2010

Investigating the effects of antioxidant vitamins on the immunocompetence of domestic turkeys (Meleagris gallopavo) infected with coccidian parasites

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by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2010

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ACKNOWLEDGMENTS

I would like to wholeheartedly acknowledge Dr. Richard Buchholz for steadfastly serving as my advisor and mentor for over two years in preparation for this undergraduate thesis. Without his generous support this research would simply not have been possible. I also acknowledge Dr. Stephen Brewer and Dr. Randy Wadkins for providing time and assistance as my thesis readers. Finally, I would like to sincerely thank the faculty and staff of the Sally McDonnell Barksdale Honors College for providing research funding as well as countless opportunities for me to develop as both a student and an individual during my years at Ole Miss.

ABSTRACT

JOSEPH GRIFFIN ORR: Investigating the effects of antioxidant vitamins on the immunocompetence of domestic turkeys (*Meleagris gallopovo*) infected with coccidian parasites
(Under the direction of Dr. Richard Buchholz)

Vitamins are organic compounds essential for healthy metabolism and growth. Additionally, some vitamins also function as antioxidants. In particular, the antioxidant vitamins A (β-carotene) and E (α-tocopherol) are known to enhance the immune response of many animals as well as humans. However, there is relatively little research linking these immune-enhancing effects to domestic turkeys facing parasitic infections, such as coccidiosis. Coccidiosis in birds is expensive to treat, costing the worldwide poultry industry hundreds of millions of dollars annually. Coccidia of the genus *Eimeria* are protozoans that parasitize poultry via the intestinal tract, causing deleterious effects to survival such as lesioning, reduced growth rate, and mortality. Twenty-two broad breasted white turkey poults (*Meleagris gallopovo*) were inoculated with 12 000 coccidian oocysts by mouth. The diet for one-half of the subjects was supplemented with high doses of vitamins A and E. Daily fecal samples from each subject were collected starting 72 hours after inoculation for six days. Feces were examined for oocyst concentration per gram of feces using a microscope. The mass of the spleen, testes, bursa of Fabricius, change in body condition, and erythrocyte and white blood cell fractions were also measured for each subject. There was a significant interaction of treatment and sex in the white blood cell fraction (ANOVA, $F = 4.716$, $p = 0.05$). Supplemented females showed a significantly higher white blood cell

fraction (t-test, $t = -3.278$, $n_1 = 3$, $n_2 = 8$, $p = 0.001$). Based on these findings, antioxidants may work in conjunction with estrogens to reduce oxidative stress in turkeys that can lead to an enhanced immune response. Future studies should focus on using antioxidants in conjunction with other proven methods to elicit a compound increase in turkey immune function.

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INTRODUCTION

Vitamins are organic compounds that are an essential part of the diet of animals and humans. Vitamins help maintain a normal metabolism and promote healthy growth. Vitamins are generally present in minute quantities in foods. Although their ingestion is only required in small doses, a lack of vitamins can lead to deficiency diseases. Many vitamins function by acting either as metabolic catalysts or as reactants in physiologically crucial reactions. A classic example is vitamin A's role in vision, in which it acts as a major component of the pigments rhodopsin and iodopsin. These two compounds are used by the photoreceptor cells rods and cones, respectively, to sense light. Vitamin A deficiency leads to night blindness, a condition in which a severe reduction in rhodopsin makes it impossible for rods to function in low light (McDowell 2000).

In addition to their physiological functions, some vitamins are also known to act as antioxidants. Antioxidants are well known for their ability to suppress oxidative damage. Active oxygen species are crucial for their roles in energy production, biosynthesis, and phagocytosis (a cellular process critical to immune function). These essential activities are accelerated or intensified during immune activation. There is increasing evidence, however, that the production of oxygen species has negative tradeoffs. Oxygen species may cause a variety of diseases by forming free radicals. Free radicals are oxidized compounds that damage tissue. In the past decade, there has been increasing interest in the contribution of antioxidants toward the effectiveness of the immune response (Noguchi and Niki 1999).

While the immuno-enhancing effects of antioxidants in human health are well known (Wu and Meydani 1999), there is relatively little research linking these effects to immunocompetence in domestic turkeys facing parasitic infections. Advances in the prevention and control of parasitic infections, such as coccidiosis, in domestic birds are of great interest to the poultry industry. Allen and Fetterer (2002 b) assert that coccidiosis has the greatest economic impact on worldwide poultry production, with annual expenses estimated at $450 million for the American broiler industry alone and over $800 million worldwide. These estimates include costs associated with treatment as well as losses due to mortality, morbidity, and poor feed conversion ratios. Coccidiosis affects a number of avian species, including both chickens (*Gallus gallus*) and turkeys (*Meleagris gallopovo*) (Levine 1982). However, the disease is less understood in the latter because turkey-infecting strains have variable morphology that makes them problematic to isolate (LeBlanc 2005), and due to a lack of research specifically to turkey coccidia (Chapman 2008).

Hypotheses and Predictions

The primary objective of this study was to observe whether antioxidants would have an effect on the severity of turkey coccidiosis. I hypothesized that the increased intake of antioxidants in the diet would directly support the ability of the turkey immune system to fight parasitic infection. I also hypothesized that increased vitamin supplementation in the diet would promote the overall health of turkey.

The most direct way to study antioxidants' effect on turkey immunocompetence in response to coccidia would be to observe how antioxidants reduce fecal oocyst shedding. Patterns of oocyst shedding include the number of oocysts shed at the peak of infection, the time it takes to reach this peak, how quickly the immune system can overcome the infection

(when minimal oocysts are shed), and the average daily parasite load. I predicted that supplemented birds would have lower values in all of these patterns in comparison to control birds.

Antioxidants may also contribute indirectly to reducing the symptoms of coccidiosis by enhancing the immune response. The bursa of Fabricius functions as the site for B-lymphocyte maturation in young birds, but disappears before reaching sexual maturity (Butcher and Miles 1991; Møller *et al.* 1997). During parasitic infections, a larger bursa of Fabricius is an indication of a greater immune response (Møller *et al.* 1997). Therefore, I predicted that supplemented birds would have a greater bursa mass than control birds. Based on this assumption, an increase in bursa mass should also correlate to an increase in lymphocyte concentration in the blood. I predicted that the white blood cell fraction would be greater in supplemented birds than in control birds. In addition to the bursa of Fabricius, another important organ to the avian immune system is the spleen. The size of the avian spleen has been generally used as an index of immune system strength. Smith and Hunt (2003) suggest that a smaller, not larger, spleen may be an indication of greater resistance to disease and thus propose that the spleen size in birds is indirectly related to immunocompetence, despite common assumptions. I predicted that supplemented birds would have a smaller spleen than control birds.

Even if antioxidants are not directly correlated to immune response, the general health benefits provided by their intake should still be evident. Body condition is an indication of a turkey's energy reserves, and can be defined as the ratio between its mass and the length of its tarsus (Buchholz 1995). I predicted that supplemented birds would grow larger, and thus have a greater change in body condition, than control birds.

Background Information

Formation of Free Radicals

In a chemical reaction, two compounds will react with one another to form new products if the products are more stable. The stability of a compound is determined by the arrangement of electrons in its chemical bonds. Atoms bond covalently by sharing pairs of valence electrons to fill their outer shell. However, many important chemical reactions occurring in living organisms react via the homolytic cleavage of a bond, forming compounds containing unpaired electrons that are known as free radicals (Bendich *et al.* 1990). Because unpaired electrons usually seek other electrons to become paired, free radicals are in general very reactive and attack other molecules (Noguchi and Niki 1999).

In order to stabilize itself, a free radical will remove a single electron from another stable compound, transforming the compound into a new free radical and starting a chain reaction. Free radicals are particularly harmful in living systems because a series of chain reactions can easily disrupt the functional bonding of tissues. Some of the most common free radicals are formed from oxygen: singlet oxygen, superoxide, and the hydroxyl radical (Bendich *et al.* 1990). Chemical equations for these reactions are shown in Figure 1.

Electron transport systems are the largest continuous source of reactive, oxygenated free radicals (Bendich *et al.* 1990). They can also be induced by light, heat, and metals (Noguchi and Niki 1999). Common intra-cellular sites of free radical generation include mitochondria, lysosomes, and peroxisomes as well as the membranes of the endoplasmic reticulum and nucleus. Free radicals generally target unsaturated bonds of lipid cellular membranes, sulfur-containing enzymes and other proteins, nucleic acids, and

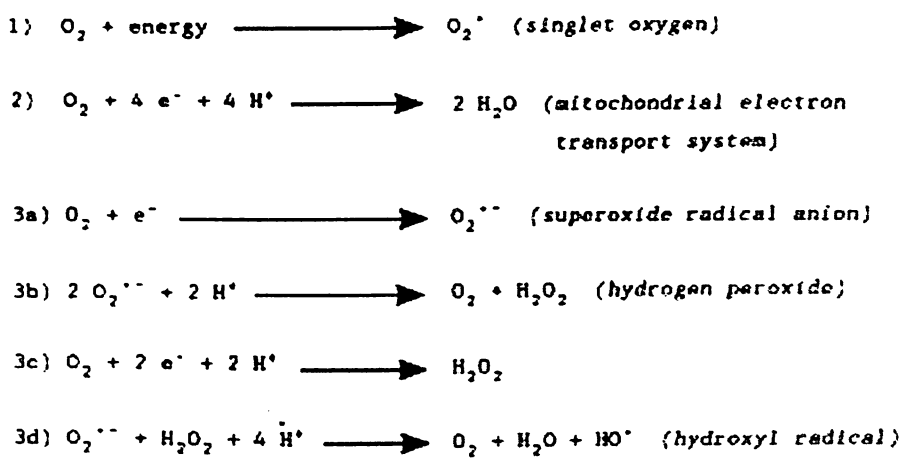

Figure 1: Reactions with oxygen resulting in more reactive species than oxygen. Source: Bendich, A., ed. *Advances in Experimental Medicine and Biology*. **262**, 171 pp. New York: Plenum Press.

endothelial tissues lining the blood vessels and lungs (Bendich, *et al.* 1990), resulting in oxidative damage such as the deterioration of foods, membrane dysfunction, protein modification, enzyme inactivation, and DNA mutation (Noguchi and Niki 1999). Lipid peroxidation is capable of reducing membrane fluidity, which may decrease the ability of lymphocytes to respond to challenges of the immune system (Wu and Meydani 1999). In conclusion, the accumulation of free radicals can result in widespread cellular damage.

Antioxidants

Antioxidants are naturally occurring compounds that can inactivate reactive free radicals, thus reducing the free radical burden. Antioxidants can, in general, enhance natural and specific immunity (Wu and Meydani 1999). There are a multitude of antioxidant types, with each of these having a variety of functions to affect the formation of free radicals at any of its reaction stages: initiation, propagation, or termination. For example, preventative

antioxidants suppress the formation of free radicals, while radical scavenging antioxidants either inhibit chain initiation or terminate chain propagation (Bendich *et al.* 1990). An increase in pro-oxidants is usually followed by a decrease in antioxidants. When there is an imbalance between these two compounds, the organism is said to be under oxidative stress (Costantini and Dell'Ormo 2006).

Radical scavenging antioxidants include vitamins A, C and E, among others (Noguchi and Niki 1999). Antioxidants that scavenge free radicals become antioxidant radicals. Unlike free radicals, however, antioxidants are stable compounds because they can react with themselves or other antioxidants to regenerate their original form. A well-known example of this can be seen in the synergistic interaction between vitamins E and C, in which α-tocopheroxyl radical is reduced by vitamin C to regenerate vitamin E (Noguchi and Niki 1999).

Vitamin E (α-tocopherol) is a major component of all cellular membranes, including those of the mitochondria, nucleus, and lymphocytes. Vitamin E acts as a major lipid-soluble antioxidant by inhibiting lipid peroxidation (Bendich *et al.* 1990). It has been shown to reduce the risk of heart disease and some cancers, as well as slowing the progression of other diseases in humans. Studies since the 1950s have shown that vitamin E supplementation improves the immune response in several animal species, including but not limited to: enhanced lymphocyte proliferation in mice, rats, and pigs; increased phagocytic ability of macrophages in rats; and improved antibody production after vaccination in rabbits. (Wu and Meydani 1999).

Another important antioxidant is vitamin A. Vitamin A (β-carotene) belongs to the class of organic pigments known as carotenoids, of which it is the most well known

compound. Carotenoids are a major component of plant pigments, responsible for the various colors seen in fruits and vegetables (Boileau *et al.* 1999). They are also a major component of colorful external tissues in animals such as birds' beaks (Constantini and Dell'Ormo 2006) and the secondary sex characteristics of some male animals. They are known to positively affect male mating success in guppies (Kodric-Brown 1989). As mentioned previously, vitamin A plays a major role in the ability of photoreceptor cells to sense light (McDowell 2000). As antioxidants, carotenoids are the most potent biological quenchers of singlet oxygen, reacting with this free radical and transferring energy to the surroundings as heat. Because it is destroyed in the reaction, abundant dietary carotenoids are necessary for good health. They have been shown to reduce membrane damage to lymphoid cells, and may enable them to act more efficiently. Carotenoids are also believed to increase the total white blood cell proliferation in HIV infected humans (Boileau *et al.* 1999). However, it should be noted that oversupplementation of carotenoids can lead to diseases as a result of toxicity in both humans and animals. Signs of toxicity of these compounds include skeletal malformations, internal hemorrhaging, loss of appetite, slowed growth, and weight loss (McDowell 2000). Thus, it is important to find a balance between vitamin A deficiency and overdose. In my study, both vitamins E and A were used as a "blanket effect" to see if antioxidants would have any effect on the turkey immune system's ability to fight coccidia infections.

Avian Immune System

In order to rationalize the effects of antioxidants the turkey's immune response, a brief review of the avian immune system is warranted. Butcher and Miles (1991) describe the avian immune system as possessing both non-specific and specific mechanisms. Non-

specific mechanisms are considered to be the innate defenses that birds possess to assist them in initially resisting disease. Prominent examples of inherent mechanisms include body temperature, skin and mucous membranes, friendly microorganisms living in the skin and gut, ciliated membranes, age, and the environment in which the birds live. Specific, or acquired, mechanisms act as the second line of defense once invading pathogens enter the organism. These are further divided into humoral and cellular components. Humoral components include the production of immunoglobulins (antibodies) and the lymphocytes that make them. Antibodies prevent antigens from binding to their target cell by blocking receptors on the invading organism (Butcher and Miles 1991). Antibodies are produced by B-lymphocytes, which originate in the embryonic liver, yolk sac, and bone marrow, but mature in the bursa of Fabricius. The bursa of Fabricius is present in young birds and gradually declines in mass. It disappears completely upon reaching sexual maturity (Møller *et al.* 1998). Cellular components involve the activity of T-lymphocytes toward the elimination of foreign pathogens. T-lymphocytes arise the same way as B-lymphocytes but mature in the thymus. The primary lymphoid organs include the thymus and the bursa of Fabricius, while secondary lymphoid tissues include the spleen, bone marrow, and lymph nodes (Harrison 2007). Understanding the importance of both non-specific and specific mechanisms of immune function is needed in order to best protect avian livestock from diseases such as coccidiosis (Butcher and Miles 1991).

Coccidia

Coccidia are protozoa that survive as intracellular parasites in a wide range of animal species, including humans. They damage their host species by proliferating within the epithelial lining of the gastrointestinal tract. Over 1500 species of coccidia encompassing 37

genera have been defined (Levine 1982). Out of these, the species of the genus *Eimeria* are of greatest importance to agriculture. *Eimeria* species are known for being very specific to their host (Chapman 2008), and even within the same host they may be restricted to specific organ systems, narrow zones within an organ system, and even to specific kinds of cells in that zone. Most coccidia inhabit the digestive tract (Schmidt and Roberts 1985). Coccidia that infect turkeys include *Eimeria meleagridis*, *E. meleagrimitis*, *E. gallopavonis*, *E. innocua*, and *E. subrotunda*. All of these species develop at specific sites in the host small intestine, with a few also developing in the cecum and rectum (Chapman 2008).

The life cycle of *Eimeria* occurs in two distinct phases: a reproduction phase within the host, and a sporulation phase outside of the host. The life cycle of *E. tenella*, a species infecting chickens, is shown in Figure 2. A mature coccidian oocyst contains up to four sporocysts, with each sporocyst containing two sporozoites. Infection begins when a host ingests mature oocysts. Exposure to bile and trypsin in the digestive tract causes the sporozoites to emerge from the oocyst and enter the intestinal wall, where they mature over 3 – 5 days (Levine 1982). All seven species of *Eimeria* that infect turkeys cause macroscopic lesions to appear in the intestine as a result of infection. In subsequent infections, these lesions can spread throughout the intestine (Chapman 2008). The newly formed oocysts are then shed in the feces. The chronology of a typical shedding cycle is shown in Figure 3. The exposure to an oxygen-rich environment causes the parasite cell to enter a reduction phase where it sporulates (Levine 1982). This sporulation process takes about 24 hours (Allen and Fetterer 2002 b). Now fully matured, it is capable of continuing the cycle of infection (Levine 1982).

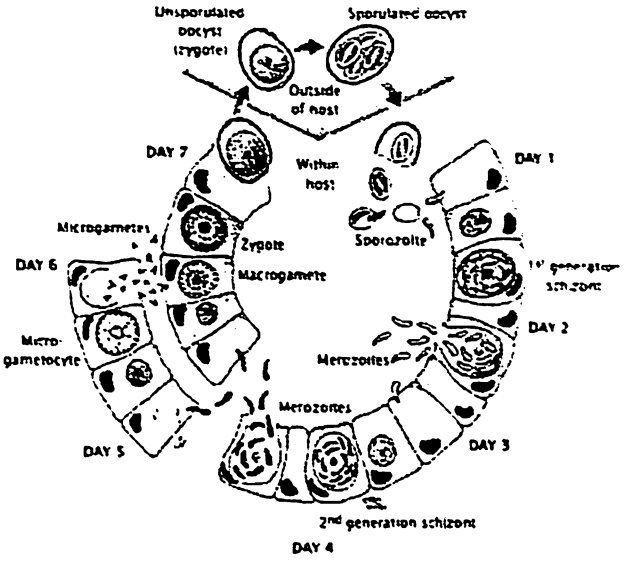

Figure 2: Overview of the life cycle of *E. tenella*, including phases in and out of the host organism. Source: Vermeulen (2004). Avian coccidiosis: a disturbed host-parasite relationship to be restored. Host-Parasite Interactions. p. 211-241. G.F. Wietgertjes and G. Flik, eds. Garland Science/BIOS Scientific Publishers: Oxford.

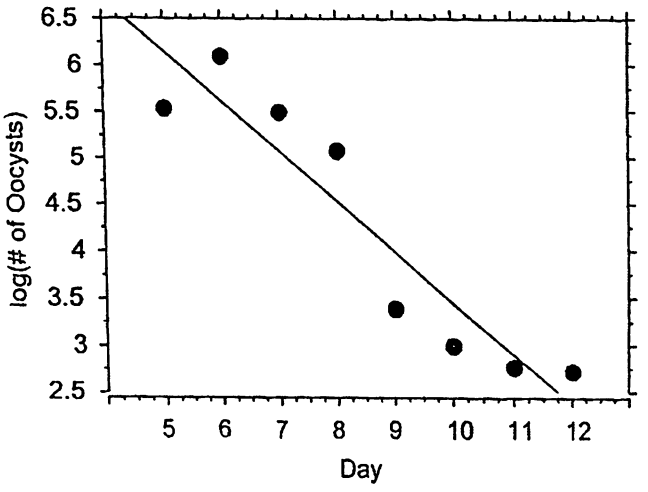

Figure 3: A typical shedding cycle for turkey coccidia. Infection usually peaks about 24-48 hours after the first oocysts are shed and then gradually declines. Source: Harrison (2007). Growth and immunocompetence in parasitized domestic turkeys (*Meleagris gallopavo*): is there a trade-off? Honor's Thesis. University of Mississippi, University, MS. 33 pp.

Coccidia infections in turkeys negatively affect host health in a variety of ways. Pathophysiologically, these effects include malabsorption, dehydration, reduced feed intake, reduced growth, poor feed conversion, and high mortality. Clinical signs of infection include loss of appetite, listlessness, huddling, drooping wings, and ruffled feathers. Additionally, bloody diarrhea may develop (Chapman 2008).

MATERIALS AND METHODS

Preparation of Pure Oocyst Strains

Harrison (2007) notes that pure genetic strains of coccidian oocysts present a more common and consistent threat to the host organism than those consisting of multiple strains. Single-species cultures from Dr. Buchholz's lab, developed originally by LeBlanc (2005), were used to prepare sporulated coccidian oocysts for use in experimental infections following the method described by Harrison (2007). First, a fecal sample containing unsporulated oocysts, about enough to fill a 532 mL plastic cup, were collected from an infected turkey. The sample was moistened with an ample amount of reverse osmosis (RO) water, then mixed with a 2.5% potassium dichromate ($K_2Cr_2O_7$) solution and strained to remove most solid matter, which was discarded. This solution was then oxygenated for no less than 48 hours by bubbling air from aquarium pumps. To isolate large numbers of oocysts, 1 mL of the fecal solution was placed into a 15 mL glass centrifuge tube and filled to the top with 14 mL of 70% sucrose solution (w/v). After being capped with a microscope cover slip (12-540B; Fisherbrand), the tubes centrifuged at 2 000 RPM for ten minutes. Oocysts are less dense than the sugar solution and will float to the top of the tube where they will stick to the cover slips. The cover slips were gently rinsed with RO water to collected sporulated oocysts at high concentrations in water. All recorded data was collected from experimental infections. All turkeys used in the experiment were euthanized under humane conditions by carbon dioxide asphyxiation. The research was conducted under IACUC approval 09-021.

Rearing Conditions

Study subjects (Ideal Poultry Breeding Farms, Cameron, TX) were reared indoors in 81 x 97 x 30 cm, electrically heated poultry brooders with raised wire floors. All birds were fed with a standard manufactured chicken feed (DuMOR Chick Starter 24%) and given water once daily. Cages were cleaned once daily in the afternoon. The birds inadvertently contracted a nematode infection soon after arrival, which made it necessary to treat them with piperazine to eliminate the nematodes prior to inoculation with coccidia.

From 4–6 weeks of age, the birds were assigned to one of two treatment groups (control, vitamin-supplemented) at random and in equal numbers (n = 11). They were individually marked and measured for weight and tarsal length. Birds assigned to different groups were housed in separate cages. The sexes of the poults were not known during assignment. The photoperiod in the room was also adjusted. According to LeBlanc (2005), peak shedding in the feces for *Eimeria* occurs in the hours immediately preceding full daylight. To allow fecal samples to be collected conveniently, the photoperiod was set so that the room was dark from 0700-1900 hours and lit from 1900-0700 hours the next day.

Treatment and Infection

On Day 1, soft gel caps of vitamins A (as β-carotene) and E (as α-tocopherol) (GNC Products, Pittsburgh) were added into the supplemented group's diet by mixing them in with the drinking water. Minimum levels of the two vitamins are 5,000 IU/kg and 12 IU/kg diet, respectively (National Research Council 1994). By supplementing their water, vitamin intake was increased to approximately 12,091 IU/kg and 29 IU/kg diet, respectively. Once treatment began, both groups were given the same quantities of feed and water at the same

time each day. On Day 6, all birds were fed approximately 12 000 oocysts by mouth from a prepared palatable sugar solution

Monitoring Procedures

Collection of fecal samples began on Day 10 between 1300 and 1900, occurring immediately following the daily cleaning of the cages. Each bird was selected at random from within its group and placed into an individual 5 gallon (19 L) plastic bucket lined with newspaper for a minimum of one hour. Due to a lack of buckets and space, all birds could not be accommodated at the same time. The group chosen first for isolation alternated with each successive day. At the end of one hour the bucket was checked to see if a fecal sample was present. If so, the bird was transferred to a holding container that was separate from the main cage until all birds from the same group had been isolated, at which time they were returned to their respective brooders. Buckets were cleared of fecal material and lined with clean newspaper between occupants. Any bird that had not produced a fecal sample after one hour was left in the bucket for an additional hour until a sample was produced. In order to increase the likelihood of producing a fecal sample within the first hour, all birds were fed approximately 30 minutes before beginning isolation procedures each day. All work performed during the dark photoperiod was done using low intensity red LED flashlights so as not to disturb the birds' photoperiod.

Analysis of Fecal Samples

Fecal samples were collected from Days 10 to 16. Entire samples were collected into small, individual plastic cups labeled for specimen number and date. Samples were weighed, mixed with an equal mass of RO water, and placed into a freezer until they could be analyzed at a later date. Upon thawing, the frozen sample was again mixed with a mass of RO water

equal to the original weight of the sample, giving the final sample a 2 : 1 ratio of water to feces. Samples were mixed well and a 1 mL sub-sample was placed in a small, stainless steel tea strainer over a clean plastic cup. Using a clean plastic spoon, the sample was pressed down firmly to express as much liquid as possible (about 1 mL). Oocysts were isolated from the strained liquid similarly to the procedures described previously. Using a microscope (Olympus BX40, 100X magnification), each sample was examined for the number of oocysts per gram of feces by means of a direct count method.

Blood Sampling and Collection of Other Tissues

On Day 17, blood samples were taken from the turkeys' left wing vein and subsequently analyzed for packed cell volume of the erythrocyte and buffy layers following the methods described by Harrison (2007). All birds were euthanized, weighed, measured for tarsal length, and then frozen. Within two days of death, the subjects were dissected to remove and weigh the spleen, bursa of Fabricius, and testes (if male).

Statistical Analyses

All statistical analyses were performed using Stat View computer software (SAS Institute Inc. 1998). Data were first analyzed with a standard normality test (Komolgorov-Smirnov) to confirm a normal distribution curve. Normally distributed data permitted the use of an analysis of variance (ANOVA), using both the sex and the assigned treatment group as factors. Any interactions that were found to be significant were then analyzed with a t-test. The dependent variables were patterns of oocyst shedding, organ mass, change in body condition, and erythrocyte and lymphocyte layer fractions.

RESULTS

Oocysts were shed in the feces starting on Day 11 for both groups (Figure 4). In the supplemented group, the infection reached a peak on Day 12 before falling steadily. The control group experienced a sudden spike in oocysts on Days 14 and 16. There was not a significant relationship between treatment and the time it took to reach minimum oocyst shedding (ANOVA, $F = 0.085$, $p = 0.7748$). Results are summarized in Table 1.

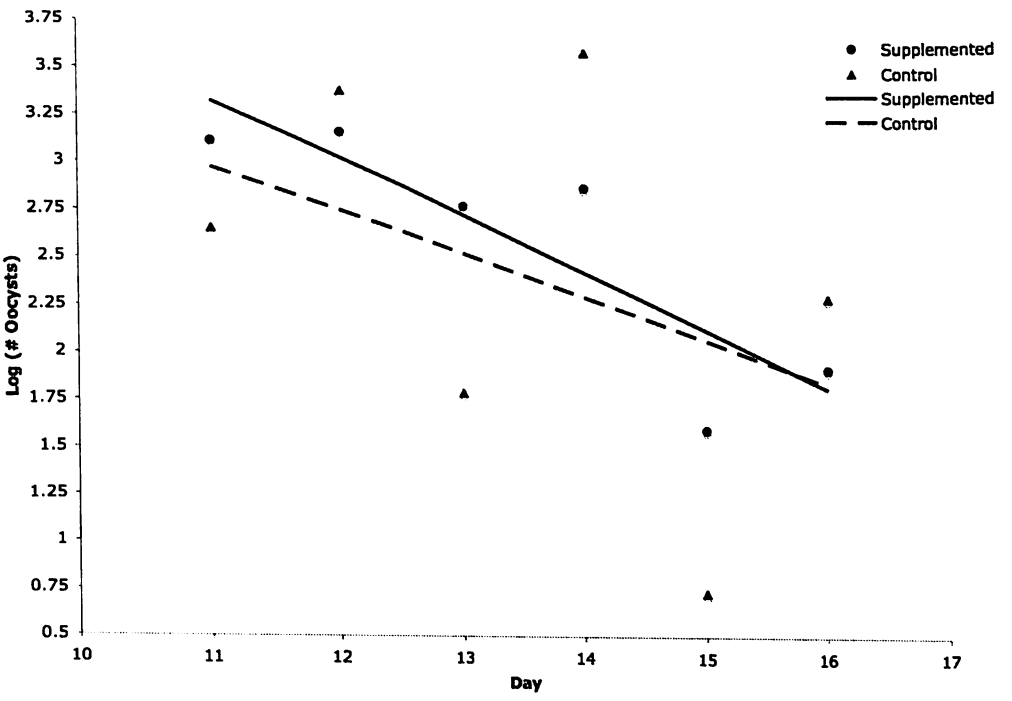

Figure 4: The control and supplemented turkeys did not differ significantly in the time it took to reach minimum oocyst shedding (ANOVA, $F = 2.872$, $p = 0.1139$).

Table 1: Measures of parasitism did not vary significantly between treatment groups.

Dependent Variable	Supplemented[a]	Control[a]	DF	F-Value	P-Value
Peak Oocysts[b]	3175 ± 705	5597 ± 2294	1, 13	0.395	0.5405
Time to Peak Shedding[c]	0.916 ± 0.039	0.857 ± 0.057	1, 13	0.402	0.5371
Time to Minimum Shedding[d]	2.364 ± 0.472	2.091 ± 0.392	1, 13	2.872	0.1139
Average Daily Oocysts[e]	689 ± 149	1256 ± 506	1, 13	0.359	0.5593

[a]Values are means ± standard error
[b]Maximum recorded value of oocysts shed
[c]Hours measured from the first recorded shed oocysts until the peak shedding
[d]Hours measured from the peak shedding until minimum shedding
[e]Average day-to-day parasite load for each subject

There was little change in body condition between the two groups (Figure 5). While the average masses of both the bursa of Fabricius and the spleen were lower in supplemented turkeys, this difference was not statistically significant (ANOVA, bursa: $F = 0.878$, $p = 0.3659$; spleen: $F = 0.003$, $p = 9588$). These results are summarized in Table 2.

Hematocrit analysis showed a significant interaction of treatment and sex on the white blood cell fraction (ANOVA, $F = 4.716$, $p = 0.05$) (Figure 5). Supplemented females showed a significantly higher white blood cell fraction (t-test, $t = -3.278$, $n_1 = 3$, $n_2 = 8$, $p = 0.001$). This suggests that sex hormones may play a synergistic role with antioxidants in lymphocyte production. There was no corresponding result with the erythrocyte fraction. Errors in data collection resulted in only 16 subjects being used in these analyses. In addition to these results, other non-significant statistical values were reported in Appendix I.

Table 2: Changes in neither growth rate nor organ mass were significantly different between treatment groups.

Dependent Variable	Supplemented[a]	Control[b]	F-Value	DF	P-Value
Change in Body Condition[b]	84.89 ± 10.09 g/cm	79.68 ± 5.37 g/cm	0.597	1, 13	0.4535
Spleen	1.22 ± 0.09 g	1.28 ± 0.12 g	0.003	1, 13	0.9588
Bursa of Fabricius	1.42 ± 0.10 g	1.46 ± 0.16 g	0.878	1, 13	0.3659

[a]Values are means ± standard error
[b]Body condition = body mass / tarsus length

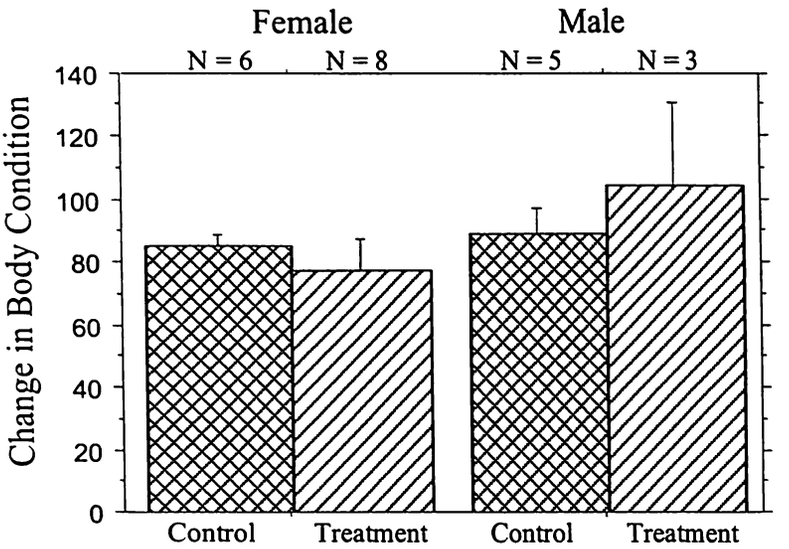

Figure 5: There was no significant difference in the change in body condition between the two treatment groups (ANOVA, F = 0.597, P = 0.4535).

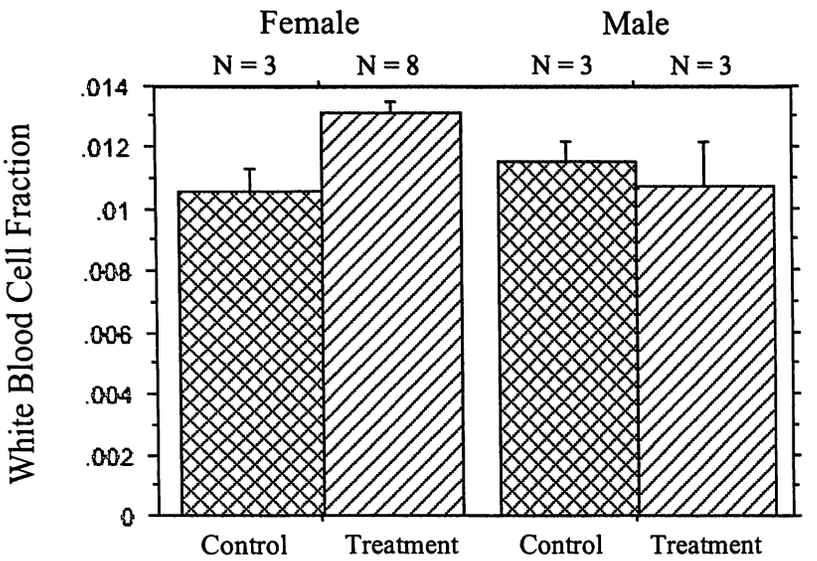

Figure 6: There was a significant interaction of treatment and sex on the white blood cell fraction (ANOVA, F = 4.716, p = 0.05). Because of errors in data collection, only 16 subjects were used in this analysis.

DISCUSSION

My first hypothesis was that antioxidants would directly improve the ability of the turkey immune system to fight parasitic infection. Antioxidants had a significant, sex-dependent effect on white blood cell proliferation. Thus my hypothesis that antioxidants would boost the immune response of turkeys was accepted only for female subjects. Furthermore, no relationships in the data supported my second hypothesis that antioxidants improved the general health of turkeys facing coccidia infections even if it did not enhance immunity.

The exact effect of antioxidants towards the treatment of coccidiosis in birds is unclear, and at times contradictory. There are studies that confirm that antioxidants have a positive correlation with immunocompetence. Bendich (1990) reports on one such experiment in which vitamin E supplementation significantly decreased mortality and increased weight gain in chickens infected with *E. tenella*. On the other hand, other research suggests that an increase in oxidative stress in birds creates an environment detrimental to parasite development. Allen and Danforth (1998) found significantly reduced lesion scores from *E. tenella* infections in chickens that were fed n-3 fatty acids and menhaden oil. These two supplements also significantly reduced plasma carotenoids in uninfected chickens, indicating that they promoted a state of oxidative stress. Another study by Allen and Fetterer (2002 a) showed that coccidiosis might cause a malabsorption of α-tocopherol, which also suggests that oxidative stress is in fact linked with this disease.

It is possible that there was no significant observed effect because the vitamin level administered was simply not large enough to make a substantial difference. The levels of vitamins A and E chosen for this study were based on twice the recommended amount for young turkey poults (National Research Council 1994). Vitamin E is known to have very low levels of toxicity because it is not stored in the liver like other fat-soluble vitamins, so I could have used higher doses. Side effects of overdose in humans are rare, even with doses over 1200 IU/day (Papas 1999).

Schildknecht and Squibb (1979) conducted an experiment similar to my study to test the effects of vitamins A, E, and K on infections of *Histomonas meleagridis*, another protozoan parasite, in young turkey poults. They used 5x and 50x the recommended dietary levels of each vitamin and found significant reductions in mortality and lesion scores when these compounds were administered in conjunction with ipronidazole. I would recommend that future studies of vitamin effects on *Eimeria* infections use similar high dose methods.

My most interesting result was the possible role of sex in the antioxidant-enhanced immune response. A possible explanation for this is that female sex hormones could work to enhance antioxidant function. Demirbag *et al.* (2005) assert that estrogens cause an increase in antioxidant vitamin levels as well as increase the activities of antioxidant enzymes themselves, thus contributing both directly and indirectly to antioxidant capacity. They cite an experiment in which total antioxidant capacity (TAC) levels were shown to be higher in premenopausal women than those in menopause as well as men with normal testosterone levels. According to the study, plasma estradiol had a positive correlation with TAC levels. As it relates to turkey coccidiosis in this experiment, estrogens may have further enabled

vitamins A and E to suppress oxidative damage by increasing the proliferation of lymphocytes.

Compared to other sex hormones, only estrogens have been found to have natural antioxidant ability. It is believed that they can inhibit the production of superoxide, but the reaction mechanism for this group of compounds has yet to be fully understood (Halifeoglu *et al.* 2003). Studies have found similar results in avian species. Halifeoglu *et al.* (2003) discovered increased levels of vitamins A, E, and C in the serum of chickens that received estrogen or testosterone, with the greatest results measured in the former. While they believed this to be related to increased estrogen binding proteins in the plasma, no conclusive findings were reported.

In comparison to estrogen, not as much research has been conducted on the relationship of testosterone towards antioxidant capacity. In terms of general health, research has shown testosterone to be immunosuppressive in some animals, including fish and mammals. The relationship of this hormone in comparison to avian immunocompetence, however, remains unclear. Some studies suggest that testosterone is immunosuppressive in birds, while other studies provide no support for such a relationship (Hudman *et al.* 2000). Huff *et al.* (1999) inoculated immunosuppressed turkeys with *E. coli* and found significantly higher development of airsacculitis and marginally higher mortalities in males than in females. This suggests that there is some difference in the stress response of female turkeys that provides protection from the effects of extreme stress on resistance to bacterial infection.

In conclusion, while there may be an antioxidant-related benefit to the turkey immune system toward coccidia infection, the advantage provided by antioxidants alone is not very obvious. Despite a variety of tests that were conducted across several measures of immune

response, the only significant increase that I observed was sex-dependent. In addition, there were no differences to suggest that antioxidants promote the overall health of the bird by themselves. Finally, some other studies that have shown correlations between antioxidants and immunocompetence toward parasitic infections did so when antioxidants were not the only factor being used in treatment.

I would recommend that future research on turkey coccidiosis focus on the use of antioxidants in conjunction with some other variable in an attempt to elicit a compound response. An example would be administering vitamin E with estrogen supplementation in female turkeys, or providing turkeys with an antioxidant-enhanced diet in addition to treating them with an anticoccidial drug. In addition to this, the existence of a possible sex-linked response to *Eimeria* infection should also be considered.

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APPENDIX I

Peak Oocysts

ANOVA Table for Peak Oocysts

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	11427121.298	11427121.298	.615	.4469	.615	.109
Treatment	1	1836060.261	1836060.261	.099	.7582	.099	.060
Sex * Treatment	1	7338067.668	7338067.668	.395	.5405	.395	.088
Residual	13	241464517.542	18574193.657				

Time to Peak Infection

ANOVA Table for Slope

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	.017	.017	.938	.3506	.938	.141
Treatment	1	.005	.005	.294	.5971	.294	.078
Sex * Treatment	1	.007	.007	.402	.5371	.402	.089
Residual	13	.242	.019				

Time to Minimum Shedding

ANOVA Table for Zero Time

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	.154	.154	.085	.7748	.085	.058
Treatment	1	.154	.154	.085	.7748	.085	.058
Sex * Treatment	1	5.191	5.191	2.872	.1139	2.872	.336
Residual	13	23.500	1.808				

Average Daily Oocysts

ANOVA Table for Average Oocysts per Day

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	860356.177	860356.177	.782	.3927	.782	.125
Treatment	1	361190.188	361190.188	.328	.5765	.328	.082
Sex * Treatment	1	395302.094	395302.094	.359	.5593	.359	.085
Residual	13	14308704.698	1100669.592				

Change in Body Condition

ANOVA Table for Condition Change

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	860.064	860.064	1.112	.3108	1.112	.158
Treatment	1	54.497	54.497	.070	.7948	.070	.057
Sex * Treatment	1	461.680	461.680	.597	.4535	.597	.107
Residual	13	10052.498	773.269				

Spleen Mass

ANOVA Table for Spleen

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	.073	.073	.594	.4548	.594	.107
Treatment	1	.087	.087	.709	.4149	.709	.118
Sex * Treatment	1	3.409E-4	3.409E-4	.003	.9588	.003	.050
Residual	13	1.595	.123				

E = x10

Bursa of Fabricius Mass

ANOVA Table for Bursa

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	.006	.006	.051	.8257	.051	.055
Treatment	1	.034	.034	.273	.6098	.273	.076
Sex * Treatment	1	.109	.109	.878	.3659	.878	.135
Residual	13	1.620	.125				

Erythrocyte Layer Percentage

ANOVA Table for Average RBC %

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	1.087E-4	1.087E-4	.233	.6375	.233	.073
Treatment	1	3.902E-4	3.902E-4	.835	.3773	.835	.131
Sex * Treatment	1	.001	.001	1.356	.2651	1.356	.182
Residual	13	.006	4.670E-4				

E = x10

Buffy Layer Percentage

ANOVA Table for Average WBC %

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Sex	1	1.769E-6	1.769E-6	.843	.3753	.843	.131
Treatment	1	2.764E-6	2.764E-6	1.317	.2718	1.317	.178
Sex * Treatment	1	9.895E-6	9.895E-6	4.716	.0490	4.716	.512
Residual	13	2.728E-5	2.098E-6				

E = x10