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Growth and Immunocomptence in Parasitized Domestic Turkeys(Meleagris gallapavo): Is There a Trade-off?

Virginia Austin Harrison

Growth and immunocompetence in parasitized domestic turkeys (*Meleagris gallopavo*): is there a trade-off?

by
Virginia Austin Harrison

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Approved by

Advisor: Associate Professor Richard Buchholz

Reader: Assistant Professor Mika Jekabsons

Reader: Associate Professor Tristan Denley

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ABSTRACT

Parasitic infection can prove to be detrimental to the condition, reproductive fitness, and survival of the host organism. During infection, an organism experiences trade-offs between functions such as growth, reproduction, and immunological activity as a result of limited resources. When challenged by infection, wild turkeys, adapted for survival, should fight the infection rather than invest in growth. Because domestic turkeys have been artificially selected to grow rapidly, I hypothesized that they would invest in growth rather than immunocompetence. To test this hypothesis, I infected domestic turkeys with coccidia and measured components of growth and immunocompetence. Turkeys were infected with the protozoan parasite *Eimeria*. Blood samples were collected prior to inoculation and both one and two weeks following inoculation. The weight and tarsus length of each turkey were also measured at the time of blood collection. Levels of plasma immunoglobulins were measured using agarose gel electrophoresis and digital densitometry. Domestic turkeys infected with coccidia experienced a trade-off between growth and immunoglobulin production, although not all individuals invested in growth. Studies such as this provide insight into how natural selection has molded the trade-off between growth and immunocompetence during infection.

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INTRODUCTION

A parasite extracts resources from its host for its own growth, maintenance, and reproduction and thereby causes damaging effects in the host organism. The extent and severity of these effects is not fully understood, but numerous studies have recently demonstrated consistent detrimental effects of parasitism on the condition, reproductive fitness, and survival of the host organism. De Lope *et al.* (1998) provides a summary of host anti-parasite defenses, which consist of a diverse range of behavioral, physiological, and immunological mechanisms. Behavioral defenses include mechanisms such as avoidance of sites harboring parasites, while physiological defenses include mechanisms such as elevated body temperatures that are unfavorable to invading parasites. Although these mechanisms significantly contribute to an organism's defenses against parasitism, it is an organism's immune system that serves as the principle weapon in directly deterring and eliminating parasitic infection (de Lope *et al.* 1998). An organism's ability to mount an effective immune response to an invading parasite is known as immunocompetence (Norris and Evans 2000). Immunocompetence can be interpreted at multiple biological levels: physiological, organismal, and evolutionary. At the physiological level, immunocompetence describes an organism's ability to detect and respond to an antigen. At the organismal level, immunocompetence describes an organism's ability to eliminate infection.

The immune system functions as an organism's primary anti-parasite defense mechanism, but investment in an effective immune response proves to be very costly to

the host organism, requiring resources such as energy and nutrients (Soler *et al.* 2003). Because immunological defense is costly, an uninfected individual circulates low levels of immune agents such as white blood cells and immunoglobulins (de Lope *et al.* 1998). In the wild, natural selection has molded the adaptation for survival; therefore, during an infection, organisms invest in immunological defense despite its costliness. Under natural conditions with limited access to energy and essential nutrients, the immune system competes with other functions such as growth, sexual signaling and ornamentation, and reproduction for resources (Tschirren and Richner 2006). Competition for limited resources during infection is predicted to result in a trade-off between immunological activity and other bodily functions (Tschirren and Richner 2006). Tschirren and Richner (2006) discovered evidence of a reduction in body mass during experimental enhancement of the immune system of nestling great tits. In a study by de Lope *et al. (1998)*, manipulation of the intensity of parasitism in house martins resulted in reduced nestling body condition and fledgling success. Verhulst *et al.* (1999) discovered a trade-off between growth and the expression of secondary sexual ornaments in domestic chickens. Functional trade-offs such as these illustrate the evolutionary level of immunocompetence in which immunocompetence describes an organism's ability to eliminate infection without excessive trade-offs.

I hypothesized that domestic turkeys (*Meleagris gallopavo*) infected with coccidia (*Eimeria gallopavonis*) would invest in growth rather than immunocompetence. I also hypothesized that as the severity of coccidia infection increased, the trade-off between growth and immunocompetence would become stronger.

Background Information

Functional Trade-offs

The extent of a functional trade-off may greatly influence not only an organism's survival, but also its growth and reproductive fitness. An organism that can effectively fight infection without depriving the body of resources necessary for allocation to other functions, and thus reduce the extent of a functional trade-off, is considered to have greater immunocompetence (Saino *et al.* 1997). Therefore, greater immunocompetence increases an organism's survival, growth potential, and reproductive fitness. Females often select their mates on the basis of secondary sexual characters, such as ornamentation size or intensity of sexual displays (Verhulst *et al.* 1999). If ornamentation is costly to produce, greater ornamentation indicates health. A highly ornamented male has access to the resources necessary for successful reproduction. Resource availability can result from (1) an absence of parasitism or (2) strong immunocompetence. A preference to mate with the most highly ornamented male, which is also the healthiest male, represents a female's attempt to increase the viability of her offspring (Verhulst *et al.* 1999, Saino *et al.* 1997). The selection of "good genes" by females provides evidence of how natural selection favors the evolution of physiological mechanisms that ensure optimal allocation of resources to competing activities (Soler *et al.* 2003). The immunological mechanisms that birds, such as turkeys, use to fight infection are located in the avian immune system.

Avian Immune System

Butcher and Miles (1991) provide a detailed description of the avian immune system, which is very complex and consists of both innate and acquired immune

mechanisms. Innate, or non-specific, immune mechanisms serve as the first line of defense against invading pathogens. These mechanisms include the physical barriers of skin and feathers, ciliated, mucous membranes, white blood cells, and a number of phagocytic cell types. Specific or acquired immune components include lymphatic vessels, lymphoid tissues, and lymphocytes. Primary lymphoid organs include the thymus and the bursa of Fabricius, and secondary lymphoid tissues include the spleen, bone marrow, and lymph nodes. Specific immune mechanisms consist of humoral and cell-mediated responses. Humoral immunity involves the production of immunoglobulins by B-lymphocytes, while cell-mediated immunity involves the activity of T-lymphocytes. Both B-lymphocytes and T-lymphocytes originate in the embryonic liver, yolk sac, and bone marrow, but mature in the bursa of Fabricius and the thymus respectively. Although B-lymphocytes have the specific role of immunoglobulin (antibody) production, the T-lymphocyte population is heterogeneous, and their role in immune response is diverse (Butcher and Miles 1991). In birds, coccidia infection induces a firm and lengthy immune response involving both humoral and cell-mediated activity (Vermeulen 2004).

Coccidia

Coccidia are single-celled, intracellular parasites that infect both vertebrates and invertebrates by residing and proliferating in epithelial cells of the host's gastro-intestinal tract. The numerous species of coccidia inhabit specific regions within the host's gastrointestinal tract. These parasites belong to the genus *Eimeria* and the order *Eucoccidiorida* (Vermeulen 2004). The coccidia that infect turkeys are monoxenous parasites. They live within a single host species during their life cycle; no intermediate host is needed for transmission (Roberts and Schmidt 1985). The life cycle, which

includes development both inside and outside of the host organism, is specific to the species of coccidia and varies in duration from 4 to 9 days. The life cycle may repeat in the same host individual resulting in chronic infection. A single coccidian oocyst contains four sporocysts which in turn each contain two sporozoites. In birds, infection occurs when mature sporocysts are ingested by the host. The sporocysts interact with bile and trypsin in the intestine and release sporozoites which become embedded in the intestinal wall. Once infected by coccidia, the host will experience gut lesions resulting from local inflammation due to parasite replication and associated cell death. An infected host will shed oocysts in its feces approximately five days after infection. This portion of the life cycle, which takes place outside of the host organism, provides the oocysts with an excess of oxygen needed to stimulate sporulation. During sporulation, oocysts release mature sporocysts, which can infect susceptible hosts (Levine 1982).

Coccidian oocysts shed in the host's feces are recovered by floating a one gram fecal sample in sugar solution. Oocysts are counted by observing of a single drop of this mixture under a microscope (LeBlanc 2005).

MATERIALS AND METHODS

In order to test my hypotheses, domestic turkeys (Cargill Turkey Products, Springdale, Arkansas) were infected with coccidian parasites. This experiment had two phases: 1) establishing pure coccidia strains and 2) measuring the immunocompetence of turkeys infected with these strains (Figure 1). First it was necessary to establish pure genetic strains of coccidian oocysts. Field samples may have multiple coccidia species in varying amounts; this presents an inconsistent threat to the host. Pure strains, on the other hand, present a common and consistent threat to the host. Oocysts used in the single oocyst isolation infections were obtained from fecal samples that Dr. Buchholz acquired from outside sources. Single oocyst infections yield few oocysts in the feces. Therefore, intermediate single strain infections were necessary to obtain the large number of oocysts needed to infect the experimental turkeys for the immunocompetence phase of the experiment. Sporulated fecal samples obtained from the intermediate infections were used to infect the experimental turkeys. All data reported here were collected from the experimental infections. All turkeys used in the experiment were euthanized humanely using carbon dioxide asphyxiation.

Experimental Infections

Because the experimental turkeys were only one week old at infection, a relatively low infection inoculum was used, 12,000 oocysts in a dilute sucrose solution. This inoculum ensured a measurable immune response in the turkeys. Oocysts were concentrated and counted, and the turkey poults received a volume of fecal sample

estimated to contain an approximate number of coccidian oocysts. Three infection treatments were used (Table 1). I hypothesized that infection resulting from multiple strain of coccidia would prove to be more challenging to the immune system of the host.

Table 1: Six turkey poults were subject to each of the infection treatments. Two controls received only dilute sucrose solution.

Infection Treatment	Infection Load	Coccidia Strains	Number of Poults Infected
Single Strain 1	12,000 oocysts	C2A	6
Single Strain 2	12,000 oocysts	C4G	6
Two Strains	6,000 each	C2A *and* C4G	6

Twenty turkey poults were used in the experimental infections. Immediately prior to infection, each turkey was banded for identification. The weight and right tarsus length were measured and recorded, and blood samples were collected from each turkey as well. Measurements and blood samples were taken again at one and two weeks following infection.

Twice daily, the turkeys were fed and watered, and the cages were cleaned. Fecal samples were collected during the A.M. cleaning for oocyst counting following the method described by Dustin LeBlanc (LeBlanc 2005). For each individual, the number of oocysts shed per day was recorded for eight days.

Blood Sampling for Measuring Immunocompetence

Blood samples were collected at three points during the experiment: immediately prior to infection, one week following infection, and two weeks following infection. The volume of blood collected from each poult was less than 1% of its body mass at the time of sampling. Blood was collected from the brachial vein under the wing, as follows. The skin was cleaned with hydrogen peroxide and the brachial vein was pricked with a

sterilized needle. Blood was collected in 70 uL micro-hematocrit capillary tubes. Multiple tubes were collected from each turkey at each of the three sampling points during the infection. The range of micro-hematocrit capillary tubes collected per turkey during a single sampling was 2 - 4. The average number of tubes collected was 3.72. All tubes were centrifuged in an IEC Micro-MB centrifuge for six minutes.

Blood Sample Analysis: White Blood Cell Fraction

For each micro-hematocrit capillary tube of blood collected, the lengths of the buffy layer and the total cellular blood content were measured using calipers. The ratio of the buffy layer to total cellular blood contents was calculated (white blood cell fraction). Ratios were averaged for multiple tubes taken from one turkey during a single sampling. After the final sampling, the ratios recorded at each of the three sampling points during the experiment were analyzed to observe changes in the white blood cell fraction during coccidian infection.

Blood Sample Analysis: Immunoglobulin Production

After centrifugation, plasma was removed with a syringe from each micro-hematocrit capillary tube collected. At each sampling, plasma from a single turkey was mixed together into a 1 mL plastic centrifuge tube labeled with the turkey's identification number. The three plasma samples collected from each turkey at each of the sampling points during the experiment were frozen at -20°C for later analysis.

Agarose Gel Electrophoresis

Electrophoresis was used to separate plasma proteins. Two standards were applied to each gel to serve as a standard of comparison for the plasma samples obtained from the experimental turkeys. The first standard was a chicken immunoglobulin standard

(MS111, Biomeda) with a concentration of 10 mg/mL. The second standard was a poult plasma standard which contained all plasma proteins. The concentration of this poult plasma standard was determined using the Bicinchoninic Acid Protein Assay Kit (Sigma, BCA 1). The concentration of proteins in the poult serum standard was determined to be approximately 46,000 ug/mL.

A 2% agarose gel was prepared (Appendix II) and placed into a Fotodyne Incorporated gel dock. Barbital/EDTA buffer was poured into the gel dock until it covered the gel. The chicken IgG standard, the poult plasma standard, and the three plasma samples obtained from a single turkey were added to lanes on the gel, as follows. On parafilm, 5 uL of 25% Bromophenol Blue tracking dye were mixed with each of the following: 3 uL of chicken IgG standard, 3 uL of poult serum standard, and 3 uL of each of the three serum samples from a single study subject. Each mixture was applied to the gel, and the gel was run at 90 volts for 150 minutes using a VWR AccuPower 300 Power Supply. The gel was immediately removed from the gel dock and placed into 0.1% Amido Black stain. After four hours, the gel was removed from the stain and placed into a 10% acetic acid destain for eight hours. After removal from the destain, the AlphaImager HP Imaging System, was used to obtain a digital image of the gel under transilluminating white light.

Spot Densitometry

Using the spot densitometry analysis tool in the Alpha Imager program, Integrated Density Values (IDV) were obtained for both IgG spots and total plasma protein spots on each gel. An IDV is the sum of all pixel values after background correction. Using the drawing tool on the toolbar, a square was drawn around the chicken IgG standard spot in

lane 1 of the gel. This square was copied to each of the IgG spots in the experimental turkey plasma lanes 3-5. IDV's were recorded for the chicken IgG standard spot and for each of the experimental turkey IgG spots. Using the drawing tool on the toolbar, a rectangle was drawn around the poult plasma protein standard spot in lane 2 of the gel. This rectangle was copied to each of the plasma protein spots in the experimental turkey lanes 3-5. IDV's were recorded for the poult plasma protein standard spot and for each of the experimental turkey plasma protein spots (Figure 2).

After obtaining IDV's for IgG and plasma protein spots on each gel, the values were adjusted to account for any discrepancy between gels. An average chicken IgG standard IDV was calculated from all of the gels (AVG Chicken IgG Standard IDV). The following equations were used to obtain an adjusted value for each experimental turkey IgG IDV:

$$\text{Adjustment Value} = \frac{\text{(AVG Chicken IgG Standard IDV} - \text{Chicken IgG Standard IDV)}}{\text{AVG Chicken IgG Standard IDV}}$$

Adjustment values were used to correct the experimental turkey IgG IDV's on each gel.

Adjusted IDV = (Adjustment Value * Turkey IgG IDV) + Adjustment Value

Plasma protein IDV's were adjusted in the same manner. An average poult plasma protein standard IDV was calculated from all of the gels (AVG Poult Plasma Protein Standard IDV). The following equations were used to obtain an adjusted value for each experimental turkey plasma protein IDV:

$$\text{Adjustment Value} = \frac{\text{(AVG Poult Plasma Protein Standard IDV} - \text{Poult Plasma Protein Standard IDV)}}{\text{AVG Poult Plasma Protein Standard IDV}}$$

Adjustment values were used to correct the experimental turkey plasma protein IDV's on each gel.

Adjusted IDV = (Adjustment Value * Turkey Plasma Protein IDV) + Adjustment Value

Using IDV's, the ratio of immunoglobulins to total plasma proteins was calculated for each turkey at each of the three sampling points during the experiment. These ratios were analyzed to observe changes in immunoglobulin production during coccidian infection.

Analyzing Blood Component Ratios

Two blood component ratios were recorded at three points during the experiment: (1) the ratio of the buffy layer to total cellular blood contents, and (2) the ratio of IgG to total plasma proteins. The changes in these ratios (final – initial) during the experiment were not accurate representations of immune system activity for all individuals; therefore, it was necessary to include ratios obtained during the second sampling. By creating simple linear regressions of an individual's ratios at the three sampling points, immune system activity during the experiment was observed by the direction and steepness of the slope. A positive slope represents increased immune system activity during the infection, while a negative slope represents decreased activity during the infection. The steepness of the slope indicates how quickly the organism responded to the infection (Figure 3).

Growth Analysis

Body condition was measured as the residual values from a simple regression of body mass plotted against tarsus length (Figure 4). Initial mass and tarsus length were used to determine initial body condition, while final mass and tarsus length were used to determine final body condition. Body condition was an indicator of muscle mass, while tarsus length was an indicator of skeletal growth.

Statistical Analyses

Normality tests confirmed that all continuous variables were normally distributed. Statistical analyses were performed using StatView. ANOVA was used to compare

treatment effects, while simple regressions were used to determine relationships between variables.

Figure 1: An overview of the development of coccidia strains used to create treatment groups.

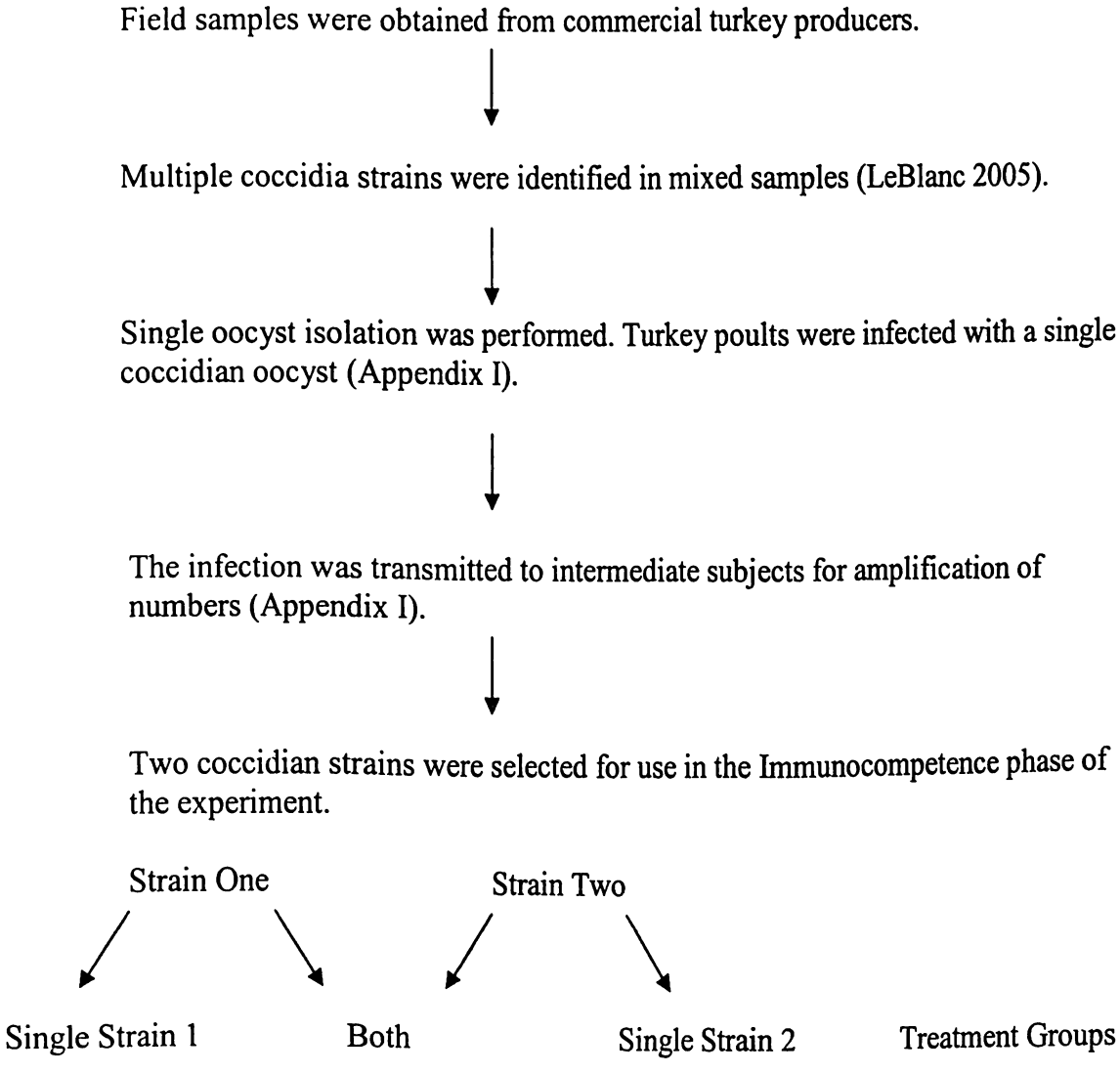


Figure 2: A gel image captured using the AlphaImager HP Imaging System. Spot densitometry was used to obtain IDV's for IgG spots and plasma protein spots. The square drawn around the chicken IgG standard spot is shown in lane 1. The rectangle drawn around the poult plasma protein standard spot is shown in lane 2.

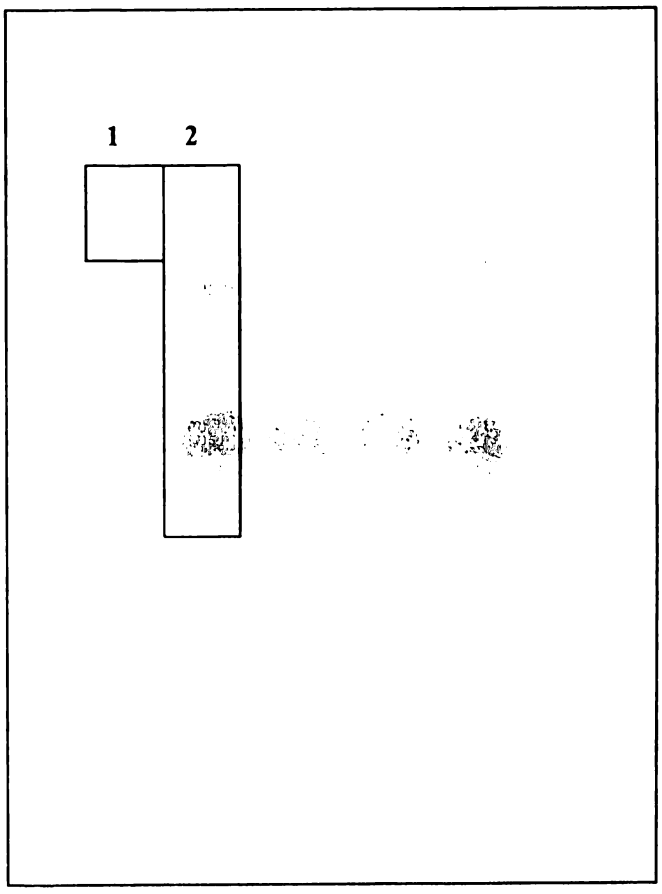
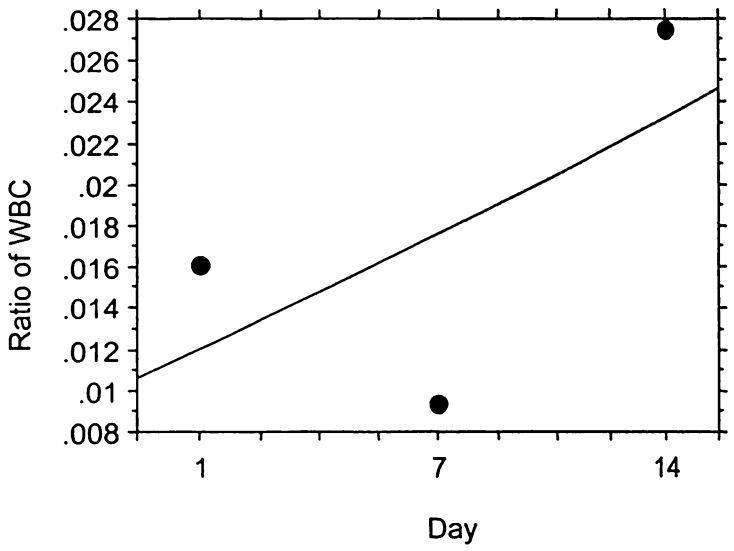


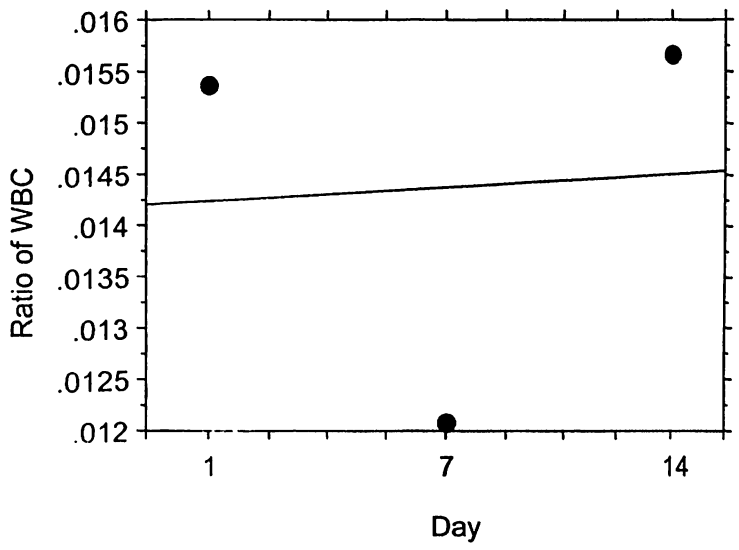
Figure 3: Examples of the different WBC responses to coccidia infection observed in infected poults. Immune responses varied among the poults. Both increased and white blood cell production was observed.

Figure 3a: White blood cell production greatly increased in response to coccidia infection.



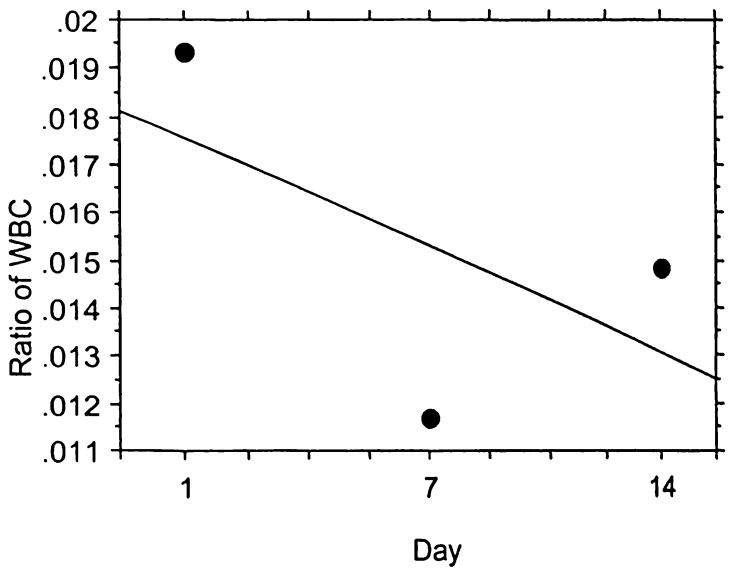
$Y = 0.01 + 0.01X$; $R^2 = 0.38$

Figure 3b: White blood cell production slightly increased in response to coccidia infection.



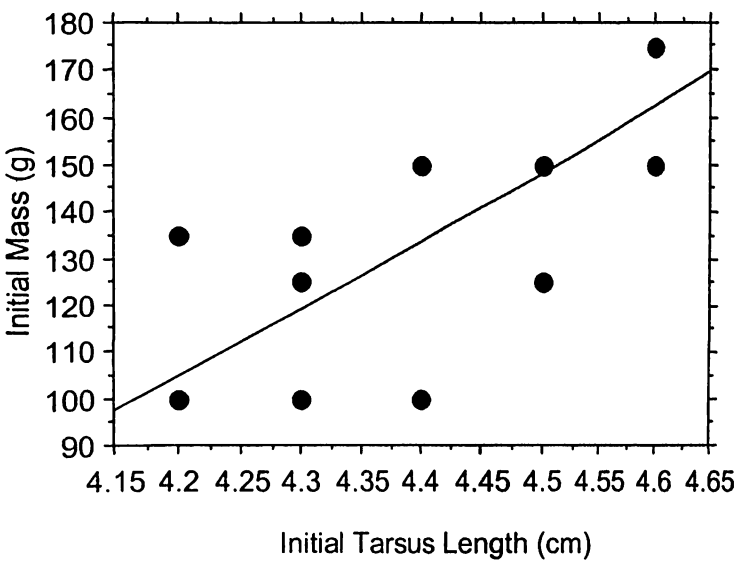
$Y = 0.01 + 1.38*10^{-4}X$; $R^2 = 0.01$

Figure 3c: White blood cell production greatly decreased in response to coccidia infection.



$Y = 0.02 - 0.01X$; $R^2 = 0.34$

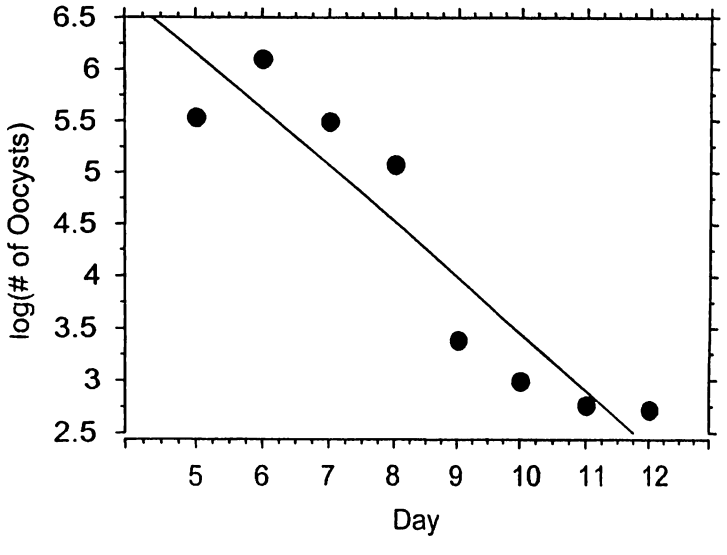
Figure 5: Body condition was measured as the residual from a simple regression of body mass plotted against tarsus length (Christe *et al.* 2000). This figure shows the initial variation in condition prior to infection.



RESULTS

All turkeys began shedding oocysts in their feces on the fifth day following inoculation. The number of oocysts shed per day by each individual was recorded for eight days thereafter. A simple linear regression of the number of oocysts shed in an individual's feces over time provides valuable information concerning parasite resistance. As shown in Figure 6, the number of oocysts shed reached a peak value on the second day of shedding and then began to decline as the host fought the infection. The peak number of oocysts shed indicates the severity of the infection. The steepness of the slope of the simple regression line represents a host's ability to control coccidia replication and thus indicates parasite resistance. Poults with steeper slopes of parasite reduction may have greater resistance than those that clear parasites more slowly and thus exhibit shallower slopes.

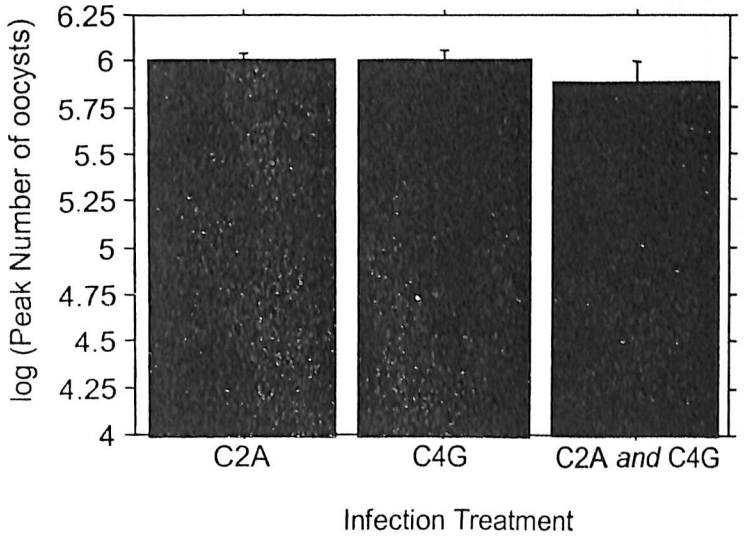
Figure 6: A larger peak number of oocysts shed indicates a more severe infection. A steeper slope indicates greater parasite resistance (Y = 6.701 - 0.541)



Infection Treatments

I hypothesized that infection resulting from multiple strains of coccidia would prove to be more challenging to the immune system of the host. One prediction that would support this hypothesis is that turkey poults infected with two strains of coccidia would shed a greater number of oocysts than those infected with only a single strain of coccidia. Although poults infected with two strains of coccidia shed fewer oocysts, there was no significant difference in the peak number of oocysts shed by poults in the three infection treatments (ANOVA, $F = 1.03$, $df = 2$, $p = 0.38$). These results do not support my hypothesis. Infection with multiple strains of coccidia does not appear to reduce a host's ability to control coccidia replication (Figure 7). Thus treatment effects were not considered in subsequent analyses.

Figure 7: The peak number of oocysts shed is not significantly different among the three infection treatments.



Coccidia Infection and Growth

Coccidia infection is expected to result in a trade-off between growth and immunocompetence. I hypothesized that domestic turkeys infected with coccidia would invest in growth rather than immunocompetence. Based on this hypothesis, I predicted that coccidia infection would not have a significant effect on the growth of infected poults. Although the distribution of the data suggests an association between skeletal growth and the peak number of oocysts shed (Figure 8), there is no significant relationship between these variables ($Y = -5.00 + 1.11X$, $R^2 = 0.19$, $p = 0.08$). Also, there is no significant relationship between body condition and the peak number of oocysts shed ($Y = -225.23 + 37.83X$, $R^2 = 0.02$, $p = 0.61$) (Figure 9).

Figure 8: Coccidia infection has no significant effect on the skeletal growth of infected poults.

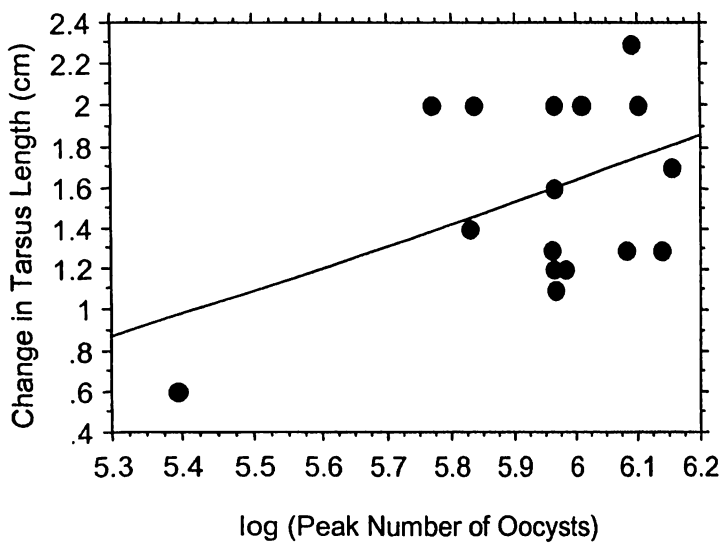
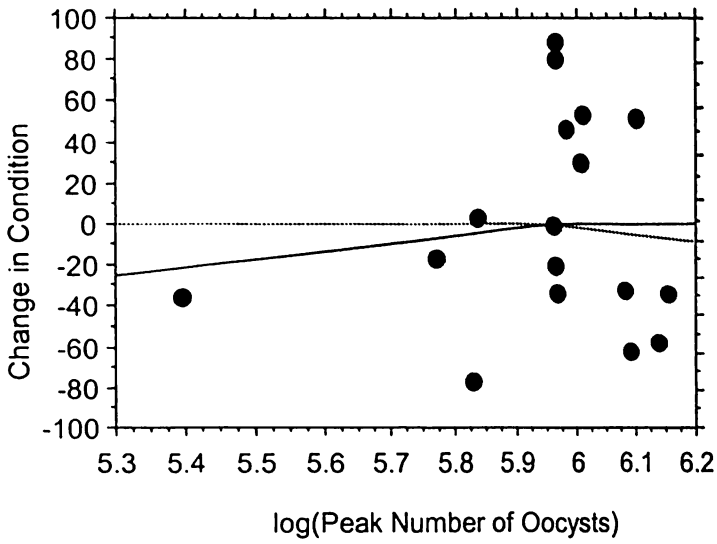


Figure 9: Coccidia infection has no significant effect on the body condition of infected poults.



The Trade-off between Growth and Immunocompetence

I hypothesized that during coccidia infection, domestic turkey poults would invest in growth rather than immunocompetence. If poults infected with coccidia invest in growth, they will enhance skeletal growth and body condition during the infection rather than increase immunoglobulin production. There was a significant negative relationship between skeletal growth and immunoglobulin production (Simple Regression, $Y = 1.65 - 0.27X$, $R^2 = 0.25$, $p = 0.04$, $N = 17$). However, there was no significant relationship between body condition and immunoglobulin production (Simple Regression, $Y = 1.44 - 6.98X$, $R^2 = 0.01$, $p = 0.66$, $N = 17$). Alternatively, investment in growth may be observed as enhanced skeletal growth and body condition rather than increased white blood cell production. There was no significant relationship between skeletal growth and white blood cell production (Simple Regression, $Y = 1.67 - 0.33X$, $R^2 = 0.12$, $p = 0.18$, $N = 17$). Also, there was no significant relationship between body condition and white

blood cell production (Simple Regression, $Y = 0.14 - 0.54X$, $R^2 = 2.67*10^{-5}$, $p = 0.98$, $N = 17$). The significant negative relationship between skeletal growth and immunoglobulin production provides support for my hypothesis that a functional trade-off exists during coccidia infection. As shown in Figure 10, some poults invested in skeletal growth, while others invested in immunoglobulin production. These results do not support my hypothesis that domestic turkeys will invest exclusively in growth. (Figures 10, 11, 12, and 13).

Figure 10: Individuals that enhanced skeletal growth did not increase immunoglobulin production in response to coccidia infection.

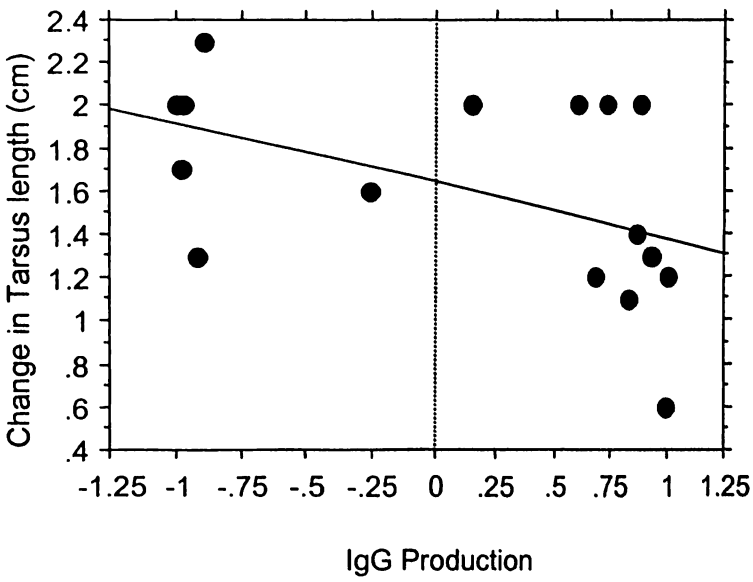


Figure 11: A trade-off does not exist between body condition and immunoglobulin production.

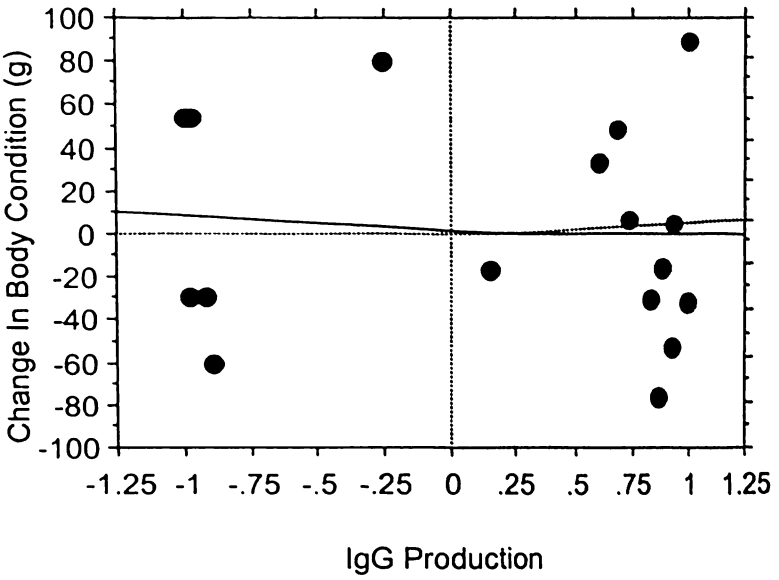


Figure 12: A trade-off does not exist between skeletal growth and white blood cell production

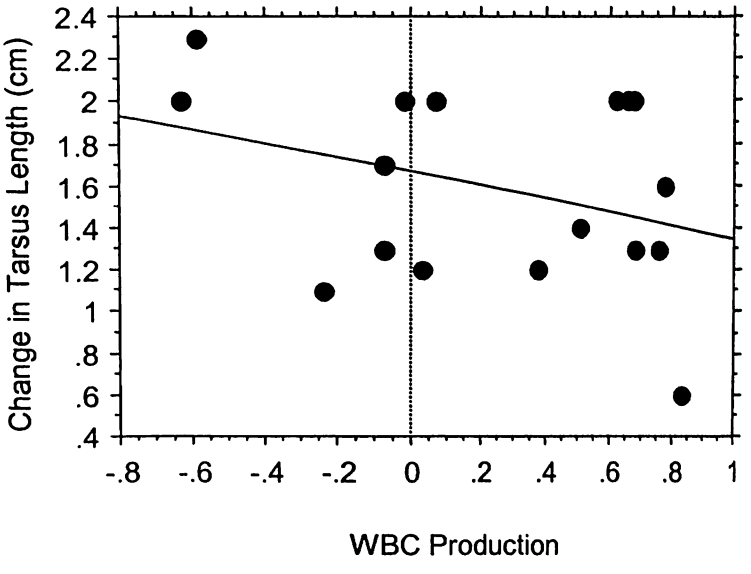
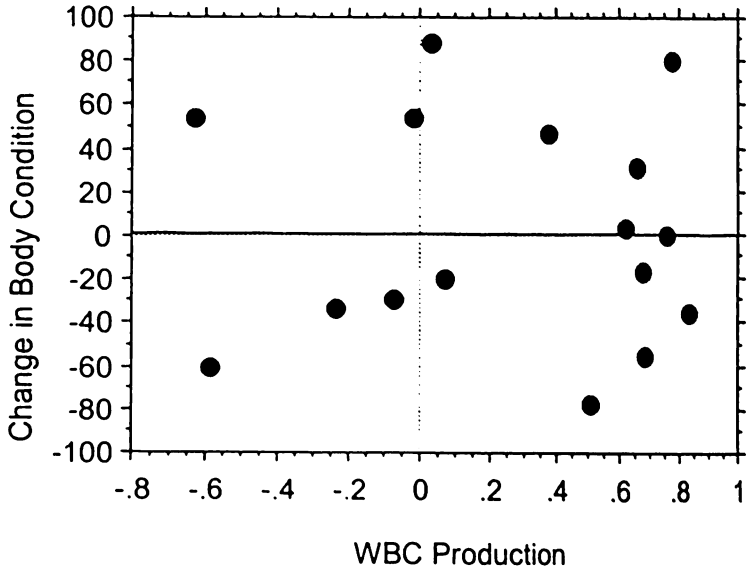


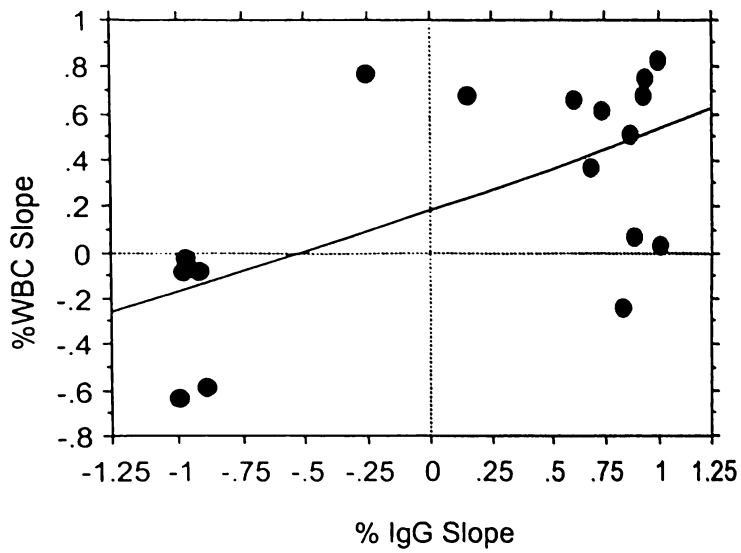
Figure 13: A trade-off does not exist between body condition and white blood cell production.



Components of Immunocompetence:

Although a trade-off was observed between skeletal growth and immunoglobulin production, a comparable trade-off was not observed between skeletal growth and white blood cell production. Despite the absence of a trade-off involving white blood cell production, it was an active component of the immune response to coccidia infection. There was a significant positive relationship between immunoglobulin production and white blood cell production ($Y = 0.18 + 0.35X$, $R^2 = 0.37$, $p = 0.01$). As immunoglobulin production increased, white blood cell production also increased (Figure 14). These results suggest that multiple components of the immune system responded to coccidia infection, although white blood cell production was not directly involved in the trade-off with growth.

Figure 14: Immunoglobulins and white blood cells were positively associated during coccidia infection.



The Extent of the Trade-off between Skeletal Growth and Immunoglobulin Production

Figure 10 provides evidence of a trade-off between skeletal growth and immunoglobulin production during coccidia infection, but not all individuals experienced an identical trade-off. What factors contributed to the strength of the trade-off? I hypothesized that as infection severity increased, the trade-off between skeletal growth and immunoglobulin production would become stronger. Trade-off strength was indicated by the residual values from a simple regression of the change in tarsus length plotted against the percent IgG slope. There was no significant relationship between the peak number of oocysts shed and the strength of the trade-off ($Y = -2.93 + 0.49X$, $R^2 = 0.05$, $p = 0.39$, $N = 17$). These results do not support my hypothesis. I also hypothesized that as parasite resistance increased, the trade-off between skeletal growth and immunoglobulin production would become weaker. There was no significant relationship between parasite resistance and the strength of trade-off ($Y = 1.35 - 1.84X$, $R^2 = 0.09$, $p =$

0.25, N = 17). Neither infection severity nor parasite resistance determined the strength of the trade-off between skeletal growth and immunoglobulin production (Figures 15 and 16).

Figure 15: Infection severity had no effect on the strength of the trade-off between skeletal growth and immunoglobulin production.

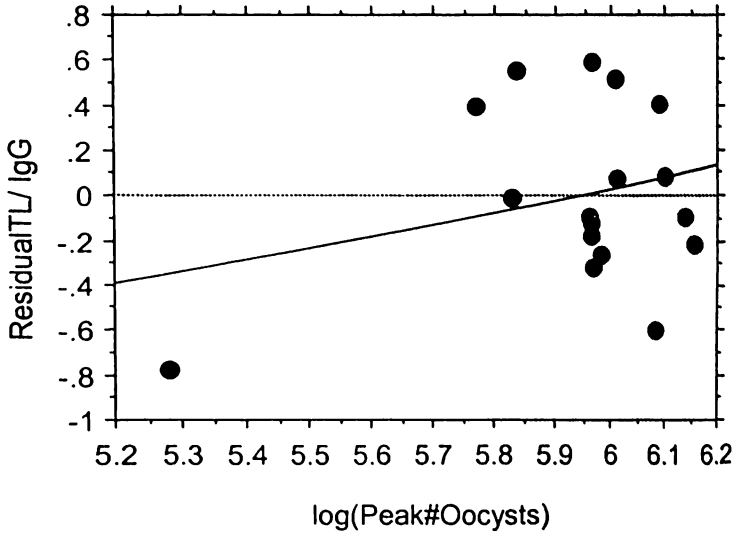
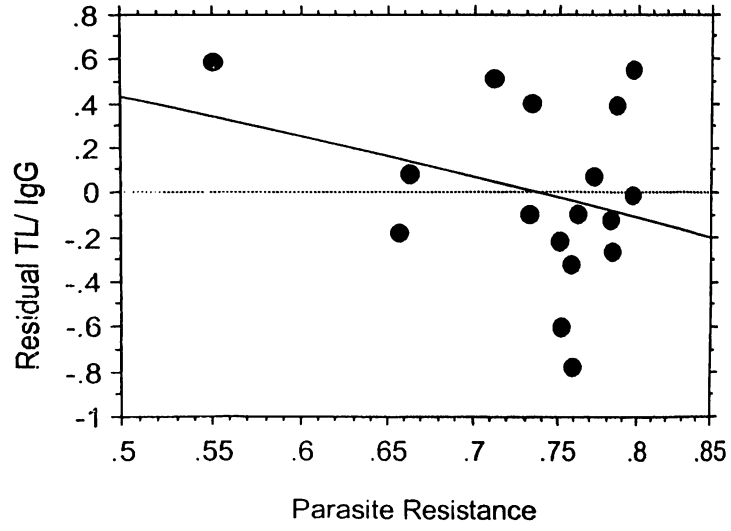


Figure 16: Parasite resistance had no effect on the strength of the trade-off between skeletal growth and immunoglobulin production.



DISCUSSION

Studies confirm that dual infection presents a greater challenge to the immune system of the host. For example, in Hill *et al.* (2004), researchers observed a greater negative effect of dual infection on feather brightness. Although three different infection treatments were used in this experiment, a comparison of the peak number of oocysts shed by individuals in the different treatment groups indicated that there was no significant difference in the control of coccidia replication among the groups. I concluded that infection resulting from multiple strains of coccidia did not present a greater challenge to the immune system of the host. This conclusion made it possible to investigate the trade-off between growth and immunocompetence without further consideration of different infection treatments.

Evidence of a trade-off between growth and immunocompetence during infection is well documented in wild species where resources are often limited. A functional trade-off is predicated based on the substantial nutritional and energetic costs associated with the maintenance of an efficient immune system and the initiation of an immune response (Mangel and Stamps 2001, Tschirren and Richner 2006). Brommer *et al.* (2004) documented a trade-off between growth and immune function in blue tits experimentally manipulated for investment in immune defense through methionine supplementation. Similarly, Klasing *et al.* (1987) documented reduced growth rates in experimentally immunized domestic chicks. However, other researchers have documented contrasting results. In a study by Buchholz *et. al.* (2004), adult wild turkey condition did not correlate

with response to antigen injection. During infection, wild turkeys are expected to invest in immunological activity and fight coccidia infection rather than grow. Domestic turkeys, on the other hand, have been artificially selected to grow rapidly for human consumption and are thus predicted to invest in growth during infection. A general concern among members of the poultry industry is that the high growth rates of domestic strains of chickens and turkeys increase their susceptibility to a variety of pathogens (Mangel and Stamps 2001). Therefore, investment in growth not only reduces a host's ability to fight infection, but also increases an organism's susceptibility to infection. I hypothesized that domestic turkeys infected with coccidia would experience a trade-off between growth and immunocompetence, demonstrated as an investment in growth rather than immunocompetence.

Based on my results, a trade-off does appear to exist between immunoglobulin production and skeletal growth in domestic turkeys infected with coccidia, but not all of the individuals invested in growth. Although white blood cell production was positively associated with immunoglobulin production, a similar trade-off was not discovered between white blood cell production and skeletal growth. White blood cells circulate in the blood at all times; however, upon an organism's initial encounter with coccidia, the immune system must immediately initiate the production of immunoglobulins specific to destroying coccidia. Fair *et al.* (1999) differentiates between the costs associated with maintaining the immune system and mounting an immune response. Mounting an immune response is more costly to an organism, and its effects may be exerted through the physiological control of metabolic and developmental pathways (Fair *et al.* 1999).

Increased activity of specific immune mechanisms upon coccidia infection may prove to be more costly to the host, resulting in the observed trade-off.

Body condition is an indicator of muscle mass, which is variable throughout life depending on resource availability. Tarsus length is an indicator of skeletal growth, which is determined during development and remains fixed throughout adulthood. In this experiment, it is possible that body condition did not suffer from increased immune activity because of an abundant food supply. Soler *et al.* (2003) suggests that experiments performed under laboratory conditions with a surplus of energy and resources may mask a possible trade-off between growth and immunocompetence. It is therefore possible that because turkeys were not limited in the amount of food they were able to consume, immune activity did not influence that muscle mass they were able to acquire. However, the long term effects of coccidia infection appear to be more damaging to the host's ability to reach it full skeletal size.

One subject suffered from a much less severe infection than the other infected subjects. After reviewing the data obtained from this individual, I decided not to remove the data from the study because of its accuracy. Removal of this data would not have significantly affected the reported results.

It appears as though domestic turkeys infected with coccidia do experience a trade-off between immunocompetence and growth, specifically immunoglobulin production and skeletal growth. The short-term effects of immunological defense are observed in functional trade-offs, but few studies have addressed the long-term effects of immune responses that occur during the developmental period (Fair *et al.* 1999). Over time, investment in immunological quality is predicted to offer strong selective

advantages that greatly contribute to the successful evolution of species (Gustafsson 1994). Increased investment in immunological defense is costly, yet this investment proves to be very beneficial to organisms living in the presence of parasites. The evolution of optimal host defense is thus governed by the fact that natural selection continues to favor the benefits associated with increased immunological defense during infection.

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APPENDICES

Appendix I

Single Oocyst Isolation Infections

Preparation for arrival of the turkeys used in the single oocyst isolation infections began one week prior to the arrival date. The experiment room was sterilized and warmed to 90°F. Cages were assembled, sterilized, and placed on shelves in the experiment room. The turkeys were approximately one to two days old upon arrival. They were kept in a single cage for one week following their arrival. At the end of week one, single oocyst isolation was performed following the method described by Dustin LeBlanc (LeBlanc 2005). At the age of one week, each turkey was infected by mouth with a plastic gel capsule containing a single oocyst. Six turkeys, identified as C4-A through C4-F, were infected with a single oocyst from fecal sample C4G, and six turkeys, identified as C2-J through C2-O, were infected with a single oocyst from fecal sample C2A. After infection, the turkeys were placed into separate cages.

Twice daily, the turkeys were fed and watered, and the cages were cleaned. Fecal samples were collected during the A.M. cleaning and checked for the presence of oocysts following the method described by Dustin Leblanc (Leblanc 2005). Five days after the infection, oocysts began to appear in the feces. The presence of oocysts in the feces confirmed the success of the single oocyst isolation infections. Fecal samples containing oocysts were sporulated and used to infect an intermediate group of turkeys.

Sporulation of Fecal Samples

Collection of feces for sporulation began five days after infection. Fecal samples were collected for four days during the A.M. cleaning and prepared using the following method. Each fecal sample collected was placed into a plastic cup and mixed with 2.5% potassium dichromate. The mixture was homogenized and filtered through cheesecloth into a second plastic cup. The mixture was then aerated to induce sporulation. Fecal samples were sporulated for forty-eight hours and then prepared for use in the intermediate infections.

Preparation of Sporulated Fecal Samples for use in the Intermediate Infections

The sporulated fecal samples were prepared for use in the intermediate infections. Each sporulated fecal sample was homogenized and poured into a 50 mL plastic centrifuge tube. The tubes were centrifuged at 3,000 RPM in a Beckman GS-6R centrifuge for eleven minutes. The supernatant was poured from each tube leaving the remaining pellet in the tube. Each tube was then filled with distilled water, and the pellet was re-suspended. This process, beginning with centrifugation, was performed three times.

Intermediate Infections

Twelve, one week old turkeys were used in the intermediate infections, which resulted from the mixture of sporulated fecal samples and turkey feed. Each turkey was assigned identification based on the sporulated fecal sample it was infected with. Sporulated fecal samples were mixed with turkey feed and fed to the turkeys for three days. Twice daily, the turkeys were fed and watered, and the cages were cleaned. Fecal samples were collected during the A.M. cleaning and checked for the presence of oocysts

following the method described by Dustin Leblanc (LeBlanc 2005). Five days after the infection, oocysts began to appear in the feces. Because several turkeys escaped from their cages during the first week of infection, it was concluded that the only turkeys C2-M and C4-C were not cross contaminated. Fecal samples were collected from C2-M and C4-C for four days during the A.M. cleaning and sporulated following the method described under *Sporulation.* After sporulation, these fecal samples were used in the experimental infections.

Appendix II

Barbital/EDTA Buffer

- 12 g Barbital Buffer
- 0.3722 g EDTA
- 1,000 mL distilled water
- Dissolve completely

2% Agarose Gel

- 1 g agarose
- 50 mL Barbital/EDTA buffer
- Heat mixture in microwave for 1 ½ minutes to dissolve completely

0.1% Amido Black Stain

- 100 mL methanol
- 25 mL acetic acid
- 0.25 g amido black
- 125 mL distilled water
- Dissolve completely

25% Bromophenol Blue Tracking Dye

- 20 g sucrose
- 50 mL distilled water
- 0.125 g BromoBlue
- Dissolve completely