statistical analyses showed that the mean performance of birds on the four rations were within the limits of normal variation. No differences between diets were noticeable in the outward appearance of the birds. Total feed intake for the growing period was increased significantly by inclusion of 4 and 6 percent cane molasses. Feed efficiency was highest in birds fed the no-molasses diet. Results from turkey experiments show that cane molasses can be used at levels from 2.5 to 5.0 percent in turkey starter feeds. The molasses was substituted for an equal amount of grain in these studies. The average weights of the birds fed 2.5 percent molasses did not differ significantly from the average weight of birds that were not fed molasses. Keshavarz et al. (1980) reported that molasses was effective as an energy source with no adverse effects on performance of growing chicks or laying hens when used in the diet at a level of 20%.

The laxative effect of molasses on chickens was found to be due primarily to presence of potassium salts in the molasses. Rosenberg and Palafox (1956b) found no adverse effects on growth and livability, but did find a depression of feed efficiency when potassium chloride and/or water were added to the experimental diets in amounts equivalent to their respective concentrations in cane molasses when the molasses was fed at 33.0 percent of total ration. In another study, Rosenberg and Palafox (1956a) observed that the comparative advantage of molasses over corn in price tended to overcome the disadvantage in efficiency of feed utilization particularly at levels of molasses ranging from 16.5 to 34.5 percent of the total ration.

Cuervo et al. (1972) determined the effect of the various sugars present in molasses as causative agents of liquid feces. Fructose, glucose and sucrose were fed individually and in all possible combinations as controls. A comparison with chicks fed molasses indicated that the sugars present in molasses are not the primary cause of diarrhea when high levels of molasses are fed. There was little difference in consumption of water among the groups receiving the various sugars. However, chicks receiving the molasses diet had a much larger water consumption than others fed the various sugars. This was thought to be due to an attempt to excrete the excess of ions, including potassium, present in molasses. Kondo and Ross (1962) earlier reported that water consumption and fecal humidity are directly related to the quantity of sodium and/or potassium present in the feed.

## The Addition of Dietary Bulk (Polyethylene)

Chicks tend to eat to meet their energy needs. It has been demonstrated with broilers that the addititon of fiber to the ration causes an elevated dry matter intake as the chick attempts to maintain a constant level of energy intake. The effect of dietary bulk upon feed consumption was demonstrated by Fisher and Weiss (1956) using normal and cropectomized chicks. This work indicated that fiber was an important factor which influenced feed consumption, independent of the energy level of the diet. The use of fiber, up to a given dietary level, stimulated feed consumption; but beyond that level, feed consumption remained relatively constant. It was found that efficiency of feed utilization was not sacrificed, but was actually improved when

fiber was added (simultaneously with fat) to high-energy diets. Dvorak and Bray (1978) added cellulose (as solka-floc$^{R}$) to the basal diet at levels graded from 10.0 to 45.0 percent, and reported a linear increase ($P < .05$) in feed intake and a linear depression in growth rate. They found that cellulose tended to lower gain per unit of basal diet consumed, indicating that the nondigestible material reduced the utilization of the basal portion of the diet.

The influence of fiber source and level upon chick growth and gastrointestinal tract parameters was demonstrated by Cannon et al. (1982). Variable results were obtained on feed intake, weight gain, and intestinal fill when polyethylene, cellulose, or hemicellulose were used at different levels. These findings suggested that when formulating chick diets the source of the fiber as well as calorie density should be considered. Consumption of alfalfa cell walls resulted in high growth rates ($P < .05$) and better feed efficiencies ($P < .05$) compared with chicks fed polyethylene (Ricke et al., 1982). Hooge and Rowland (1978) observed that the use of 6% builder sand in broiler diet improved feed efficiency without influencing three-week body weights. Andrews et al. (1972) recommended the use of sand over solka-floc$^{R}$ as dietary diluent. Although the diets without a diluent were more concentrated, the results obtained with them were not significantly higher than those with diluents. The diets containing sand resulted in the most uniform data. Solka-floc$^{R}$ appeared to depress growth.

On the response of broiler chicks to diets varying in nutrient density content, Waldroup et al. (1976) concluded that growth rate and efficiency of feed utilization were almost direct functions of the

dietary nutrient density level. Poor efficiency of feed utilization was noticed by Classen and Campbell (1982) with decreasing nutrient density of broiler diets. Broilers fed diets containing 3080 Kcal of metabolizable energy per kg of starter diet (24.0% protein) and 3135 Kcal per kg of finisher diet (22.0% protein) had significantly poorer body weights and feed conversions than did broilers fed diets containing metabolizable energy levels of 3160 (24.8% protein) Kcal per kg of starter diet and 3245 (22.8% protein) Kcal per kg of finisher diet (Cherry et al., 1978).

## Nutrition and Host-Helminth Interactions

### Mineral Content of The Host Diets

Deo and Srivastava in 1963 found that calcium deficiency affected the resistance of chickens to Heterakis gallinarum. The number of worms recovered from the calcium-deficient group of chickens was significantly more than that obtained from the control group maintained on an adequate basal diet. The length of worms in the calcium-deficient group of birds, though not statistically significant, was less than that of worms obtained in the control group. This decrease in length is attributed to the effect of over-crowding of worms in the caeca. The deficiency of the mineral matter, particularly calcium and phosphorus, significantly affected the fate of the Heterakis gallinarum. As a result of this deficiency the chicks ceased to grow though they ate well for a time (Clapham, 1934b). Gaafar and Ackert in 1953 studied the effect of calcium and phosphorus deficiency on the resistance of fowl to Ascaridia galli infection. In four

experiments there were fewer and shorter A. galli recovered from the fowls kept on a ration deficient in phosphorus or calcium than from the control groups maintained on adequate diets. Chickens on the phosphorus deficient diet manifested typical deficiency symptoms of general debility; similarly the fowls on the calcium deficient rations showed typically retarded growth, pliable beaks, and usually rickets. The resistance of growing chickens to the fowl nematode, A. galli, appears not to be affected by a deficiency of phosphorus or of calcium in the diet of the host. In contrast Cuca et al. in 1968 reported a decrease in length and ability of A. galli to survive as the level of calcium was increased from 0.3 to 2.5% in the diet of the host. They observed that the lower level of calcium used, which was well below the need of the host, was sufficient for normal development of embryonated eggs and growth of the parasite. The chicks exposed to this parasite gained less than the non-exposed.

Shumard et al. in 1956 studied the resistance of lambs on pasture to the effects of Haemonchus contortus and other nematodes when fed additions of trace minerals, dicalcium phosphate, phenothiazine, and combination of these. Lambs receiving trace-mineralized salt, without dicalcium phosphate or phenothiazine, were most severely affected by the nematode infection. Eighty percent of these lambs died, and they contaminated the pasture with the largest number of nematode eggs. Addition of dicalcium phosphate to the diet containing trace-mineralized salt produced an added resistance. Although these lambs were more heavily infected, they gained 12.0 percent more weight than lambs receiving iodized salt. More lambs receiving trace-mineralized

salt passed worm eggs the first few weeks than did lambs not receiving this salt, regardless of further addition of phenothizine or dicalcium-phosphate. Analyses of worm egg counts, number of worms recovered at necropsy, and hemoglobin concentrations of infected lambs seemed to support the contention that a phenothiazine, trace mineral-dicalcium phosphate combination was superior to any of these alone when Haemonchus contortus is present. Threlkeld et al. in 1956 indicated that lambs fed cobalt carried heavier worm burdens than did deficient lambs, but these lambs made better gains in weight when both groups were exposed to the same number of infective Haemonchus contortus larvae. When compared at necropsy to lambs maintained on the basal ration only, those which received cobalt also had more Haemonchus contortus; more of the female parasites were gravid and the average length of these females was greater than those in control animals.

Many endoparasitic nematodes of animals are known to survive for long periods in various concentrations of sodium chloride in vitro (Fenwick, 1939). Studies on the survival of Ascaridia galli at ambient temperature in different concentrations of sodium chloride were undertaken by Narain (1973). He reported that the longest survival time and minimum death rate were observed in 0.8 - 0.9 g NaCl/100 ml of water at all temperatures. Death rate was higher and survival time shorter in concentrations below the optimum (0.1 - 0.7) than in those above the optimum (1.0 - 2.0) g NaCl/100 ml. He observed that gravid females died earlier than others, and that they were able to lay eggs in 0.2 - 1.0% NaCl solutions but not at concentrations outside these limits.

## Carbohydrate Content of the Host Diets

Helminths are strongly affected by the source of dietary energy of their hosts. Significant differences were noted in the number of worms that developed and in the length of individual worms when glucose was substituted for starch as a source of energy in diets of rats exposed to Hymenolepis diminuta infection (Komuniecki and Roberts, 1975). Dunkley and Mettrick in 1969 studied the effect of the quality of host dietary carbohydrate on the growth of H. diminuta in rats. In this study, the effect of sugar quality in the host diet was compared using glucose, maltose, sucrose, galactose, and dextrin; taking glucose as a standard, worm dry weight from rats fed on a maltose diet showed a decrease of 3.9%, dextrin a decrease of 11.8%, and sucrose a decrease of 22.8%. Galactose did not support worm growth. It is normally absorbed through the intestinal mucosa and transported to the liver for final conversion into glucose. Thus, if galactose is used as the sole dietary carbohydrate source it should be absorbed by the intestinal wall and no exogenous glucose should be available to the worms. If they are strongly dependent on glucose, under such circumstances one would expect the worms to show the effects of carbohydrate deficiency (Hager, 1941). The effect of starch quality in the diet of rats on the growth of H. diminuta was also compared using potato, corn (maize), and wheat starch. With potato starch as a control, the increase in worm dry weight was 22.0% on wheat starch and 30.0% on corn starch; changes in host body and liver weight were shown to be influenced by the different diets. In all the experiments the availability of glucose to the worm in the intestinal lumen appears

to be the limiting factor controlling parasite growth (Dunkley and Mettrick, 1969). Rats infected with the acanthocephalan Moniliformis were fed on five purified diets which contained 0.0, 1.0, 2.0, 3.0, and 4.0% starch, respectively. The growth of the rats and the worms was shown to be related to the amount of carbohydrate in the diet (Crompton and Nesheim, 1977).

Protein and Vitamin Content of the Host Diets

Ackert and Beach (1933) reported that infected chicks on a diet deficient in proteins of animal origin were less resistant to infections by Ascaridia galli than were birds fed a vegetable diet supplemented with either skim milk or meat meal. Riedel and Ackert (1950) were of the opinion that the beneficial action of skim milk might be due to the ease of digestibility of milk casein or to the high concentrations of lysine and tryptophane present in skim milk. Using a highly purified simi-synthetic diet, it was demonstrated that a deficiency of pteroylglutamic acid (PGA) lowered resistance of chicks to A. galli infections (Sadun et al., 1949, 1950). Cuca et al. (1968) reported a decrease in worm size as the lysine level increased from 0.65 to 2.05%. At 4 weeks after exposure, the number of worms was singificantly greater in chicks fed low levels of lysine. These results show that the need of A. galli for lysine as measured in the diet of the host is less than 0.65% which is well below the optimal (1.20%) level for the diet of the host. The effect of aureomycin and vitamin $B_{12}$ used in combination as a feed supplement on the resistance of chickens to the fowl ascarid, Ascaridia galli, were investigated by Hansen,

Norris and Ackert (1953). Chicks fed the supplemented ration showed a lower mortality and infection rate and harbored significantly fewer worms than did the chicks fed only the basal ration; superior weight gains were made by those chicks fed the fortified diet. Small differences in feed efficiencies of the various diets was noted.

The effect of continuing ingestion of eggs of Ascaridia galli on growth rates of chickens placed on different nutritional levels was studied by Ikeme (1971a). When repeated doses of 10, 100 and 1000 infective eggs/day were fed with increasing amounts of protein in feed, there occurred significant weight differences between infected groups; the highest protein levels fed to chicks resulted in longest duration in weight depression due to infection. On the other hand, assuming adequate nutrition for the host, the greatest effects on growth rates were seen in chickens receiving daily doses of 1000 eggs and the lowest in chickens receiving 10 eggs. The prepatent periods in the groups which received ten eggs daily for 36 days were not influenced by differences in diet of the hosts (Ikeme, 1971a).

On the role of host nutrition in the pathogenesis of ovine fascioliasis, Berry and Dargie (1976) found that sheep on the lower protein ration developed anemia more rapidly than controls. In addition they experienced hypoalbuminemia and weight loss, and died earlier than their better fed counterparts exposed to the same number of Fasciola hepatica metacercariae; fluke burdens were comparable in both groups of lambs at necropsy.

# Chapter III

## MATERIALS AND METHODS

### The Experimental Design

An experiment was designed to see if sodium chloride level, energy source, or dietary diluent would affect the development of Heterakis gallinarum or the performance of its host.

A total of 168 chicks were randomized into 7 groups of 24 chicks each, as follows: G1, G2, G3, G4, G5, G6, and G7. Each group was subdivided further into 3 random lots of 8 chicks each. The basal diet contained the normal dietary level of sodium chloride (0.5%) for growing chicks; and corn and cornstarch were used as the major sources of energy. This diet was fed to two dietary control groups (G1 and G2). One dietary control group (G1) was left unexposed to H. gallinarum to serve as an environmental control. Chicks in the remaining six groups were all exposed to H. gallinarum by giving each bird 100 infective eggs of the nematode per os. Three groups (G3, G4, and G5) were fed the same basal diet as controls except for different levels of sodium chloride (1.5, 2.9, and 4.3%, respectively). The two remaining groups (G6 and G7) received diets that contained 0.5% sodium chloride but differed from the basal diet as follows: G6 contained molasses, replacing most of the cornstarch of the basal diet as energy source and that of G7 contained polyethylene as an undigestible dietary

diluent. This polyethylene was added to the basal diet as feed additive.

The following criteria were studied.

1. Host factors
   - A - Weight gain of individual birds--determined at weekly intervals for the six-week duration of the experiment.
   - B - Feed consumption and efficiency of utilization -- determined for groups of chicks.
2. Parasite factors
   - A - Prepatent period of infection.
   - B - Number and sex ratio of worms recovered at necropsy.
   - C - Length and weight of worms recovered at necropsy.

## Preparation of Infective Material

Pure infections of Heterakis gallinarum were established in the laboratory by exposing parasite-free donor chicks to embryonated eggs obtained from Doctor M. Ruff of the United States Department of Agriculture, Beltsville, Maryland. Donor chicks were shown by fecal flotation technique to be parasite free before exposure to the infective eggs. After exposure of the donor chicks to Heterakis infection, feces were collected periodically and examined until about the time of expected patency. When the resulting infections became patent, feces were pooled and mixed with 2-3 times the volume of water in a blender for about 20 seconds. The mixture was screened with a fine sieve (0.42 mm opening). The supernatant portion was then collected and allowed to stand overnight. The water was decanted and the sediment

resuspended in a concentrated salt solution ($NaNO_3$) and centrifuged at 5000 rpm for 3 min. The supernatant fluid containing eggs was collected, diluted 20 times its volume with water and allowed to stand overnight to enable the eggs to settle. The supernatant fluid was aspirated off and the remaining sediment containing the eggs was resuspended in an equal volume of 1.0 N sodium hydroxide, mixed and left for 30 min. to dissolve the sticky albuminous coat of the eggs. Removal of the albuminous coat prevented eggs from clumping and made uniform mixing possible. Following the de-coating procedure, the eggs were concentrated by centrifugation, and were washed three times by resuspending in water and repeating centrifugation. For embryonation they were resuspended in $NaCO_3$ salt solution and centrifuged. The supernatant fluid containing the eggs was collected and diluted with about 20 times its volume of embryonating fluid (0.5% formalin). This fluid containing the eggs was then centrifuged at 5000 rpm for 3 min., and the eggs were washed twice in the embryonating fluid. Thereafter, the fluid containing the eggs was distributed in amounts of 50 ml each into 250 ml flasks. The eggs were stored in a thin layer of the embryonation fluid (about 2 cm. depth) to insure adequate oxygen was available for embryonation (Ikeme 1971a).

The eggs prepared were incubated at room temperature for about 21 days. Once embryonated, eggs were refrigerated at about 4°C until administered to experimental chicks.

## Preparation of Rations

Six different rations were prepared for this experiment. They

were designated as follows: R1, R2, R3, R4, R5, and R6. The components of the basal ration (R1) are shown in Table I. It contained yellow corn and cornstarch as the major sources of energy. In this ration sodium chloride was used in the normal dietary level (0.5%) for growing chickens. Rations 2,3, and 4 were the same as R1 in terms of energy sources (corn and cornstarch), but they differed in content of sodium chloride. They contained 1.5, 2.9, and 4.3% NaCl, respectively.

Rations 5 and 6, like R1, each contained 0.5% NaCl. Ration 5 differed from R1 in its energy sources. It contained corn and molasses as major sources of energy. The molasses replaced most of the cornstarch and was used at a level of 29.5% (Table I). In R6, energy sources were the same as R1 (corn and cornstarch), but it differed from R1 by the addition of 13% polyethylene. The polyethylene was added to the basal ration as undigestible dietary diluent. Therefore R5 contained lower energy per kg of feed than R6. Both rations contained the lowest amount of energy per kg of feed among the six rations prepared (Table II).

Soybean meal and meat and bone meal were used as the major sources of protein in formulating all six rations. Other nutrients like trace minerals, vitamin mix, and essential amino acids were adjusted according to specifications from the National Academy of Sciences, National Research Council Publication, 1977: 1, Nutritional Requirements of Poultry.

Rations were balanced for the caloric to protein (c/p) ratio, but they were not isocaloric, i.e., the same weight from each did not provide the same amount of energy and protein.

TABLE I

RATION FORMULATIONS

| Ingredient | RATION | | | | | |
|---|---|---|---|---|---|---|
| | R1 % | R2 % | R3 % | R4 % | R5 % | R6 % |
| Tallow, Feed grade | 5.0 | 4.9 | 4.8 | 4.8 | 4.2 | 4.4 |
| Yellow corn, ground | 29.3 | 29.0 | 28.6 | 28.2 | 24.7 | 25.5 |
| Cornstarch | 20.2 | 20.0 | 19.7 | 19.4 | 3.3 | 17.4 |
| Soybean meal 44% | 33.0 | 32.8 | 32.6 | 31.7 | 27.7 | 28.7 |
| Meat and bone meal 50% | 5.0 | 4.9 | 4.9 | 4.8 | 4.2 | 4.4 |
| Alfalfa meal 17% | 3.0 | 2.9 | 2.9 | 2.9 | 2.5 | 2.6 |
| D-L Methionine | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 |
| Lysine | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| $CaCO_3$ | 1.0 | 1.0 | 1.0 | 1.0 | 0.8 | 0.9 |
| Phos-Supp $Ca_{20}$-$P_{18}$ | 1.6 | 1.6 | 1.6 | 1.5 | 1.4 | 1.4 |
| Trace mineral | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Salt | 0.5 | 1.5 | 2.9 | 4.3 | 0.5 | 0.5 |
| Vitamin mix | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 |
| Molasses | 0.0 | 0.0 | 0.0 | 0.0 | 29.5 | 0.0 |
| Polyethylene | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13.0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

TABLE II

RATION FORMULATION ANALYSIS

| Nutrient | RATION | | | | | |
|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 |
| Dry matter (%) | 90.5 | 90.4 | 90.8 | 90.9 | 86.0 | 91.7 |
| ME[a] KCal/Kg | 3056 | 3025 | 2983 | 2939 | 2574 | 2658 |
| Protein (%) | 20.0 | 19.8 | 19.5 | 19.3 | 17.0 | 17.4 |
| Caloric/Protein (ratio)[b] | 69.4 | 69.3 | 69.4 | 69.4 | 69.4 | 69.4 |
| Calcium (%) | 1.3 | 1.3 | 1.3 | 1.2 | 1.2 | 1.2 |
| Phosphorus (%) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |

[a] Metabolizable Energy

[b] Kilocalories for every 1% of protein

Grain was ground and all ingredients were mixed at the Poultry Research Center, Department of Animal Science, Oklahoma State University.

A large platform balance was used to weigh macroingredients in pounds; whereas, gram levels of microingredients were weighed on a double pan balance. The microingredients were added to approximately 10 pounds of ground grain and mixed for a few minutes. The resulting "premix" was then blended into the remainder of the 100 pounds of macroingredients and mixed for 5 min. in a 100-pound capacity horizontal paddle mixer. Each 100 pound diet was divided into three lots, and put into paper bags, labeled, and stored at the Oklahoma State University Poultry Farm. Feed from the paper bags was weighed into the diet storage cans, and the amounts were recorded in kg. Feed was added to the feeders every day. At the end of each one-week period, the feed remaining in the feeders was emptied into the storage cans and weighed again. The amount of feed consumed per lot of chicks per week was calculated, i.e., the difference in these weights was determined. Feed utilization, calculated as kilograms of dry matter of feed consumed per kilogram of gain $(\frac{\text{Intake (Kg)}}{\text{Gain (Kg)}})$, was determined for each lot of chicks.

## Experimental Chicks

A total of 168 one-day-old White Leghorn male chicks were used in this experiment. The chicks were wing banded and randomized into 7 groups of 24 chicks each. Electrically heated batteries were used to rear the chicks through the first 4 weeks of age, after which they

were left in the same batteries, but without supplemental heat until the end of the 6-week experimental period. Feed and water were provided *ad libitum*. The experimental diets were given to the chicks from day one of age. The chicks were weighed individually at the beginning of the experiment and weekly thereafter. Weekly feed consumption was recorded on a dry weight basis for each lot of 8 chicks.

## Exposure of Chicks to Infective Eggs

At the age of 2 weeks, the chicks which had been raised under parasite-free conditions were each given 100 embryonated *Heterakis gallinarum* eggs *per os*. A McMaster's chamber was used for counting the eggs in a suspension of 0.5% formalin solution, the storage fluid. The chamber is 1.0 $cm.^2$ with a grid etched into the upper cover and having depth of 0.15 cm. The grid is divided into six columns to facilitate counting, i.e., the accuracy of counts is improved by having the field subdivided into columns.

The storage fluid (stock solution) containing the eggs was stirred thoroughly until homogeneous egg suspension was achieved. An aliquot of this solution was placed in the chamber, and left for 1-2 min. to allow the eggs to settle. The total number of eggs within the grid was counted in all columns under the low power objective (10x), which gives a total magnification of 100x. Since the depth of the chamber is 0.15 cm., the total number of eggs in 1 sq. cm. was multiplied by 6.7 ($\frac{1.00 \text{ cm}}{0.15 \text{ cm}} = 6.7$) to determine the number of eggs in a $cm.^3$ (1 ml). The average egg count for 5-6 aliquots was used in determining the total number of eggs in 1 ml of the stock solution. Finally the volume of

the stock solution was adjusted so that each ml contained 100 embryonated eggs.

Administration of nematode eggs to the experimental chicks was done by using a 2 ml syringe, with a catheter tube which was inserted into the crop. One group of chicks (three lots of 8) was left un-exposed as an environmental control. Each of the 144 remaining chicks in G2 - G7 was given a single dose of 100 embryonated H. gallinarum eggs in 1 ml of the stock solution by the method described above. Proper protocol would require that the environmental control group be given 1 ml of the incubation fluid free of parasite eggs, but, owing to oversight, this was not done.

## Determination of Prepatent Period

Fecal samples were collected from each lot of chicks for 14 consecutive days beginning 18 days after inoculation and continuing until the experiment was completed 31 days post inoculation. A random sample of approximately 50 g of fresh feces was collected from each lot of chicks each day. Samples were placed in labeled plastic bags and refrigerated until examined. The remaining feces in the trays from each lot of chicks was collected and placed in paper bags to avoid contamination of the environment. The fecal trays were cleaned and lined with paper in preparation for the next day's collection.

A simple fecal flotation test was conducted using saturated sodium nitrate levitation solution (Sp. Gr. 1.40). The procedure was conducted by taking a random amount of 5-10 grams of feces from the fecal mass and mixing it with approximately 20 ml of the saturated salt solution.

The mixture was stirred thoroughly in a 50 ml paper cup by tongue depressor, strained through gauze and then poured into a vial. The fluid level was brought to slightly above the rim of the vial, and a coverslip was gently placed on top in contact with the liquid. After 10 min., the coverslip was carefully removed and placed on a glass microscope slide. This slide was systematically examined microscopically under the 10x objective; the total magnification was 100x. When heterakid eggs were first observed by this method, infections were considered to be patent.

## Necropsy of Chicks and Recovery of Adult Worms

Four weeks after inoculation with Heterakis gallinarum, chicks from each lot were killed randomly by cervical dislocation on 3 consecutive days. Each day 3 chicks from each lot were killed (2 the last day). Chicks were dissected immediately, and the ceca and large intestine removed.

Ceca and large intestine from each chick were opened and the contents washed into separate containers with distilled water. The containers were then filled with distilled water and left for 15 min. while the worms settled. The water was decanted and the sediment examined for worms. The cecal worms were then collected and counted in a petri-dish containing 0.75% saline solution. Worms collected each day were identified as to bird origin and refrigerated in saline until all birds had been necropsied.

## Weighing and Storage of Worms

Worms that were collected and refrigerated as described above, were washed with distilled water in a petri-dish and transferred onto a paper towel to absorb excess fluid from the worms' body. After drying, the worms were placed on a piece of wax paper and weighed using a Mettler $H_{30}$ analytical balance. The wax paper with worms was transferred to Beltsville nematode fixative (Chitwood, 1961) and stored until other measurements were completed. Preservation of worms in this fixative was done without removing the helminths from the wax paper because they stuck to it and might have been damaged if removed.

## Determination of Length and Sex of Worms

Worms that had been preserved in Beltsville nematode fixative were mounted temporarily with this fixative on a slide and examined microscopically to determine length and sex. A Bausch and Lomb microprojector was used for measuring the length. Worms were placed under the 10x objective power of the microprojector. The magnified image of each worm from the slide was shown on a white screen. Since many worms are curved rather than straight, a thread was needed for measuring their magnified length directly from the screen. The length of the thread, representing the magnified length of a worm, was then measured with a metric ruler. The magnification factor was determined by projecting the image of a stage micrometer on the white screen and measuring it directly. The magnification factor was then used to calculate the actual length of the worms in mm. by dividing the magnified length of helminths by the magnification factor.

After measuring the length, the slide containing the temporarily mounted worms was transferred to a compound light microscope and the sex of worms determined by observing the posterior end of each worm. The tail of the male has wide alae (expansions of the cuticle) and papillae in addition to the two spicules. The tail of female worms is pointed and has no cuticular expansions.

## Analysis of Data

A Duncan's Multiple Range Test was utilized to test for differences in treatment means to assess the response of birds and their parasites to the dietary sodium chloride level and energy sources. A comparison between the non-exposed and the exposed control group of birds was made. The mean and standard deviation of each parameter was determined.

# CHAPTER IV

# RESULTS

## Pre-exposure Period

Results from a period of two weeks before exposing parasite-free chicks to the single dose of Heterakis gallinarum eggs indicate that growth rates of the chicks did not differ among groups fed different diets (Fig. 1 and 2). Statistical analyses on body weight gain and feed utilization of chicks showed no significant differences during the first two weeks of the six-week experiment. The highest body weight gain by chicks was 110.2 g, the average weight of chicks fed molasses (G6); the lowest was 103.2 g, chicks fed a diet containing 4.3% NaCl (G5). Feed utilization from these two groups were 2.07 and 2.04, respectively.

## Post-exposure Period

A summary of the major results of the parasitologic studies, the last four weeks of the experiment, are recorded in Tables III and IV.

### Host Factors

Weight Gain. Chicks fed the basal diet and exposed to 100 embryonated Heterakis gallinarum eggs (G2) showed a significant reduction ($p < .05$) in weight gain in comparison with controls

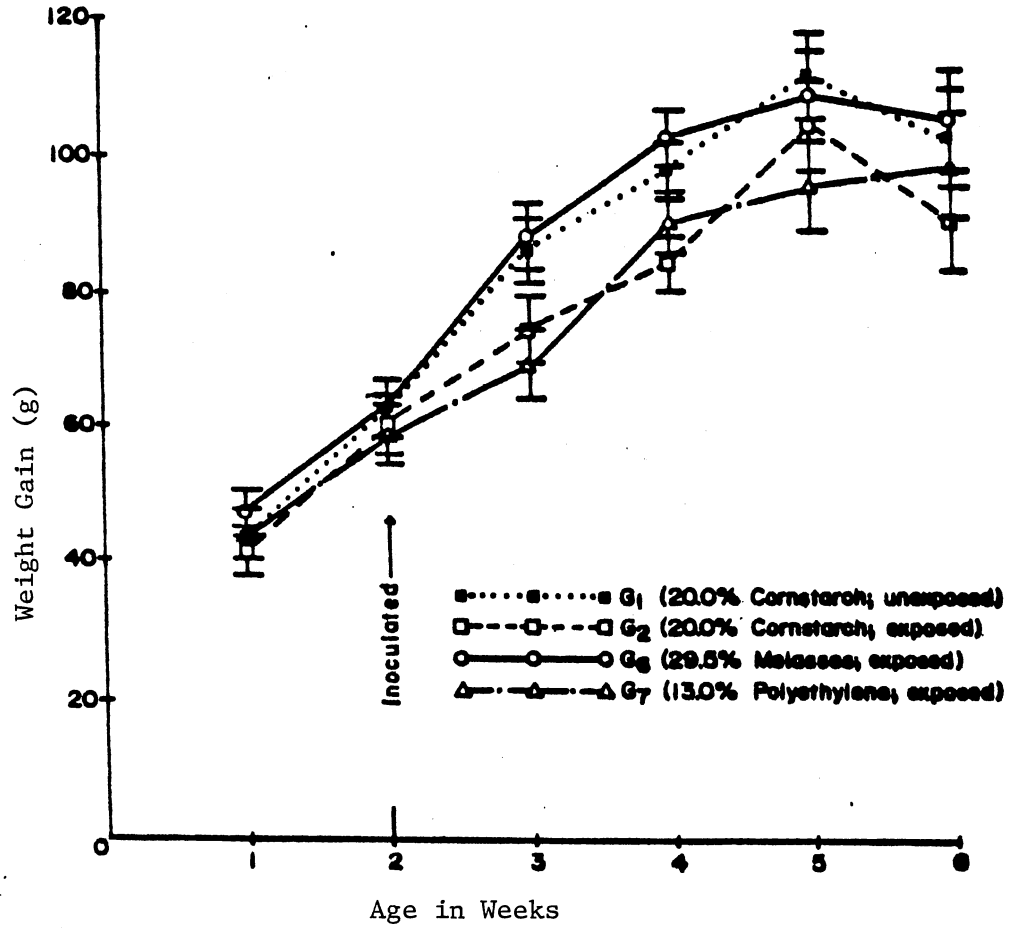


Figure 1. Weight Gain of Heterakis-infected and Non-infected Chicks Fed Diets Containing 0.5% NaCl and either Cornstarch, Molasses or Polyethylene. Each Point Represents the Mean of 24 Birds. Bars Represent the Standard Error of the Mean.

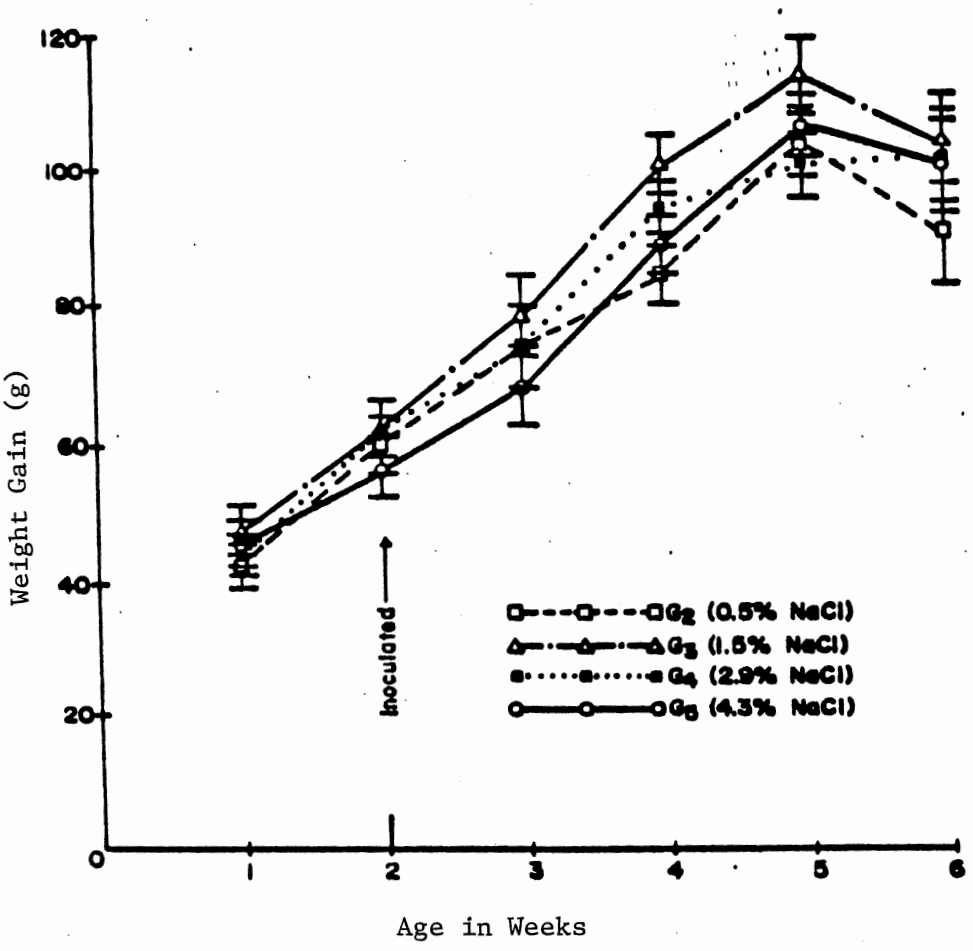


Figure 2. Weight Gain of Chicks Inoculated with 100 Eggs of Heterakis gallinarum and Fed a Basal Diet which Varied in NaCl Concentration. Each Point Represents the Mean of 24 Birds. Bars Represent the Standard Error of the Mean.

TABLE III

EFFECTS OF DIETARY NaCl LEVELS, MOLASSES AND POLYETHYLENE ON WEIGHT GAIN AND FEED CONSUMPTION IN CHICKS INFECTED WITH 100 HETERAKIS GALLINARUM EGGS

| Parameter[b] | Treatment Groups[a] (% NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Non-infected control | Infected | | | | | |
| | NaCl (0.5) | NaCl (0.5) | NaCl (1.5) | NaCl (2.9) | NaCl (4.3) | 29.5% Molas. (0.5) | 13% Polyet. (0.5) |
| Weight gain (g) | $400.1^{AB}$ | $355.0^{C}$ | $400.3^{AB}$ | $373.1^{ABC}$ | $367.3^{BC}$ | $407.4^{A}$ | $351.0^{C}$ |
| Feed consumption (Kg) | $7.3^{AB}$ | $6.7^{A}$ | $7.3^{AB}$ | $7.1^{A}$ | $7.0^{A}$ | $8.1^{C}$ | $7.8^{BC}$ |
| Feed utilization[c] | $2.28^{A}$ | $2.38^{AB}$ | $2.28^{A}$ | $2.38^{AB}$ | $2.40^{AB}$ | $2.50^{B}$ | $2.79^{C}$ |

[a] Twenty-four chicks raised in lots of 8 chicks.

[b] Means with different superscripts showed statistically significant differences ($P < 0.05$).

[c] Computed as $\frac{\text{Feed intake}}{\text{Weight gain}}$.

TABLE IV

EFFECTS OF DIETARY NaCl LEVELS, MOLASSES AND POLYETHYLENE ON THE PREPATENT PERIOD, WORM COUNTS, WEIGHT (BIOMASS), SEX RATIO, AND LENGTH OF WORMS IN CHICKS INOCULATED WITH 100 HETERAKIS GALLINARUM EGGS

| Parameter[b] | Treatment Groups[a] (% NaCl) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Non-infected control | Infected | | | | | |
| | NaCl (0.5) | NaCl (0.5) | NaCl (1.5) | NaCl (2.9) | NaCl (4.3) | 29.5% molas. (0.5) | 13% polyet. (0.5) |
| Prepatent period (days) | NA[c] | $30.0^{A}$ | $26.7^{AB}$ | $24.0^{BC}$ | $27.3^{AB}$ | $22.3^{C}$ | $27.0^{AB}$ |
| Worm count | 0.0 | $4.0^{A}$ | $12.4^{A}$ | $8.9^{A}$ | $7.3^{A}$ | $6.7^{A}$ | $10.4^{A}$ |
| Worm weight (mg) | NA | $3.6^{A}$ | $6.5^{A}$ | $5.9^{A}$ | $6.2^{A}$ | $4.2^{A}$ | $6.7^{A}$ |
| Sex ratio (M/F) | NA | $1.1^{A}$ | $0.9^{A}$ | $1.0^{A}$ | $1.0^{A}$ | $0.6^{A}$ | $1.3^{A}$ |
| Length of male (mm) | NA | $6.4^{C}$ | $6.5^{BC}$ | $6.7^{ABC}$ | $6.9^{AB}$ | $6.9^{AB}$ | $7.0^{A}$ |
| Length of female (mm) | NA | $7.5^{A}$ | $7.5^{A}$ | $8.3^{A}$ | $8.5^{A}$ | $8.0^{A}$ | $8.5^{A}$ |

[a] Twenty-four chicks raised in lots of 8 chicks.

[b] Means with different superscripts showed statistically significant differences ($P < 0.05$).

[c] NA = Not applicable.

(G1), Fig. 1. Mean weight gain of exposed chicks was 355.0 g; that of controls was 400.1 g (Table III). Differences in weight gains were observed in the parasitized groups when chicks were fed a diet that differed only in having higher levels of sodium chloride (Fig. 2). Infected chicks whose diet was supplemented with 1.5% NaCl (G3) had greater weight gain (11.3%) than did infected chicks fed the basal diet (G2). In G4 and G5 supplementation of 2.9 and 4.3% NaCl, respectively, enhanced body weight gains by 5.1 and 3.4%, respectively. Parasitized chicks fed a diet containing molasses and 0.5% NaCl (G6) gained more weight than birds in the other groups including the non-exposed controls (Fig. 1). The average weight gain made by the individual chicks fed molasses was 407.4 g (Table III). Infected chicks fed polyethylene (G7) gained the least weight (351.0 g) of any group of chicks in the experiment (Table III). The range of weight gain for individual birds within this group was 270.0 to 462.0 g; that of infected chicks fed the basal diet was 188.0 to 437.0 g. No statistically significant effect ($p > .1$) on body weight gain was observed when polyethylene was fed (G7).

Feed Consumption and Utilizaton. Feed utilization or conversion was calculated by lot of chicks as shown earlier; the grams of feed consumed was divided by the grams of weight gained. The average quantities of feed consumed by the various groups of chicks during the last 4 weeks of the experiment are shown in Fig. 3. Non-exposed chicks (G1) consumed more feed (7.3 kg) than did exposed chicks fed the same basal diet (6.7 kg; Table III). There was little difference in efficiency of feed utilization between these two groups (Table III).

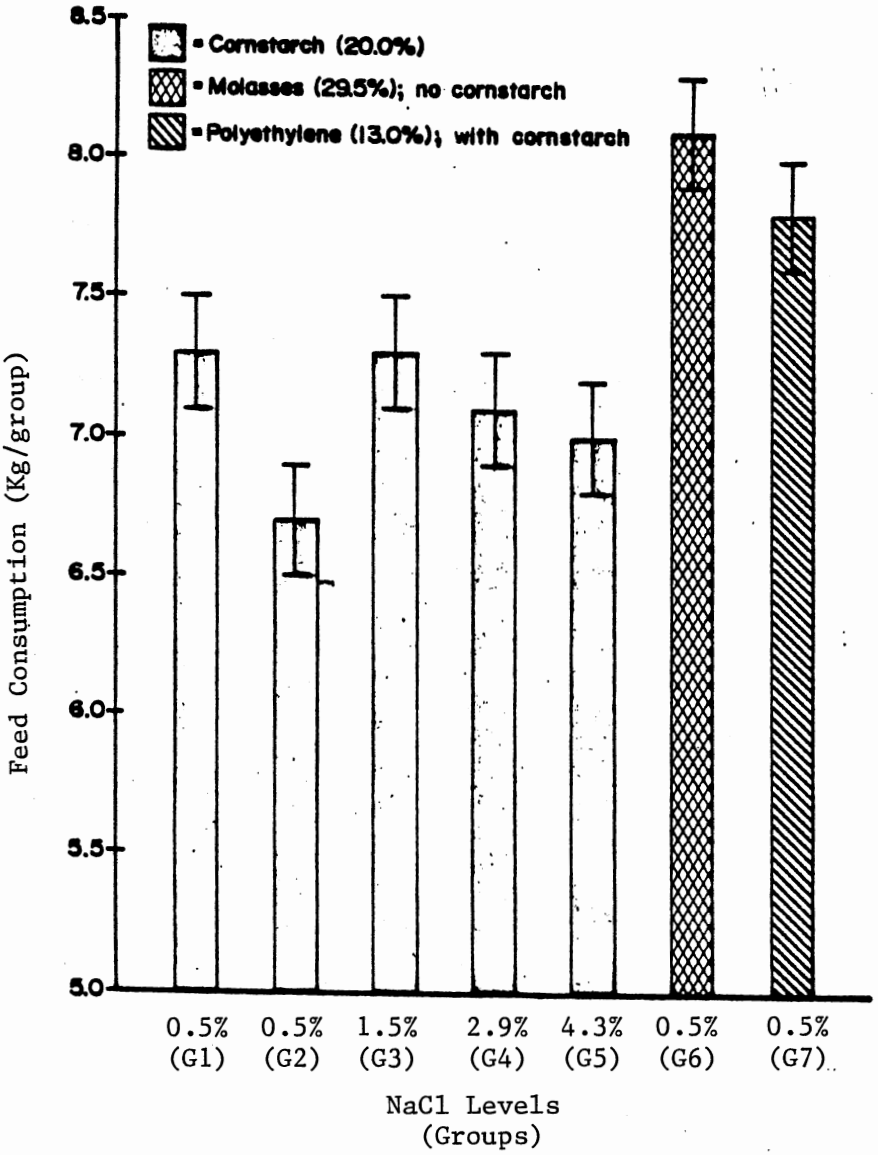


Figure 3. Feed Consumed by Heterakis-parasitized Chicks (24/group) During the Last 4 Weeks of the Experiment. Percentages Represent NaCl Levels in the Feed for Each Group. Group 1 was an Unexposed Control. Vertical Lines Indicate the Standard Error of the Mean.

When high but non toxic levels of NaCl were fed to infected chicks in G3, G4, and G5, no significant differences were noted in feed consumption and utilization compared to that of infected chicks fed the normal level (G2). When 1.5% NaCl was fed to chicks in G3 more feed was consumed (7.3 kg) and better efficiency of feed utilization (2.28) occurred compared to that of infected chicks fed 0.5% NaCl. Little more feed was consumed when chicks were fed 2.9% (G4) and 4.3% (G5) NaCl compared to those fed the normal level (G2) (Table III). Efficiency of feed utilization among these groups was almost the same.

When molasses was used (G6) as a source of energy replacing cornstarch in the basal diet, feed consumption increased. This group consumed the greatest amount of feed (8.1 kg) in the experiment (Fig. 3). When molasses was fed the efficiency of feed utilization was 2.50. This was lower than that recorded for chicks fed the basal diet (2.38), but the difference was not statistically significant (Table III). The poorest ($p < .05$) efficiency of feed utilization (2.79) occurred when chicks were fed polyethylene (G7); the amount of feed consumed by this group of chicks was 7.8 kg.

## Parasite Factors

Prepatent Period of Infection. The prepatent period is defined as the time from exposure of a host to a particular parasite until evidence of the infection can be detected. In this study, the infection was considered to have become patent when parasite eggs were recovered from a feces by a fecal flotation test. Flotation data for composite

fecal samples collected from individual lots of birds within groups are shown in Table V. The prepatent period for each group (made up of 3 lots) of chicks was then calculated as an average of 3 lots of that group. When the normal level (0.5%) of NaCl was fed, the prepatent period averaged 30 days; eggs were not recovered from one lot until the experiment was terminated. Although adult worms were recovered from this lot of chicks at necropsy, microscopic examination of the females revealed no eggs were present in their uteri. When higher (1.5, 2.9, and 4.3%) levels of NaCl were used the prepatent periods were 26.6, 24 and 27.3 days, respectively. When chicks were fed molasses rather than cornstarch as an energy source and the NaCl level at 0.5% the prepatent period was the shortest observed, i.e., 22.3 days (Fig. 4). One lot of chicks in this group showed a positive fecal flotation test when its fecal sample was examined for the presence of Heterakis eggs on day 18 after exposure, the first day of the fecal flotation test.

The non-exposed environmental control group was negative to the fecal flotation test throughout the experiment (Table V), and no adult worms were recovered from chicks in this group at necropsy (Table VI).

Number and Sex Ratio of Worms Recovered at Necropsy. The average numbers of worms recovered per chick in the various parasitized groups are shown in Table VII. No significant differences were noted in worm numbers among the different groups of chicks. Chicks in G3 (1.5% NaCl) harbored an average of 12.4 worms, and the total number of worms recovered was 297 (Table VII). This was the largest number of worms recovered from any group of chicks in the experiment; the range per bird was 0 to 48. On the other hand, the smallest number of worms (97)

TABLE V

PREPATENT PERIODS IN DAYS OF HETERAKIS GALLINARUM INFECTIONS IN CHICKS INOCULATED WITH 100 EGGS AND FED RATIONS WITH VARIOUS LEVELS OF NaCl, MOLASSES OR POLYETHYLENE

| Group | Lot[a] | | | Mean[b] Prepatent Period |
|---|---|---|---|---|
| | A | B | C | |
| Non-infected Control[c] | | | | |
| G1 (0.5% NaCl) | - | - | - | |
| Infected | | | | |
| G2 (0.5% NaCl) | 31 | —[d] | 27 | 30[A] |
| G3 (1.5% NaCl) | 26 | 26 | 28 | 26.6[AB] |
| G4 (2.9% NaCl) | 22 | 24 | 26 | 24.0[BC] |
| G5 (4.3% NaCl) | 27 | 27 | 28 | 27.3[AB] |
| G6 (29.5% Molasses; 0.5% NaCl) | 23 | 18 | 26 | 22.3[C] |
| G7 (13% Polyethylene; 0.5% NaCl) | 26 | 28 | 27 | 27.0[AB] |

[a] Comprised of 8 chicks each.

[b] Means with different superscript letters are significantly different ($P < 0.05$).

[c] This group was negative for the fecal flotation test.

[d] No evidence of infection was observed in this lot; a prepatent period of 32 days was assigned for statistical purposes.

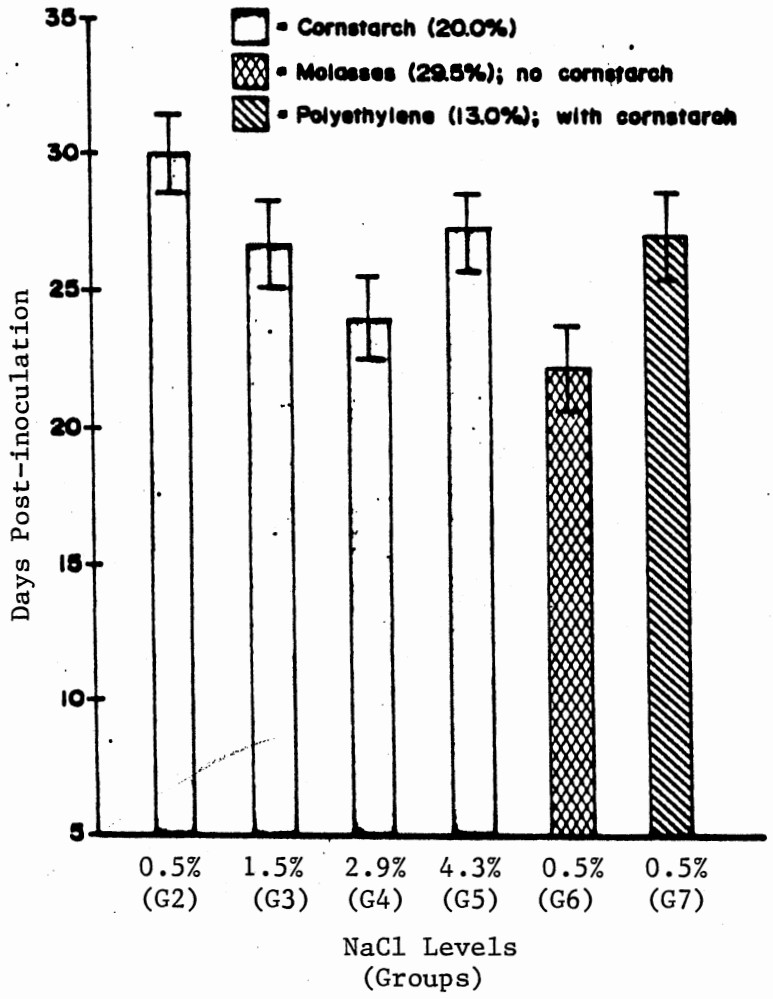


Figure 4. Prepatent Period in Chicks Inoculated with 100 Eggs of Heterakis gallinarum and Fed Diets Varying in NaCl Levels, Energy Source and Inert Bulky Material. Vertical Lines Indicate the Standard Error of the Mean.

TABLE VI

NUMBERS OF HETERAKIS GALLINARUM MALES AND FEMALES RECOVERED AT NECROPSY AND RANGES IN WORM COUNTS FROM LOTS OF 8 CHICKS

| Group | Lot | Number of Worms | | | | Sex ratio |
|---|---|---|---|---|---|---|
| | | Total | Range[a] | Males | Females | (M/F) |
| Non-infected | | | | | | |
| G1 (0.5% NaCl) | A | 0 | NA[b] | NA | NA | NA |
| | B | 0 | = | = | = | = |
| | C | 0 | = | = | = | = |
| Infected | | | | | | |
| G2 (0.5% NaCl) | A | 36 | 0 - 18 | 25 | 11 | 2.3:1 |
| | B | 17 | 0 - 11 | 5 | 12 | 0.4:1 |
| | C | 44 | 0 - 23 | 16 | 28 | 0.6:1 |
| G3 (1.5% NaCl) | A | 62 | 0 - 28 | 27 | 35 | 0.8:1 |
| | B | 160 | 0 - 48 | 72 | 88 | 0.8:1 |
| | C | 75 | 0 - 29 | 41 | 34 | 1.2:1 |
| G4 (2.9% NaCl) | A | 44 | 0 - 25 | 24 | 20 | 1.2:1 |
| | B | 61 | 0 - 22 | 25 | 36 | 0.7:1 |
| | C | 109 | 4 - 25 | 58 | 51 | 1.1:1 |
| G5 (4.3% NaCl) | A | 66 | 0 - 25 | 31 | 35 | 0.9:1 |
| | B | 86 | 0 - 40 | 48 | 38 | 1.3:1 |
| | C | 23 | 0 - 19 | 11 | 12 | 0.9:1 |
| G6 (29.5% Molasses; 0.5% NaCl) | A | 85 | 0 - 25 | 39 | 46 | 0.8:1 |
| | B | 31 | 0 - 7 | 12 | 19 | 0.6:1 |
| | C | 44 | 0 - 14 | 14 | 30 | 0.5:1 |
| G7 (13% Polyethylene; 0.5% NaCl) | A | 71 | 0 - 27 | 40 | 31 | 1.3:1 |
| | B | 120 | 0 - 34 | 63 | 57 | 1.1:1 |
| | C | 59 | 0 - 17 | 35 | 24 | 1.5:1 |

[a] Range refers to the numbers of helminths in individual birds within lots.

[b] NA = Not applicable.

TABLE VII

NUMBERS OF HETERAKIS GALLINARUM RECOVERED FROM THE VARIOUS GROUPS OF 24 CHICKS EACH AT NECROPSY

| Group | Total | Average[a] | Range[b] |
|---|---|---|---|
| Non-infected Control | | | |
| G1 (0.5% NaCl) | 0 | NA[c] | NA |
| Infected | | | |
| G2 (0.5% NaCl) | 97 | 4.0 | 0 - 23 |
| G3 (1.5% NaCl) | 297 | 12.4 | 0 - 48 |
| G4 (2.9% NaCl) | 214 | 8.9 | 0 - 25 |
| G5 (4.3% NaCl) | 174 | 7.3 | 0 - 40 |
| G6 (29.5% Molasses; 0.5% NaCl) | 160 | 6.7 | 0 - 25 |
| G7 (13% Polyethylene; 0.5% NaCl) | 250 | 10.4 | 0 - 34 |

[a] Exposed birds that showed no evidence of infection at necropsy were also included in calculating the average number of worms per bird.

[b] The range represents the number of worms in individual birds.

[c] NA = Not applicable

was recovered from chicks in G2 and the range for individual birds in this group was 0 to 23 (Table VII). The mean number of worms obtained per exposed chick (4.0) in this group was the smallest of any in the experiment. Of the 24 chicks exposed to the parasite in this group, only 11 were found carrying worms at necropsy, and the average among infected birds was 8.8 (Table VIII).

Of the 144 chicks exposed to H. gallinarum in the experiment, only 98 were found carrying worms at necropsy. A total of 1,193 worms were recovered and the range of worm burden was 0 to 48. The average number of worms recovered from 144 exposed chicks was 8.3, and that of chicks determined to be infected at necropsy was 12.2 (Table IX).

The numbers of male and female worms recovered from each lot of parasitized chicks are shown in Table VI. The highest male to female ratio was 2.3:1 observed in lot 2A; the lowest was 0.4:1, from lot 2B. The average male to female ratio of parasite from the various groups of chicks is shown in Table IV.

Length and Weight of Worms Recovered at Necropsy. The average length of male worms, but not females, were observed to be statistically different among the six parasitized groups of chicks fed various diets (Table IV). The shortest male recovered from any bird was 2.1 mm, and it was recovered from a chick in lot 5A. The longest male was 9.6 mm, and it was recovered from a chick in lot 6C (Table X). On the other hand, the average length of the shortest male was 6.4 mm, recovered from chicks fed the normal level of NaCl (G2). In this group, the range of the length of male worms was from 4.2 to 8.2 mm. The longest worms recovered, measuring an average of 7.0 mm, were from chicks fed

TABLE VIII

SUMMARY OF TOTAL WEIGHT OF *HETERAKIS GALLINARUM* SPECIMENS RECOVERED FROM THE VARIOUS GROUPS OF CHICKS AT NECROPSY

| Group | No. of Infected Birds | Total No. of Worms | Mean No./Bird | Total Weight (mg) | Mean Weight per Worm (mg) |
|---|---|---|---|---|---|
| Non-infected Control | | | | | |
| G1 (0.5% NaCl) | 0 | 0 | - | - | - |
| Infected | | | | | |
| G2 (0.5% NaCl) | 11 | 97 | 8.8 | 41.0 | 0.4 |
| G3 (1.5% NaCl) | 18 | 297 | 16.5 | 139.0 | 0.5 |
| G4 (2.9% NaCl) | 17 | 214 | 12.6 | 101.0 | 0.5 |
| G5 (4.3% NaCl) | 15 | 175 | 11.7 | 88.0 | 0.5 |
| G6 (29.5% Molasses; 0.5% NaCl) | 19 | 160 | 8.4 | 82.0 | 0.5 |
| G7 (13% Polyethylene; 0.5% NaCl) | 18 | 250 | 13.9 | 122.0 | 0.5 |

TABLE IX

NUMBER OF CHICKS THAT WERE CARRYING WORMS AT NECROPSY AND THE MEAN NUMBER OF WORMS PER BIRD EXPOSED TO 100 EMBRYONATED *HETERAKIS GALLINARUM* EGGS AND FED VARIOUS DIETS

| Group | Lot | No. Birds Exposed | No. Birds Infected | No. Birds Uninfected | Number of Worms Recovered | | |
|---|---|---|---|---|---|---|---|
| | | | | | Total | Mean/ Infected Bird | Mean/Bird |
| Non-infected | | | | | | | |
| G1 (0.5% NaCl) | A | 0 | 0 | 8 | 0 | 0 | 0 |
| | B | 0 | 0 | 8 | 0 | 0 | 0 |
| | C | 0 | 0 | 8 | 0 | 0 | 0 |
| Infected | | | | | | | |
| G2 (0.5% NaCl) | A | 8 | 4 | 4 | 36 | 9.0 | 4.5 |
| | B | 8 | 3 | 5 | 17 | 5.7 | 2.1 |
| | C | 8 | 4 | 4 | 44 | 11.0 | 5.5 |
| G3 (1.5% NaCl) | A | 8 | 5 | 3 | 62 | 12.4 | 7.8 |
| | B | 8 | 7 | 1 | 160 | 22.9 | 20.0 |
| | C | 8 | 6 | 2 | 75 | 12.5 | 9.4 |
| G4 (2.9% NaCl) | A | 8 | 4 | 4 | 44 | 11.0 | 5.5 |
| | B | 8 | 5 | 3 | 61 | 12.2 | 7.6 |
| | C | 8 | 8 | 0 | 109 | 13.6 | 13.6 |

TABLE IX (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| G5 (4.3% NaCl) | A | 8 | 6 | 2 | 66 | 11.0 | 8.3 |
| | B[a] | 8 | 6 | 2 | 86 | 14.3 | 10.8 |
| | C | 8 | 3 | 5 | 23 | 7.7 | 2.9 |
| G6 (29.5% Molasses; 0.5% NaCl) | A | 8 | 7 | 1 | 85 | 12.1 | 10.6 |
| | B | 8 | 6 | 2 | 31 | 5.2 | 3.9 |
| | C | 8 | 6 | 2 | 44 | 7.3 | 5.5 |
| G7 (13% Polyethylene; | A | 8 | 5 | 3 | 71 | 14.2 | 8.9 |
| | B | 8 | 7 | 1 | 120 | 17.1 | 15.0 |
| | C | 8 | 6 | 2 | 59 | 9.8 | 7.4 |
| Total | 21 | 144 | 98 | 70 | 1,193 | 12.2[b] | 8.3[c] |

[a] One bird in lot 5B died at day 13 after exposure; it was dissected and immature worms were recovered and counted.

[b] Average number of worms per individual chick experimentally exposed to the parasite excluding individuals negative at necropsy.

[c] Average number of worms per individual chick experimentally exposed to the parasite including the individuals negative at necropsy.

TABLE X

THE MEAN LENGTH OF HETERAKIS GALLINARUM MALES AND FEMALES AND THE RANGE OF LENGTH MEASUREMENTS FOR THE VARIOUS LOTS OF 8 CHICKS

| Group | Lot | No. Worms Recovered | Male Worms | | | Female Worms | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total Number | Range of Lengths (mm) | Average Length (mm) | Total Number | Range of Lengths (mm) | Average Length (mm) |
| Non-infected Control | | | | | | | | |
| G1 (0.5% NaCl) | A | 0 | - | - | - | - | - | - |
| | B | 0 | - | - | - | - | - | - |
| | C | 0 | - | - | - | - | - | - |
| Infected | | | | | | | | |
| G2 (0.5% NaCl) | A | 36 | 25 | 4.2-8.0 | 6.5 | 11 | 4.8-9.4 | 7.6 |
| | B | 17 | 5 | 5.4-7.4 | 6.2 | 12 | 4.1-8.6 | 6.9 |
| | C | 44 | 16 | 4.2-8.2 | 6.6 | 28 | 5.1-9.9 | 8.2 |
| G3 (1.5% NaCl) | A | 62 | 27 | 3.7-7.7 | 6.5 | 35 | 5.2-9.1 | 7.5 |
| | B | 160 | 72 | 3.4-7.9 | 6.9 | 88 | 5.4-10.4 | 8.1 |
| | C | 75 | 41 | 3.6-8.3 | 6.2 | 34 | 3.9-10.0 | 6.9 |
| G4 (2.9% NaCl) | A | 44 | 24 | 2.6-8.9 | 6.2 | 20 | 4.1-9.5 | 7.6 |
| | B | 61 | 25 | 4.3-8.3 | 6.9 | 36 | 4.7-10.9 | 8.7 |
| | C | 109 | 58 | 5.1-8.4 | 6.9 | 51 | 6.0-10.9 | 8.8 |

TABLE X (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G5 (4.3% NaCl) | A | 66 | 31 | 2.1-8.4 | 7.1 | 35 | 4.4-9.7 | 8.5 |
| | B | 86 | 48 | 2.5-8.9 | 7.0 | 38 | 2.2-11.4 | 8.2 |
| | C | 23 | 11 | 5.1-7.7 | 6.9 | 12 | 5.9-10.5 | 8.9 |
| G6 (29.5% Molasses; 0.5% NaCl) | A | 85 | 39 | 4.1-8.6 | 6.6 | 46 | 4.4-11.1 | 8.3 |
| | B | 31 | 12 | 5.9-8.0 | 6.9 | 19 | 5.5-9.2 | 7.2 |
| | C | 44 | 14 | 4.2-9.6 | 7.4 | 30 | 2.7-11.5 | 8.6 |
| G7 (13% Polyethylene; 0.5% NaCl) | A | 71 | 40 | 4.5-8.3 | 7.1 | 31 | 5.7-10.0 | 8.6 |
| | B | 120 | 63 | 3.7-8.9 | 7.0 | 57 | 4.1-10.5 | 8.6 |
| | C | 59 | 35 | 4.9-8.7 | 7.1 | 24 | 3.8-10.2 | 8.2 |

polyethylene (G7). In this group, the range of length of male worms was from 3.7 to 8.9 mm (Table XI). The difference in average length between the worms of G2 and G7 was significant ($p < .05$); no other statistically significant length differences were observed. In no case was the length of female worms affected to a statistically significant extent by dietary variables of the host (Table IV).

The total weights of worms recovered from birds in the different groups of parasitized chicks are shown in Table VIII. The highest weight was 139.0 mg. This was the weight of 297 worms recovered from 18 birds, out of 24 birds exposed, in group 3; the average weight of a single worm in this group was 0.5 mg. On the other hand, of the aggregate of helminths recovered from a group, the lowest weight was 41.0 mg; this was the weight of 97 worms recovered from 11 infected birds in G2 and the average weight of a single worm in this group was 0.4 mg. The average weights of worms per infected bird in the various parasitized groups of chicks is shown in Table IV.

TABLE XI

THE AVERAGE LENGTH OF THE HETERAKIS MALES AND FEMALES AND THE RANGE OF THESE LENGTHS FOR THE VARIOUS GROUPS OF CHICKS

| Group | No. Birds Exposed | Total No. Worms Recovered | Male Worms | | | Female Worms | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total Number | Range[a] of Lengths (mm) | Average Length (mm) | Total Number | Range[a] of Lengths (mm) | Average Length (mm) |
| Non-infected Control | | | | | | | | |
| G1 (0.5% NaCl) | 0 | 0 | - | - | - | - | - | - |
| Infected | | | | | | | | |
| G2 (0.5% NaCl) | 24 | 97 | 46 | 4.2-8.2 | 6.4 | 51 | 4.1-9.9 | 7.5 |
| G3 (1.5% NaCl) | 24 | 297 | 140 | 4.3-8.3 | 6.5 | 157 | 3.9-10.4 | 7.5 |
| G4 (2.9% NaCl) | 24 | 214 | 107 | 2.6-8.9 | 6.6 | 107 | 4.1-10.9 | 8.3 |
| G5 (4.3% NaCl) | 24 | 175[b] | 86 | 2.1-8.9 | 6.9 | 81 | 2.2-11.4 | 8.5 |
| G6 (29.5% Molasses; 0.5% NaCl) | 24 | 160 | 65 | 4.1-9.6 | 6.9 | 95 | 2.7-11.5 | 8.0 |
| G7 (13% Polyethylene; 0.5% NaCl) | 24 | 250 | 138 | 3.7-8.9 | 7.0 | 112 | 3.8-10.5 | 8.4 |

[a] The range of males' length between groups is from 2.1 to 9.6 mm. Females' range is from 2.2 to 11.5 mm.

[b] In G5, the total number of male and female worms (86 + 81) does not equal 175. Total includes eight additional worms not classified as male or female; they were immature when recovered because their host died at day 13 after exposure.

# CHAPTER V

# DISCUSSION

## Pre-exposure Period

Results indicate that growth responses by chicks to the different diets were almost identical among the various groups during the first two weeks (Fig. 1 and 2). It appears that all diets supported normal growth, comparing favorably with the basal one. This is not surprising, perhaps, given that all diets were formulated to be complete and balanced for all nutrients required by growing chicks. Differences in weight gain and feed utilization were minimal and were not statistically significant during the first two weeks, the period before chicks were exposed to Heterakis gallinarum.

## Post-exposure Period

### Exposed vs Non-exposed

From the results obtained in this study on Heterakis gallinarum it can be generally stated that when the chicken host receives a single dose of 100 ± eggs there is a significant depression in body weight gains of the host. This depression may indicate a deterimental effect of Heterakis infection on the growth of young chicks, probably reflecting the response of chicks to the presence of parasites,

including the mechanical action of the larval stages on the caecal mucosa. This interpretation agrees with the observation of Rudek (1970) who found that the periodic body weight gains of Heterakis-parasitized chickens in the first 20 days after infection were significantly lower than those of control birds. He concluded that the parasite in its tissue phase, up to 12 days after infection, does exert an inhibiting effect on the growth rate of chickens. Stoimenov (1972) studied the effect of Heterakis infection on weight gain in chickens of different ages (15th, 30th, 45th, and 60th day of life). Birds proved most likely to experience adverse effects when exposed on the 15th or 30th day of life; weight gains for such birds were 327 and 278 g, respectively, lower than that of the unexposed controls. Ikeme (1971b) concluded that the effect of repeated infection of Ascaridia galli upon the growth of chicks is similar to that of a single infection except that growth of the birds is even more depressed when birds are subjected to repeated exposure. This worker concluded that the growth inhibition was a result of long-lasting diarrhea and the pathogenic effect of the larvae inhibited by immune response resulting from earlier exposures. In contrast, Clapham (1934a) reported very little difference in final weight of young chicks, 16 days old, fed 300 H. gallinarum eggs compared with that of unexposed controls. A similar view was expressed by Cuca et al. (1968), Robert and Edgar (1971), and Dubinsky et al. (1973); none of these workers, all of whom studied experimentally the effect of A. galli infection on the growth of chicks, could detect any harmful effect of the parasite on body weight gain.

From the above findings it is clear that there are differing views on the effect of helminth parasites on the weight gain of chicks. There are likely multiple reasons for the disparity of opinion. For example, not only were various dose rates of infective eggs used by the several authors, but also in some cases multiple exposures of the birds were made; age of birds and duration of the experiments also varied among the studies. All these factors can work, either singly or in combination, to affect host response to the presence of the parasite and may account for the differing opinions.

Host Factors

The results of the present study indicated that body weight gain of infected chicks was improved to equal that of the uninfected controls by feeding them higher than normal levels of NaCl (0.5%). Higher levels of NaCl (1.5, 2.9, and 4.3%) did not retard worm development but appeared to serve, somewhat, to protect chicks against effects of heterakid parasitism. These levels of dietary NaCl are higher than optimal nutritionally but appeared to be non-toxic to chicks; the chicks grew normally and there was almost no mortality. Infected chicks fed a diet containing 1.5% NaCl were heavier than those fed diets containing 2.9 or 4.3% NaCl; this may indicate that 1.5% NaCl is the optimal level for parasitized birds. Barlow et al. (1948) reported that levels of 5% or more of added salt may depress growth rate and increase mortality in young chicks. They reported linear increase in water consumption when the percent of NaCl added to the diet was increased from 0-10%. Barlow et al. also demonstrated that the chicken has an amazing ability

to maintain isotonicity of its body fluids and to maintain a constant chloride concentration in the blood plasma in spite of great differences in salt consumption.

Many endoparasitic nematodes of animals are known to survive for long periods in various concentrations of sodium chloride *in vitro*. Sodium chloride is an essential constituent of the chemical micro-environment of endoparasites, and adjustment to changing sodium chloride concentration in the medium forms a major part of their osmoregulatory mechanism (Fenwick 1939; Stoll 1940; and von Brand and Simpson 1942).

The significant increase in body weight gain by infected chicks that were fed molasses as energy source in place of cornstarch of the basal diet may demonstrate the importance of energy source of the diet for the growth of young chicks suffering from heterakid infection. The importance of molasses as an energy source in chicken nutrition was also demonstrated by previous investigators. Konda and Ross (1962), Cuervo et al. (1972), and Keshavarz et al. (1980) used molasses at rates of 15, 20, and 30% in chick diets. They concluded that molasses was effective as an energy source with no adverse effect on performance of growing chicks. In the present study chicks fed molasses consumed significantly larger amounts of feed and showed lower, though not significantly so, efficiency of feed utilization (2.50 vs 2.38) than those fed the basal diet, Table III. Therefore, the increase in body weight gain of these chicks was probably due to the increased consumption of feed which may have resulted from its palatability.

It is apparent from the results of this study that inclusion of 13% polyethylene, a diluent or filler material, in the diet did not

influence body weight gain of infected chicks compared to those fed the basal diet (Table III). It appears that, within limits, birds can meet their requirements for the various nutrients when they are fed low nutrient density diets; they apparently do so by adjusting their level of feed intake. Cannon et al. (1982) reported an increase in the amount of feed consumed by chicks, to a certain limit, as the level of dietary filler (polyethylene, cellulose, and hemicellulose) is increased. Sellers et al. (1979) studied the effects of various dietary fillers and clay on broiler performance. Five compounds (kaolin, sodium bentonite, attapulgite clay, rice hulls, and sand) were added to the basal diet at levels of 2.5 and 5.0% by these workers. They reported no significant differences in body weight and feed efficiency between treatments. It appears that for economic reasons, reducing the nutrient density of diets to a certain level by adding diluent materials is quite a common technique used in commercial feeding of chickens. In the present study, dietary polyethylene apparently had no effect on the body weight gain of infected chicks. On the other hand, a comparison of these birds with the non-exposed controls showed that body weight gain of infected chicks fed polyethylene was significantly lower (351.0 g vs 400.1 g), Table III. This difference in body weight gain may reflect the effect of heterakid parasitism in combination with dietary diluent, but not dietary polyethylene alone, on the growth of young chicks.

In this study, the quantities of feed consumed by various groups of chicks were calculated on dry matter basis. No significant differences were noticed in these quantities when various levels of

NaCl were fed. Although a little more feed was consumed by chicks when higher than normal levels of NaCl were fed, feed utilization was not affected. Barlow et al. (1948) reported that when graded levels (0-10%) of salt were added to the diet, feed consumption was not affected. Koreleski et al. (1976) stated that the ability of chickens to utilize starch was reduced when a restricted level of NaCl (0.25%) was fed. He found a higher level of intact starch in the intestinal contents of chickens fed the restricted level than in that of controls. From these observations it appears that the use of not less than the optimal level (0.5%) of NaCl is important for energy utilization from the diet. So long as the levels are not toxic, excessive amounts of NaCl like those used in the present study (1.5, 2.9, and 4.3%) in the diets are unlikely to influence energy utilization; in turn, feed efficiency may not be appreciably influenced.

Large quantities of feed were consumed when chicks were fed molasses or polyethylene. Feed efficiency was not impaired significantly by inclusion of molasses in the diet, even though a significantly larger quantity of feed was consumed by chicks in this group compared to those fed the basal diet. Infected chicks fed molasses were able to grow and to develop better gains than any others in the study, including the non-parasitized controls. Therefore, the large quantity of feed consumed by chicks in this case probably helped to offset effects of heterakid parasitism. The effect of molasses on efficiency of feed utilization may have been influenced by a variety of factors. For example, molasses increases the rate of passage of feed through the digestive tract due to its laxative effect on the

bird; frequent voiding of intestinal aliment may reduce efficiency of feed utilization. A lower level of energy was provided to birds per unit of feed in the diet containing molasses (Table II), than in those containing cornstarch; birds with this low energy level consumed more feed, apparently in an attempt to meet their energy requirements. Water in cane molasses also impairs efficiency of feed utilization by reducing the caloric value of the diet (Cuervo et al., 1972). In the present study, feed consumption was calculated on dry matter basis; therefore, factors other than water content of molasses may have influenced utilization of this diet. Feed utilization was significantly impaired by the addition of polyethylene, a diluent agent, to the diet. The large quantity of feed consumed and the impaired feed utilization by chicks fed polyethylene may reflect the effect of the low nutrient density and energy level resulting from inclusion of polyethylene in the diet. Investigators of poultry nutrition have observed an improvement in feed utilization by chicks with increasing caloric density of the diet (Malone et al., 1980 and Brake and McDaniel, 1981).

Parasite Factors

Egg production by Heterakis females, from various lots of chicks, was found (with one exception) to begin as early as 18 and as late as 31 days after infection; the single exception was one of three lots (G2) that showed no evidence of patency before the termination of the experiment 31 days after exposure. These results suggest that under some circumstances egg production by Heterakis gallinarum will not begin before 31 days after infection, but what factors may have

influenced this are not known. It is difficult to explain why one lot of birds among 18 exposed would show patency at 18 days and another lot (in a different group) be delayed for so long. Olsen (1974) found that Heterakis worms are fully grown in 14 days but females often do not begin ovipositing until 24 to 36 days after infection.

It appears from the present study that maturation rates of H. gallinarum may have been influenced by dietary factors such as the use of higher than normal levels of NaCl or the use of molasses as energy source, in place of cornstarch in the diet. A statistically significant increase in maturation rate (reduction in the prepatent period) of this parasite was observed in some groups of birds that were fed higher than normal levels of NaCl. This reduction in the prepatent period was evident for all lots in the groups. It appeared that the prepatent period of Heterakis gallinarum decreased as the level of NaCl in the diets of the various groups of chicks was increased from normal (0.5%) until 2.9% NaCl was fed (Table V). On the other hand, the longest prepatent period, as an average of 3 lots comprising a group, observed in this study occurred when the hosts were fed the normal level (0.5%) of NaCl (G2). The results may be skewed, however, because the infection in one lot of chicks in this group did not become patent, and the female worms recovered from birds in this lot at necropsy were not gravid. Upon consultation with a statistician a prepatent period of 32 days was assigned for this lot. It is difficult to explain why female worms from this lot were not gravid even at the end of the experiment. It should be noted, however, that helminths in the other two lots in this group behaved about the same; infections

in one of them became patent at day 31 after exposure, the last day in the experiment; and the third lot on day 27. Based upon these results, one may suggest that NaCl in the feed of the host influences the rate of maturation of H. gallinarum. Other investigators have reported that mineral content of host diet influences helminth parasite egg production. For example when lambs infected with Haemonchus contortus were fed cobalt, in trace-mineralized salt or "cobaltized" salt, the worms matured more rapidly and their egg production was greatly increased (Richard et al., 1954, Threlkeld et al. 1956, and Shumard et al., 1956). Gaafar and Ackert (1953) found that phosphorus content in a fowl ration influenced the viability and growth of Ascaridia galli; more and longer worms were collected from chickens fed a high phosphorus ration than from the control fowls. From the above observations, it appears that certain helminth parasites respond to the mineral content of their host's diets. To reiterate, based upon the present study, it appears that the mineral NaCl may influence maturation of H. gallinarum. Furthermore, the rapid maturation of worms and consequent reduction in the prepatent period of the parasite in birds fed higher than normal levels of NaCl may reflect a heterakid requirement for this mineral which is well above that of the host. These findings are of particular interest because no published data are available on the effects of host dietary NaCl level on development of this parasitic helminth.

The greater reduction in the helminth's maturation time was observed when chicks were fed molasses rather than cornstarch as an energy source though the sodium chloride level was 0.5% as in the basal

diet. Chemical analysis of molasses has shown the total sugar content to be 60.5% (Cuervo et al., 1972), composed almost entirely of sucrose, glucose and fructose which were present at levels of 34.05%, 14.05% and 12.10% of the molasses, respectively. In addition to the sugar, however, significant mineral content was found. Potassium was present at a level of 3.45%; and magnesium and sodium comprised 0.45 and 0.41% of the molasses, respectively (Cuervo et al., 1972). It may be that inclusion of molasses in the host's diet, providing some minerals and simple carbohydrate to both host and parasite, influences rate of maturation and egg production of parasites. For example, among the three lots comprising group 6 in which chicks were fed molasses, the prepatent period was an average of 22.3 days. Again, the results may be skewed, however, because infection in one lot (6B) in this group became patent no later than day 18 after exposure. Heterakis eggs were recovered from feces from this lot on the first day of the fecal flotation test. It is difficult to explain why the prepatent period was so short in this group. Suggestions include (1) the indirect increase in sodium level of the diet (as a result of added molasses) may have affected the prepatent period; (2) the presence of high levels of potassium in molasses may have had an effect on maturation of Heterakis; (3) molasses contains simple sugars that may be more readily available to the parasite than in cornstarch. Whether potassium level or ready availability of simple sugars would, in fact, influence prepatent period is beyond the scope of this study. The total amount of energy provided to chicks that were fed molasses was actually lower than that provided to those fed cornstarch; therefore, the source of

energy may be a more important influence on maturation rate of Heterakis than is the amount of energy (Table II).

It might be important to mention in connection with the short prepatent period for lot 6B that accidental exposure of chicks to H. gallinarum may have occurred. It is highly unlikely but remotely possible that birds in lot 6B ingested contaminated feed or water before the experimental exposure. Infections in the other two lots of chicks in this group that were fed molasses became patent 23 and 26 days, respectively after exposure. If naturally infected birds (or other sources of infective material) are available in the vacinity, infective eggs from the environment could be transferred to experimental birds in cages by houseflies or other insects that can carry these eggs on their feet and thus contaminate chicks' feed or water. It is pertinent to state that the environmental controls remained free of parasites and no birds in exposed lots had more parasites than would be expected given the infective dose used.

Further investigation is required to determine which factor(s) is (are) responsible for the apparent early maturation of Heterakis gallinarum when molasses is used as an energy source in the host's diet. It is highly probable that the prepatent periods observed were accurately recorded; nevertheless, one cannot positively rule out the possibility of experimental error.

It is interesting to look concurrently at the body weight gain of chicks and the prepatent period of their parasites. Highest gains occurred when the prepatent period was the shortest. This could mean that the gains made by birds suffering from heterakid infections are

closely related to the activity of the immature stages of the parasite. That is, as suggested by Rudek (1970), the tissue phase of the parasite may have been extended with resultant inhibition of host growth.

In the present study, the use of polyethylene as a bulky or diluent material in the host's diet resulted in a signficant increase in length of male worms. The use of polyethylene as undigestible dietary material may reduce the digestibility or the availability of certain nutrients in the diet to the host. Dvorak and Bray (1978) stated that nondigestible material in feed reduced utilization of the basal portion of the diet. It is possible, therefore, that adding polyethylene to the diet caused the host some nutritional stress which was reflected in better development of parasites, specifically by increase in length of male worms. After review of numerous studies on the effect of the host's diet on the well-being of parasites, Noble and Noble (1982) concluded that a dietary deficiency will render a host more vulnerable to parasitic infection. In referring to intestinal parasites, they concluded that the intestinal environment becomes more favorable to the parasite during periods of malnutrition of the host. Gerald (1954), reported that chicks on a purified semi-synthetic diet, deficient in both pteroylglutamic acid (PGA) and vitamin $B_{12}$ harbored more and larger Ascaridia galli than birds fed a complete diet. His results provided additional data to substantiate a generalized concept that a deficiency of any vitamin would lower the resistance of chicks against infection of A. galli. Platzer and Roberts (1970) conducted studies with the rat tapeworm, Hymenolepis diminuta; they recovered larger worms from rats fed a riboflavin-deficient diet than from the controls.

An alteration in intestinal physiology favorable to H. diminuta may have occurred, e.g., glucose may have been more available to the worm since glucose absorption is impaired in rats on riboflavin-deficient diets (Althausen et al., 1946). In the present study, the effect of polyethylene on Heterakis was probably indirect and the signficant increase in the length of male worms in birds fed this material was likely attributable to the effect of polyethylene on the availability of some essential nutrient(s) to the host.

The number of worms recovered at necropsy did not differ significantly among groups of chicks fed various diets. The fewest worms were recovered when chicks were fed the basal diet that contained the normal level of NaCl (0.5%), and about one-half of the birds in this group were carrying no worms at necropsy. Therefore, host nutrition as one of the important environmental factors may have influenced host-parasite relationship in this respect. When a host is exposed to a stress factor such as being fed either higher or lower levels of certain nutrients than are normally required, it may affect the host's ability to withstand a particular parasitic infection. In the present study, it seems likely that the high levels of NaCl fed to some groups of chicks and the high level of molasses or polyethylene fed to others, may have exerted stress on chicks. As a result of this dietary stress chicks may have been slightly more susceptible to Heterakis infection. Evidence for such a conclusion is suggested by increase in number of worms that developed compared to others exposed to the same dose of Heterakis eggs; although this increase was not statistically significant, it may be biologically important.

In regard to the number of worms developing and ability of chicks to achieve proper weight gains, Todd and Hansen (1951) studied the percentage development and size of Ascaridia galli in chickens. They concluded that chicks with the fewest worms at post-mortem possessed the greatest natural resistance; positive action directed against the parasite by these chicks reduced their ability to achieve proper weight gains. These authors used their data to support a thesis that energy employed by animals resisting infection, measured by decreased percentage development and size of the parasite, prevents maximally efficient weight gains. In contrast, Reid and James (1958) concluded that the more worms present, the more weight gain of the bird is depressed. Thus they did not substantiate in their study the findings of Todd and Hansen in regard to weight gain. In the present study, chicks in G2 in which fewest worms were recovered at necropsy made the second lowest weight gains, just better than those fed polyethylene. Other groups with larger numbers of worms consistently had better weight gains (Tables III and IV). These findings are in agreement with the observations of Todd and Hansen in regard to weight gain of parasitized birds. It appears that host ability to gain weight properly may be influenced by a variety of factors, including the number of worms establishing. In the present study it is not possible to know, of course, the absolute number of helminths that may have become established. We can draw conclusions only on the basis of the numbers recovered at necropsy because birds were not housed individually and no attempt was made to determine whether helminths were shed spontaneously by chicks. However, no helminths were ever observed in

feces collected for determination of prepatent period.

Neither weight (biomass) of parasites nor sex ratio of Heterakis gallinarum appeared to be affected by the various diets in this study (Table IV). On the growth and development of the rat tapeworm Hymenolepis diminuta, Komuniecki and Roberts (1975) reported that worms from rats fed a starch diet with a 6% roughage component were heavier and had more proglottids than those from rats fed the starch or combination of sugars and starch diets. These authors described the effect of roughage as a secondary one, exerted by altering one or more of the digestive parameters of the rat (gastric emptying, intestinal mixing, or transit time, etc.). Alteration of these parameters might make more glucose available to the worm, which in turn could facilitate worm growth. In the present study the exact effect of such alterations of the digestive parameters of the chick, in response to dietary materials, on growth and development of Heterakis is not known; experiments to make such determinations were beyond the scope of this research.

# CHAPTER VI

## SUMMARY

Two-week-old chicks maintained on one of several different dietary regimens were exposed to a single dose of 100 embryonated Heterakis gallinarum eggs to determine the effects of sodium chloride level, molasses, and polyethylene in the host's diet on the growth of parasites and on the performance of the host. The prepatent period of parasite and the length of adult worms as well as weight gain and feed utilization of the host were significantly affected by certain dietary factors. Rapid maturation of worms and consequent reduction in the prepatent period was observed when 1.5% or 2.9% sodium chloride was used in chick diets, rather than the standard 0.5% employed in the basal diet. Even greater reduction in the prepatent period was observed when chicks were fed molasses rather than cornstarch as an energy source though the sodium chloride level was 0.5% as in the basal diet. Furthermore, an increase in the length of male worms, but not females, was noticed when polyethylene was added to the basal diet as undigestible dietary diluent. This increase in length was statistically significant when compared to males collected from chicks fed the basal diet.

Chicks not exposed to helmiths and receiving a normal sodium chloride level (0.5%) in the basal diet gained more weight and utilized

feed more efficiently than the infected group of chicks receiving the same diet. The latter group, in turn, gained less than other groups that were infected but fed a diet that differed only in having higher levels of sodium chloride. Feed utilization was not significantly different. On the other hand, chicks fed a diet containing molasses and 0.5% sodium chloride gained more weight than other groups, including the un-infected control group; the infected group fed polyethylene gained the least weight of any group in the experiment. The poorest feed utilization also occurred in the group fed polyethylene.

It appears from this study that sodium chloride level and the energy source in the host's diet may influence the growth and reproduction of H. gallinarum. On the other hand, weight gain and feed utilization by infected chicks were improved when higher than usual levels of sodium chloride (e.g., 1.5% vs 0.5%) or molasses was used in their diets. Therefore, sodium chloride level and energy source in the host's diet may influence helminth growth and maturation and affect the chick's performance when Heterakis gallinarum is present.

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