EFFECTS OF THE ANTICOCCIDIAL DRUG AMPROLIUM ON BROILER BREEDER PERFORMANCE AND ENTERIC HEALTH FOLLOWING COCCIDIOSIS VACCINATION

A Thesis

by

SAMANTHA KAYE POHL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	David J. Caldwell
Committee Members,	James A. Byrd
	Jason T. Lee
	Jose Linares
Head of Department,	John B. Carey

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ABSTRACT

Effects of the Anticoccidial Drug Amprolium on Broiler Breeder Performance and Enteric Health Following Coccidiosis Vaccination. (December 2010)

Samantha Kaye Pohl, B.S., Texas A&M University

Chair of Advisory Committee: Dr. David J. Caldwell

Two experiments were performed to evaluate effects of amprolium administration at specific times and concentrations in replacement broiler breeders of three genetic lines vaccinated against coccidiosis. Effects on performance parameters including body weight and flock uniformity, and post-vaccination oocyst cycling patterns were evaluated in addition to development of immunity following clinical *Eimeria* challenge according to gross and microscopic lesion scoring, post-challenge body weight gain (BWG), and total oocyst output. Experiment one was conducted on fresh pine shavings while experiment two was conducted on used litter remaining in treatment pens from the first trial.

No significant differences were seen among treatment groups with regard to body weight in either trial. Increased magnitude of oocyst shedding was observed in trial one, Line A with the group receiving amprolium on day 10. Trends in the data indicated increased uniformity in Line A related to amprolium administration following day 21. The group in Line A receiving amprolium at day 10 showed a significantly lower degree of total oocyst output following challenge than the other medicated groups. The group

receiving amprolium on day 10 in Line B showed significant reduction in post-vaccination oocyst shedding following treatment in both trials while all shedding was delayed in trial two when compared to the first trial. Effects on uniformity in Line B pullets varied between trials with trends indicating it being advantageous when used litter was a factor. Higher post-challenge BWG was observed in Line B pullets administered the low concentration at day 16 than the controls. Reductions in gross lesion development were seen in Line B pullets in both trials. Line C pullets receiving the highest concentration of amprolium at day 16 showed significantly less uniformity in trial one while the controls appeared to perform better than all medicated groups in trial two. All medicated groups in Line C exhibited delayed and increased magnitudes of oocyst shedding in trial two. These data indicate that the effects of amprolium on performance and immunity development are variable according to genetic strain and indicated that administration may be influenced by litter condition.

DEDICATION

I would like to dedicate this to my family. You are and forever will be the most important thing in my life. Without you and your support throughout the years, none of this would have been possible nor would it have mattered without you to share it, and I cannot thank you enough for that.

To my parents, you have provided unending love, countless amounts of encouragement, never once doubting my abilities, and sometimes perhaps having more faith in me than I, myself, did…

To Alex, my sister/cousin/best friend, you have forever been there to help me realize why it is that I do the things that I do and to provide the loving support and laughter that only a true sister can supply…

To all of my aunts, uncles, and cousins, you have been there every step of the way, never failing to remind me where I come from and why I love that so much…

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. David Caldwell, and my committee members, Dr. Jason Lee, Dr. J. Allen Byrd, and Dr. Jose Linares, for their guidance, support, and patience throughout the course of this research. Throughout the course of my studies I have come to recognize all of you as close friends more than advisors, and I believe that is due to the caring and humble nature with which you present yourselves. Thank all of you for setting an example of whom and what I hope to one day be. Dave, you have taught me many things, both in the classroom and out, of which I will be forever grateful. I can honestly say that I would not be where I am today without you and the opportunities that you have provided for me throughout the past four years.

I would also like to thank all of those who have supported my efforts throughout the course of my time here. Dr. Morgan Farnell, Leslee Oden, T.J. Klein, Kendre Stringfellow, Sadie Horrocks, Amanda Jordan, Joe Klein, Stephanie Iselt, Kevin Ellis, Jacob Coppedge, Steven Gottselig and Sid Anderson—all of you have, at one time or another, fought through early morning workdays, filth, and feces, and (almost) always with a smile on your face. We have, together, created many memories which will remain with me forever. I thank each and every one of you from the bottom of my heart for your unending support, laughter, advice, and most importantly, your friendship. Without all of you none of this would have been possible.

Special thanks are also offered to Dale Hyatt, Melvin Carter, Jacob Prukop, and the rest of the farm crew at the Texas A&M Poultry Research Center. Thank you all for keeping our facilities going and for always helping me make sure the birds were taken care of.

Last, but certainly not least, thanks to my family and friends for their unending support and love. Without you all none of this would have been possible.

TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

TABLE Page

LIST OF FIGURES

FIGURES Page

CHAPTER I

INTRODUCTION

Avian coccidiosis is an enteric disease affecting the commercial poultry industry. This disease is caused by obligate intracellular protozoan parasites of the genus, *Eimeria*. To date, nine species of *Eimeria* have been found to infect chickens including *Eimeria maxima*, *E. acervulina*, *E. mitis*, *E. praecox*, *E. mivati*, *E. tenella*, *E. brunetti*, *E. hagani*, and *E. necatrix* (McDougald and Fitz-Coy, 2008). These parasites are responsible for invading the epithelial lining of the intestinal mucosa, resulting in pathological changes ranging from local destruction of the mucosa and underlying tissues to systemic effects such as blood loss, shock syndrome, and in some cases death (Vermeulen et al., 2001). Coccidiosis infection within the industry results in dramatic economic losses each year, costing in excess of 800 million dollars (Allen and Fetterer, 2002). Costs are largely attributed to vaccination, prophylactic medication, and production losses associated with morbidity and mortality (Dalloul and Lillehoj, 2005; Williams, 1998). Issues with avian coccidiosis have existed for more than sixty years, and despite constant advances in therapy and prevention these issues continue to plague the industry for a number of reasons. The primary reasons being the nature of the parasite including its life cycle and mode of transmission in combination with host behavior and rearing environment. The coccidian life cycle consist of three primary phases including sporogony, merogony, and gametogony (Lillehoj and Lillehoj, 2000). The infective oocyst resides in the intestine of the bird and is shed in the feces wherein it

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This thesis follows the style of Poultry Science.

undergoes sporogony to develop into the infective stage of a sporozoite. Each oocyst leads to the development of four individual sporozoites contained within the sporulated oocyst. This sporulated oocyst is ingested by the bird from the litter, the individual sporozoites are released following ingestion and subsequently develop into oocysts within the digestive tract that are shed in the feces once more. The specifics of the life cycle of each species of *Eimeria* can often be used as a valuable diagnostic tool when determining what species is responsible for the infection present (McDougald, 1998).

Multiple routes of control are currently available to the poultry industry including various types of anticoccidial drugs that can be administered via feed or water, as well as various types of vaccination. A number of anticoccidial drugs have been employed at different times throughout the past 50 to 60 years, and include both ionophorous antibiotics produced by fermentation and synthetic compounds produced via chemical synthesis (Chapman, 1999).

Vaccination has been used since the 1950s as an effective means of control for avian coccidiosis (Edgar, 1958; Shirley and Bellatti, 1988). In the modern poultry industry, vaccination is most commonly utilized in broiler breeders and laying flocks. Its use in commercial broiler operations has thus far been limited due to the negative effects on growth and feed conversion initially seen in vaccinated birds. The mode of action behind coccidiosis vaccination is the induction of an immune response that is responsible for enabling the birds to resist future challenges with virulent strains of *Eimeria* spp. without causing detrimental levels of infection (Chapman et al., 2005). A single initial infection induced in an immunocompetent bird can be responsible for a

certain degree of immunity to reinfection, and is the reasoning behind coccidiosis vaccination (Rose & Long, 1962). For complete protective immunity to be conferred, birds must be reexposed to the same strain of *Eimeria* initially used in vaccination. This is achieved in the litter environment as the birds are exposed to the sporulated oocysts released in the feces following the initial cycle and shedding (Chapman et al., 2005).

One chemical anticoccidial that has been used for control and prevention of coccidiosis is the chemical amprolium which acts as a thiamine analog that competitively inhibits the active transport of thiamine, negatively affecting *Eimeria* species without harming the bird due to the comparatively greater sensitivity of the parasite than the host to this exclusion. Use of this chemical in pullet replacement flocks via water application has shown to be effective in alleviating the symptoms caused by coccidiosis infection without negatively affecting immunity development (Ruff and Chute, 1991). The primary objective of this research was to determine what type of effect the administration of amprolium following industry standard methods of coccidiosis vaccination would have on the development of immunity and flock performance, body weight gain and uniformity, within a replacement pullet flock.

CHAPTER II

REVIEW OF LITERATURE

In today's poultry industry, *Eimeria* species are the cause of extreme economic losses that have plagued the industry for many years. Various aspects of the organism including its life cycle, host environment, and the resilient nature of the parasite have led to recurring issues associated with losses in body weight gain (BWG), decreases in flock uniformity, and decreases in feed efficiency in addition to other performance parameters associated with egg producing breeds such as laying stock and replacement breeders. Research is and has been constantly taking place in effort to discover new and improved ways to control and alleviate the symptoms resulting from infection by this parasite.

The issues associated with coccidiosis in replacement broiler breeders have led to a variety of treatment and prevention methods including both vaccination and anticoccidial programs. In some cases, the two of these are combined in order for the anticoccidial therapy to alleviate the performance losses associated with coccidiosis vaccination. The following review discusses the specifics of the organism including life cycle, specificity, and pathogenicity, along with coccidiosis vaccination and anticoccidial treatments.

History

Coccidia possess a somewhat complicated history in the story of how they came to be a part of the taxonomic classification of which they are currently recognized. The first coccidia were observed by Leeuwenhoek in the late 17th century and consisted of oocysts that were found in rabbit bile (Levine, 1982). As a whole, the genus known as

Eimeria is the largest of the Eimeriidae family and belongs to the phylum Apicomplexa of the subkingdom Protozoa which is characterized by the presence of an apical complex in the sporozoite stage of the parasite. All apicomplexans are characterized as intracellular parasites (Levine, 1982; McDougald and Fitz-Coy, 2008). Members of the genus, *Eimeria*, are classified as having oocysts with four sporocysts, each with two sporozoites, and are considered homoxenous, meaning that all endogenous stages occur within a single host. Of this genus there are approximately 1200 named species, capable of infecting and causing disease in a wide range of host organisms (Current et al., 1990). Coccidia of this genus are primarily host-specific with certain species infecting only a single host species or a group of closely associated hosts (Conway and McKenzie, 2007). Originally, the disease in chickens was believed to be caused by a single species, *Eimeria avium* (Edgar, 1958). However, research performed by Tyzzer (1929) elucidated the fact that multiple species of *Eimeria* were capable of causing the disease in chickens as well as in other species. There are currently nine species of *Eimeria* known to parasitize chickens: *Eimeria acervulina, E. brunetti, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox, E. hagani*, and *E. tenella* (McDougald and Fitz-Coy, 2008).

Life Cycle

The nature of the coccidial disease contrasts with that of diseases caused by bacteria and viruses due to its self-limiting nature (McDougald and Fitz-Coy, 2008). An example of this characteristic of coccidiosis was seen in surveys for coccidia present in broiler houses in Georgia when oocysts were shown to build up during the initial growth

of a flock and then to decrease as the birds gradually gained immunity to further infection (Reyna et al., 1983). The omnipresent nature of poultry coccidia precludes the possibility of elimination from the environment or prevention of exposure by quarantine, disinfection, or sanitation (Calnek, 1997).

The life cycle is comprised of asexual and sexual as well as parasitic and non-parasitic phases and consists of both internal and external stages of development (Trees, 2002; Hafez, 2008). Life cycles of the various *Eimeria* species affecting chickens vary slightly in the number of asexual generations and the time required for each of the developmental stages, but are, in general, rather typical among all species (McDougald and Fitz-Coy, 2008). The three main phases comprising the life cycle include that of the exogenous sporogony, and the endogenous merogony and gametogony (Hammond and Long, 1973).

The internal stage is composed of all those parts occurring within the body of the host, beginning with the initial ingestion of the infective oocyst and ending with the excretion of the resulting oocysts in the fecal contents and includes the phases of schizogony (merogony) and gametogony. Following the ingestion, the sporulated oocyst is crushed in the gizzard leading to the release of the formed sporocysts contained within. Following their release, the actions of trypsin and bile in the duodenum serve to activate and release the sporozoites which then invade the epithelium of their designated region of the intestinal tract to develop and become trophozoites (McDougald, 1998). Prior to the clinical phase of infection, the parasite proceeds to the schizogony phase where multiple generations of daughter parasites known as schizonts are formed to move

onto the sexual phase of the life cycle. The number of generations formed is dependent upon genetically predetermined characteristics of each individual species of *Eimeria* which can vary from two to four depending on the actual species (Current et al., 1990; Trees, 2002). These gametocytes differentiate into biflagellated microgametocytes and macrogametocytes. The microgametocytes divide asexually by multiple fission to form a large number of flagellated microgametes which migrate to the macrogametocytes in order to form macrogametes—this is the phase of gametogony (Levine, 1982). These macrogametes then go on to be fertilized to form zygotes in the intestinal epithelium. Following the maturation of the formed zygote and the formation of the oocyst wall from the intracytoplasmic granules coalesced among the periphery of the oocyst; the mature oocyst is released into the intestine and excreted in the fecal contents of the bird (Trees, 2002; McDougald, 1998).

The external stage is centered on the excreted oocyst and its development into an infective sporulated oocyst. This part of the *Eimeria* life cycle is imperative, as reinfection cannot occur if the oocysts are not sporulated prior to ingestion (Hafez, 2008). After approximately 24 hours in the warm moist litter of the poultry house, oocysts sporulate and enter their infective state which consists of an oocyst containing four cysts known as sporocysts which each contain two infective parasites known as sporozoites (Fetterer and Barfield, 2003). In order for an oocyst to undergo sporulation there are certain conditions that are required. These conditions center on warmth, moisture, and oxygen. For optimal sporulation conditions, the ideal temperature range is from 25 to 30°C, while freezing and heat in excess of 56°C are lethal (Trees, 2002).

While the ideal temperature range is relatively narrow, the additional conditions required for optimal growth are not as specific which leads to oocysts remaining viable in poultry litter for many months, and in some cases years (Hafez, 2008). It is generally accepted that conditions of moist litter are favored for sporulation to take place (Card and Nesheim, 1972). However, studies conducted to compare the optimal level of litter moisture have shown that this is not always the case. Results have shown that oocysts of *Eimeria acervulina* vary only slightly in their rate of sporulation according to relative humidity (Graat et al., 1994). Additionally, Waldenstedt and colleagues (2001) performed similar studies regarding the sporulation of oocysts of *E. maxima* which showed that their sporulation actually favored drier conditions.

In theory, according to the number of sporozoites in the mature, sporulated oocyst and taking into account the number of asexual and sexual reproduction stages that are involved in the life cycle of the *Eimeria* species, each oocyst is capable of producing 2,520,000 second generation merozoites. Each of these is able to develop into a macro- or microgamont, but the actual number of oocysts produced per oocyst fed is considerably lower than that which is possible (Levine, 1982).

Due to the nature of the life cycle of *Eimeria* species, the most common route of transmission is direct fecal-oral transfer, wherein the sporulated oocysts are ingested from the environment. Sporulated oocysts may also be transmitted via mechanical routes by wild birds, insects or rodents that may be present in the environment, and by contaminated clothing, footwear, equipment, or dust (McDougald and Fitz-Coy, 2008).

Eimeria are both host specific, with the denoted species affecting only domestic chickens, and site specific, meaning that certain species parasitize specific regions of the gut and intestinal epithelia. Certain exceptions have been noted, but only under experimental conditions such as those demonstrated by McLoughlin (1969) in his attempts to transmit *E. tenella* to turkeys and *E. meleagrimitis* to chickens. The physiological reasoning behind the host specificity associated with this parasite is largely unknown, but generally understood to be a combination of genetic, nutritional/biochemical, and immune factors (Yun et al., 2000). It should also be noted that while species of *Eimeria* are site specific and vary in the severity of their pathogenicity, interactions between different species as well as the condition of the host can have variable effects on the actual level of pathogenicity exhibited (Fernando, 1982). Investigations of this topic have led to the conclusions that while combined infections of varying concentrations of *E. acervulina*, *E. brunetti*, and *E. maxima* led to increased weight loss, competition between the species led to reduced oocyst production by individual species except in the instance that the species possessed entirely different infection sites (Hein, 1976).

One of the most common species of *Eimeria* encountered in commercial poultry is *E. acervulina* which primarily attacks older chickens or replacement hens and possesses a low reproductive potential, meaning that the immunity which develops following infection is relatively weak (Pellérdy, 1974; Conway and McKenzie, 2007). This species manifests primarily in the mucosal epithelial cells of the duodenal loop, with gross lesions in light infections limited to that specific area. During heavy

infections, however, it is possible for lesions to be found lower in the intestinal tract and to result in the destruction of the villous tips (McDougald and Fitz-Coy, 2008). *E. acervulina* is reported to have two basic effects on the gut: an alteration of the intestinal structure and activity which leads to disturbances in absorption and intestinal permeability, as well as an indirect effect leading to reductions in feed and water consumption (Yvoré, 1972). Mortality associated with *E. acervulina* infections is typically low, but due to the fact that infection disrupts normal digestive functions, affected birds show weight loss and reduced egg production (Pellérdy, 1974). Another species which has a tendency to colonize the upper region of the small intestine is *E. mivati*. This species was originally identified as, and continues to be, incorrectly diagnosed as a strain of *E. acervulina*, but was later named a separate species (Edgar and Seibold, 1964; Conway and McKenzie, 2007). While *E. mivati* is considered to primarily infect the upper duodenal region of the intestine, this species is also commonly found throughout the intestinal tract extending even as far as the ceca and cloaca in the most severe infections. *E. mivati*, like *E. acervulina,* affects the mucosal cells of the intestinal villi, but unlike *E. acervulina*, can be commonly found through the entire length of the villi (McDougald and Fitz-Coy, 2008). Although infections of *E. praecox* are not typically known to result in notable gross lesion development, this species is recognized as one which affects the epithelial cells of the sides of the villi inhabiting the duodenal loop (McDougald and Fitz-Coy, 2008).

There are two species of *Eimeria* that are noted for their infection in the mid-small intestine. *E. maxima* is considered one of the most commonly occurring species in

chickens while *E. necatrix* is referred to as one of the most virulent species afflicting chickens. Both occur in the midgut, however *E. maxima* is most often found superficially in the epithelial cells of the intestinal mucosa with hemorrhages being found near the tips of the villi. *E. necatrix* is a more invasive species with affected birds showing submucosa and lamina propria containing stages of coccidial development (McDougald and Fitz-Coy, 2008). Another interesting characteristic of *E. necatrix* is its tendency to show different stages of development in different regions of the intestine, with the schizogonous generations occurring within the epithelium of the ileum and gametogony taking place in the surface epithelial cells of the ceca (Gregory, 1990).

The species of *Eimeria* most notably affecting the lower region of the gut is that of *E. tenella*. This is one of the most commonly known and often considered one of the most pathogenic species of coccidia due to its ability to cause large amounts of loss in commercial broilers as well as for its propensity of resulting in relatively spectacular gross lesion development. *E. tenella* is generally associated with infections in younger chickens, and in very severe situations can lead to mortality as early as 5 to 6 days of age (Pellérdy, 1974). It is only rarely that older birds develop infections from this species due to repeated exposure leading to a series of small infections which confer effective active immunity (Pellérdy, 1974). This species is known to inhabit the villar epithelial cells and submucosa of the ceca of the chicken with the schizonts developing deep in the lamina propria, disrupting the mucosa and associated blood vessels, often destroying the muscularis mucosa (Joyner, 1982; McDougald and Fitz-Coy, 2008).

Although there are inherent factors associated with each species of *Eimeria* that distinguish their respective levels of pathogenicity, a number of other factors must also be taken into account when evaluating the level of pathogenicity associated with individual species. These consist of, but are not limited to, factors associated with the internal environment of the host as well as the outside environment acting on the parasite in addition to the factors associated with the genetic details and immunological status of the host (Joyner, 1982). For example, the reaction of the host to infection is directly related to the number of sporulated oocysts which are ingested by the animal (Joyner, 1982). Increases in the number of oocysts ingested typically accompany an increase in the severity of infection (Long, 1973). Several environmental factors such as litter moisture, outside temperature, and oxygen availability are capable of affecting the viability and infectivity of oocysts as well due to the fact that sporulation, which requires certain environmental conditions, is required for the oocyst to become infective.

Diagnosis and differential identification of individual species of *Eimeria* parasitizing chickens can depend on several factors including the following: area of the intestinal tract being parasitized, the location of the parasite within the intestinal epithelium, gross appearance of the lesions resulting from infection, sporulation time, prepatent period, and morphology of the infective oocysts including size shape and color (Conway and McKenzie, 2007; McDougald and Fitz-Coy, 2008). As stated previously, the appearance of the gross lesions resulting from coccidial infection vary according to species. Lesions of *E. tenella* and *E. necatrix* are often considered to be the most notable of avian *Eimeria* species. *E. tenella* lesions typically around five days post infection and

appear as hemorrhagic or whitish lesions in the ceca. The contents of the ceca are known to coalesce, forming a semisolid core that can remain in the ceca for several days (Gregory, 1990). This is often indication of a severe infection. Lesions of *E. necatrix* occur throughout the intestine, mostly in the midgut, and often are observed as greatly hemorrhagic lesions with large amounts of blood and mucous accumulation in the intestine (Gregory, 1990). *E. maxima* is known to create a certain amount of petechial hemorrhaging in the midgut that can often be observed from the serosal surface of the intestinal tract (Gregory, 1990). Characteristic lesions of *E. acervulina* are discrete compared to certain other species, and are observed as whitish transverse lesions in the duodenal region of the intestine (Gregory, 1990).

The oocysts included in the species of *Eimeria* known to affect chickens range in their size, shape, and morphology. Sizes range from approximately 11×10μm up to 21×17μm. Of the commonly encountered species, *E. mivati* and *E. acervulina* are generally recognized as being the smallest in size; however *E. acervulina* oocysts tend to be more ovoid than spherical in shape as compared to *E. mivati*. The largest oocysts belong to the species of *E. maxima*, *E. tenella*, and *E. brunetti*. Of these three *E. maxima* and *E. brunetti* are more similarly ovoid in shape with the *E. maxima* oocysts possessing a slight yellowish shade when viewed microscopically, while *E. tenella* tends to have a more spherical appearance without color (McDougald and Fitz-Coy, 2008).

Vaccination

Options for *Eimeria* control in commercial poultry flocks are numerous, and consist of feed-based and drinking water-based anticoccidial drugs—ionophores as well

as synthetic chemicals—and live oocyst vaccination (Dalloul and Lillehoj, 2006). Problems of drug resistance, lack of new anticoccidial drugs, and consumer pressure to move away from drug use in animal feeds have forced the industry to focus more intently on the use of vaccination to combat coccidiosis (Hafez, 2008). Live oocyst vaccination was developed in the 1950s and has since been used consistently in poultry production (Edgar, 1958). Vaccines are currently used extensively in the rearing of broiler breeders and replacement layer stock (Chapman, 2000; Chapman et al., 2002). The purpose of vaccination is to induce an immune response that is capable of enabling birds to resist challenges with virulent, heterologous infections—whether they be natural or experimental (Chapman et al., 2005). Options for immunization vary widely; however, there are a limited number that are recognized as commonly used methods of control within the commercial poultry industry (Trees, 2002). The methods available include: 1) chemically modulated, natural infection wherein layer and breeder stock are raised on litter prior to sexual maturity to encourage coccidial exposure while modulating infection with anticoccidial drugs; 2) live, unattenuated vaccines administered in water that contain mixtures of unattenuated lines of oocysts of the species considered important; 3) live, attenuated vaccines that are available with all important precocious *Eimeria* species of chickens having been attenuated so that they have lost virulence, yet retain immunogenicity; 4) killed or non-living vaccines which have been an important topic of research and involve the use of vaccines containing important antigens of certain species of *Eimeria* to confer immunity to exposed birds (Trees, 2002). No matter the type of immunization administered, the objectives to be

achieved by vaccination should: induce protective immunity, be safe for target species, not pose an environmental hazard, consist of parasites of normal or low virulence that remain viable through optimal storage conditions, protect against field strains from different geographical areas, be administered by practical methods, have no negative effects on performance, be compatible with other vaccines administered, be free from all types of contaminants, be cost effective, and include drug sensitive lines in order to reduce drug resistance (Chapman et al., 2005).

Due to the fact that the immunity conferred by these vaccines is incredibly species specific, it is necessary to incorporate multiple species of *Eimeria* within the vaccine. For birds reared for extended periods of time such as broiler breeders and laying replacements, it is necessary to include certain species that might not be necessary for birds such as broiler flocks that do not live for an extended period of time. Such species include *E. brunetti*, and *E. necatrix* due to their lack of manifestation in younger flocks of birds, as well as *E. praecox* which is considered less pathogenic than other species (Chapman et al., 2005). Broiler breeders are more likely to encounter certain species of *Eimeria*, and should therefore be immunized against all possible coccidial species (Shirley & Millard, 1986; Williams, 1998).

Live, unattenuated vaccines have been suggested to induce long-lasting protective immunity by stimulating a range of immune responses found to take place when birds are naturally infected (Chapman et al., 2005); however, drawbacks of their use include the inherent pathogenicity and the fact that only limited amounts can be safely administered to young chicks. To address this issue, live, attenuated vaccines

were developed. Attenuated vaccines function under the same mechanisms as non-attenuated vaccines except the oocysts included in the attenuated vaccines include those which have been characterized as precocious by exhibiting reduced prepatent periods, decreased reproductive potential, and diminished infectivity (McDougald et al., 1986). Attenuation is most commonly achieved by *in vivo* passage of parental strains, selection for early oocyst development, and parasite irradiation (Lillehoj and Lillehoj, 2000). Killed vaccines can contain a large number of immunogens responsible for and capable of inducing humoral immune responses, however they typically lack the critical components associated with intracellular developmental stages that are needed to activate cell mediated immunity (Yun et al., 2000).

The premise behind live oocyst vaccine use is the stimulation of host immunity that develops from low level *Eimeria* infection (Yun et al., 2000). For this immunity to effectively develop it is necessary that the parasite be introduced into the gastrointestinal system of the bird for merogony and gametogony to occur, the oocyst be shed onto the litter, sporulated, and re-ingested by the bird (Chapman and Cherry, 1997).

Methods of application for live oocyst vaccines are numerous and include spray application either in the hatchery or at the farm prior to placement, inclusion in edible gel that is provided at the hatchery or farm, spraying onto the first feed given after placement, injection into amniotic fluid at late stages of embryonic development, injection into yolk sac of freshly hatched chicks, ocular administration or drinking water administration (Chapman et al., 2005). However, the more common route of application in commercial hatcheries is that in which oocysts are introduced to the bird via spray

application in a commercial spray cabinet of a predetermined volume of liquid suspension containing the oocysts (Chapman et al., 2002). Birds are then allowed to preen and ingest the oocysts. Approximately one week following vaccination, viable oocysts are excreted onto the litter for sporulation and re-ingestion, a process necessary for solid immunity development (Chapman, 2000). One of the most important criteria for choosing the best method of vaccination is to ensure that all birds receive the appropriate intended dose of the vaccine (Chapman et al., 2002).

Although live oocyst vaccination has been a successful means for the control of coccidiosis, especially in breeders and laying stock, there are concerns associated with use in the industry. One of the primary disadvantages associated with the use of coccidiosis vaccination is their association with depression of performance parameters including BWG and feed conversion early on in bird development (Mathis, 1999). This period of performance loss is associated with the mild infection that is necessary in the bird in order to stimulate immunity development (Danforth et al., 1997). Another drawback associated with use of non-attenuated vaccines is the development of clinical coccidiosis when the number of oocysts in the environment increases at a pace which exceeds that which is controllable by the development of acquired immunity (Chapman, 2000). One solution to these problems may be the concomitant administration of an anticoccidial drug which is capable of controlling the clinical infection without suppressing the development of immunity within the host (Chapman, 2000; Williams, 2002). It has been said that the combination of chemoprophylaxis with vaccination could be useful due to the fact that drug resistant strains which may be present in the

environment would be controlled by vaccination while the sensitive strains would be controlled by the drug (Danforth, 1998). There are even reports of certain vaccine manufacturers that have been shown to recommend subsequent treatment with anticoccidial drugs following vaccination in order to suppress post-vaccinal reactions (Chapman and Cherry, 1997). Another option for alleviating the decline in performance associated with coccidiosis vaccination is the use of attenuated vaccines with precocious strains of *Eimeria* organisms. These vaccines are capable of inducing protective immunity while avoiding the decline in performance that is occasionally seen in more commonly used vaccination strategies (Crouch et al., 2003).

While concomitant use of anticoccidial drugs and coccidiosis vaccination can be advantageous in certain instances, improper timing or excessive administration of anticoccidial compounds can have negative effects on the performance of coccidiosis vaccines. As stated, immunity development is reliant upon the successful cycling of the *Eimerian* parasite within the host organism, and if the parasites are incapacitated prior to the completion of their life cycle in its entirety, that immunity development is inhibited. Therefore, it is imperative that any anticoccidials administered with the intentions of alleviating the negative effects of vaccine administration be carefully employed.

Anticoccidials

It was originally discovered that certain drugs could be used in the treatment of coccidiosis in 1939 when Levine discovered the successful administration of sulfanilamide in chickens; however, treatment was not applied widely across the agricultural industry until after the Second World War (McDougald, 1982). At this point

in time, changes in the chemical industry allowed decreases in chemical production which led to successful implementation of sulfaquinoxaline for the prevention of coccidiosis in chickens (Grumbles et al., 1948). Since then, innumerable amounts of anticoccidials have been implemented at one point or another within the poultry industry. While the mode of action, method of administration, and resulting effect on the disease may vary, all of the drugs have been developed and used with the common intent of alleviating the burdens to the industry that coccidiosis has presented. Since their inception, these anticoccidial agents have led to a major growth in the poultry industry due to their ability to achieve this goal (Allen and Fetterer, 2002).

There are two separate types of anticoccidials used within the industry including synthetic drugs produced by chemical synthesis and ionophores, which are produced via fermentation. While synthetic anticoccidials operate via a specific mode of action associated with parasite metabolism, ionophores act through alteration of membrane ion transport which leads to disruption of osmotic balance within the parasite (Chapman, 1999; Allen and Fetterer, 2002). Another difference between the two classes of anticoccidials is the stage of the *Eimerian* life cycle that they are typically effective in controlling. Generally speaking, anticoccidials are known to act against the asexual stages of the parasite (Chapman, 1993). Ionophores typically target sporozoites, but merozoites have shown to be affected in certain instances as well. Effects against sporozoites are a result of the interruption of important physiological balances such as that of sodium and potassium ions (McDougald, 1990). Additionally, chemical anticoccidials are typically thought to have action against the later stages of the *Eimerian*

life cycle. Several examples exist in terms of approved drugs for both types of chemotherapy. For instance, some synthetic anticoccidials may include amprolium, nitrobenzamides, as well as folate antagonists and inhibitors while examples of ionophores include monensin, narasin, and salinomycin. Generally speaking, most drugs used in *Eimeria* control are effective against only certain species of the parasite, while not as effective against others. This has resulted in the use of a combination of drugs in many instances.

Although anticoccidials have historically proven to be effective in the battle against coccidiosis in the broiler industry, one issue that continues to contradict this effectiveness is that of drug resistance which is defined as the ability of a parasite strain to multiply or survive in the presence of concentrations of a drug that would typically destroy parasites of the same species or prevent their multiplication. Resistance has been shown to develop wherever chemicals are used extensively to control the disease, and due to the fact that anticoccidials are constantly implemented in poultry production, this is an industry that has experienced its share of issues regarding drug resistance. Medication through feed and/or drinking water is considered cost effective and convenient in poultry production, and is therefore used in almost all production in the United States. Two types of resistance have been identified over time and include an acquired resistance that consists of a gradual decline in the level of sensitivity to a certain drug, as well as an innate resistance. The innate resistance constitutes a certain strain or species' lack of sensitivity to certain drugs (Chapman, 1997). McDougald (1982) has reported that subtle differences exist in regard to various ionophores against

different species of *Eimeria*. In the present poultry industry nearly every drug that has been discovered and developed to combat avian coccidiosis has, at some point, produced a certain level of drug resistance (Chapman, 1997).

There are several programs that have been implemented within the young layer and replacement breeder areas including feeding suboptimal concentrations of certain anticoccidials on alternate days in restricted feeding programs which allowed for the shedding of enough oocysts to impart immunity in affected birds while preventing detrimental outbreaks, using drugs that have lost effectiveness, using 'step-down' programs in which the concentration of the drug being administered is gradually decreased, using a drug intermittently, or using drugs only in therapeutic instances when birds are showing signs of clinical coccidiosis infection (Reid et al., 1968; Long, 1979). Anticoccidial programs which allow for immunity development are vital in these classes of birds—especially when they are in a floor-rearing situation and thus have access to feces—due to the fact that any anticoccidial drugs that are being administered are subsequently removed at the point when the bird enters its laying period (Chapman, 1999).

Amprolium Treatment

Since its introduction in 1960 that the chemical anticoccidial amprolium possessed a high level of effectiveness against various species of *Eimeria*, namely *E. tenella*, *E. necatrix*, and to a lesser extent, *E. maxima* (Cuckler et al., 1960, McDougald, 1982). Amprolium is effective in the prevention of production, sporulation, and infectivity of *Eimeria* oocysts (Ruff et al., 1993). Additionally, amprolium

administration has been shown to protect affected birds from the negative effects on BWG associated with coccidial infection and to prevent mortality in affected birds while conferring complete protection against various levels of *Eimeria* infection (Singh and Gill, 1976; Prasad et al., 1986; Chapman, 1989). Amprolium acts as a selective thiamine antagonist, competing with the parasite for the absorption of thiamine, and thereby controlling its proliferation in the host (Ryley and Betts, 1973; McDougald, 2003).

It has been suggested that amprolium is a suitable option in the instance of rearing replacement breeder flocks due to the fact that it would allow immunity development when high build up of infection is available in floor pens (Chapman, 1999). In fact, amprolium is one of two drugs in the United States whose use is suggested for the development of active immunity, and for which various step-down programs using different concentrations of drug are approved for this purpose (Anon, 1997). The concern resides in situations where lower levels of infection are present, leading to the impediment of immunity development (Singh and Gill, 1976). Evidence of this issue was brought to light by Bajwa and Gill (1977), who administered amprolium at a rate of 0.024% via drinking water, and found that a possible interference with the development of immunity to infections with small numbers of oocysts actually did exist. Counter to those results, however, are those reported by Hu and colleagues (2000) who found that amprolium administration took place without interfering appreciably with protective immunity development in broilers. Additionally, Ruff and colleagues reported that when amprolium was administered via water while feed intake was restricted, protection against coccidiosis was provided while immunity development was not hindered (Ruff

and Chute, 1980; Ruff and Chute, 1991). With regard to this information, there remains a degree of disagreement in the timing of liquid amprolium administration for the use of controlling possible pathogenic effects of the non-attenuated vaccines. Select sources state that amprolium should be administered beginning ten days after vaccine administration (Chapman, 1999; 2000; Chapman and Cherry, 1997) while others believe that administration of amprolium should begin 16 – 17 days after vaccination in order to reduce the reaction to infective oocysts without disrupting the cycling of the parasite and the development of immunity (Chapman et al., 2002).

Therefore the objective of this research trial is to determine and compare the effects of amprolium administration at different times and concentrations in vaccinated replacement broiler breeders on BWG, flock uniformity, oocyst cycling, and immunity development. Information obtained from these trials could benefit the industry by providing knowledge about the interactions of vaccination and chemical anticoccidial administration and how they impact the development of immunity to clinical *Eimeria* infections in commercial production.

CHAPTER III

IMPACT OF AMPROLIUM ADMINISTRATION ON OOCYST CYCLING AND PERFORMANCE PARAMETERS IN COCCIDIOSIS VACCINATED REPLACEMENT BROILER BREEDERS

Introduction

Coccidiosis is a major disease condition affecting the poultry industry, resulting in significant economic losses in excess of 800 million dollars each year (Allen and Fetterer, 2002). The majority of these costs are associated with performance losses in the form of growth depression and decreased feed efficiency due to the organism's invasion of the intestinal epithelium of the host which results in decreased nutrient absorption. Due to the nature of the organism and its ubiquitous nature in the rearing environment, this is not a disease that will soon be eradicated (Yun et al., 2000). The best option available to today's poultry industry is to implement all available and feasible control measures for treatment and prevention.

Current options for coccidiosis control include vaccination and anticoccidial drug administration. In replacement breeding stock vaccines are used extensively to control coccidiosis (Chapman, 2000; Chapman et al., 2002). The idea behind live oocyst vaccination is to impart a low level of *Eimeria* infection in order to stimulate immunity (Yun et al., 2000). Live, unattenuated vaccines have been suggested to induce long-lasting protective immunity by stimulating a range of immune responses when birds are infected by vaccine strain *Eimeria* (Chapman et al., 2005). For immunity to develop it is necessary to introduce the parasite into the host's system, complete its life cycle, and be

excreted onto the litter for re-ingestion by the host (Chapman and Cherry, 1997). The primary disadvantage associated with coccidiosis vaccination is the associated period of performance loss associated with the mild infection that is necessary for immunity development (Mathis, 1999; Danforth et al., 1997).

The reason for concern associated with live oocyst vaccination in replacement flocks is due to this possibility of negative implications on body weight gain (BWG) and feed efficiency. There are several factors to consider in the rearing and successful management of replacement broiler breeders, with the most important being control of average body weight, uniformity, and disease development. This is due to the early rearing period being a critical time for the establishment of appropriate frame and body weight of the bird (Hudson et al., 2001). If these details are not taken into careful consideration, negative effects can be observed by the level of reproductive efficiency following the onset of sexual maturity.

One option for alleviating some of the performance losses associated with live oocyst vaccination in breeders involves the concomitant administration of an anticoccidial drug capable of controlling the clinical infection without suppressing the development of host immunity (Chapman, 2000; Williams, 2002). Certain vaccine manufacturers have gone as far as to recommend treatment with anticoccidials following vaccination in order to suppress post-vaccinal reactions (Chapman and Cherry, 1997). Amprolium administration has been shown to protect affected birds from the negative effects resulting from coccidial infection while allowing for complete immunity development against *Eimeria* infection (Singh and Gill, 1976; Prasad et al., 1986;

Chapman, 1989). However, while previous research has been performed to address this topic, a great deal of time has passed since, and with that several changes in bird genetics, nutrition and feeding, breeder management practices, and changes in the parasite have come as well. These facts have led to the need for further investigation of the topic. Therefore, the objective of the current study was to determine and compare the effects of different concentrations of amprolium administered at different time points via drinking water on BWG, flock uniformity, and oocyst cycling in coccidiosis vaccinated replacement broiler breeders from placement to 35 days of age.

Materials and Methods

Experimental Design

This experiment was conducted in an environmentally controlled dark-out growing facility at the Texas A&M University Poultry Science Teaching, Research, and Extension Center in College Station, TX. Animal care and husbandry were provided in accordance with an approved Texas A&M Institutional Animal Care and Use (IACUC) protocol. Both trials of this experiment followed an experimental design consisting of four treatment groups present in each of three genetic lines of birds. Five replicate pens were placed for each treatment group for a total of 60 pens. The three genetic lines of the replacement broiler breeder females evaluated in these trials are denoted as Lines A, B, and C. Of the four treatment groups (Table 3-1), one group was administered amprolium from days 10 through 12 at a concentration of 0.006%, another was administered from day 16 through 18 at the same concentration, while the other was administered from day 16 through 18 at a concentration of 0.012%. A negative control

was maintained for each genetic line in which access to the municipal water source used on our research farm was maintained for the duration of the trial. Experimental parameters evaluated included BWG, flock uniformity, and oocyst shedding associated with vaccination (oocysts shed per gram (OPG) of feces).

Body Weight Gain and Flock Uniformity

Performance was assessed according to individual bird weights and compared to line specific target body weights (Table 3-1) at day of placement, as well as 7, 10, 14, 16, 21, 28, and 35 days post placement. Individual body weights were also used to determine variability by pen and to assess total flock uniformity.

[Table 3-1] Target body weights (g) for all genetic lines investigated, according to age, up to 35 days.

	Age (d)				
	7	14	21	28	35
Line A	109	213	309	400	490
Line B	159	281	400	522	622
Line C	136	272	363	454	545

Oocyst Shedding (OPG Determination)

Beginning on day 5 of each trial, and continuing on an every-other-day basis through termination of each trial, feces were collected from four pens from each of the treatment groups. These four samples were then pooled into two pen samples for examination and quantification of oocysts present per each gram of fecal contents. Prior

to analysis, each pooled fecal sample was homogenized, weighed, and diluted at a 3:1 ratio of water to fecal matter. From each sample, 10 µL of fecal suspension were extracted and loaded into a hemacytometer using a 200µL pipette to be observed microscopically for oocyst presence. A standard light microscope and a 20× objective were used to quantify non-sporulated oocysts present in each sample for oocyst per gram of feces (OPG) calculations.

Experimental Animals and Rearing

Broiler breeder pullets of each line evaluated were obtained at day of hatch from commercial hatcheries. Following normal services and processing, chicks were transported to the research rearing facility, wing-banded for identification, weighed individually, and randomized according to weight. Randomization was performed by removing the heaviest and lightest 5% of each strain of birds and then randomly distributing the remaining pullet chicks to allow for an evenly distributed starting pen weight for each pen. Chicks were placed on fresh pine shavings for initiation of the first trial. In the second trial, chicks were obtained and placed on used shavings remaining in rearing pens from the previous trial. Chicks were placed at a density of 1.5 ft^2/bird, in agreement with industry standards. For the first 14 days of each trial, chicks were allowed access to only half of the final pen space to mimic half house brooding. On day 14 all barriers were removed and birds were allowed full pen access through 35 days of age. Each pen was equipped with appropriate feeders and commercial-style nipple drinking systems.

In both trials chicks were provided appropriate supplemental heat, water, and breeder specific starter diets according to their respective feeding schedules (Table 3-2) in order to maintain breeder recommended target weights. All strains were fed ad libitum through 14 days of age, fed daily allocations through 28 days of age, and subjected to a 3/4 skip-a-day feeding regime for the remainder of the trial. Breed specific starter diets were fed through day 28, after which breed specific grower diets were fed.

[Table 3-2] Feed allocation on a per bird, per day basis for each genetic line, by week, through 5 weeks of age.

		Age (wk)		
Line	1-2	3	4	5
		Trial 1		
Line A	*ad libitum*	32g	35g	38g
Line B	*ad libitum*	36g	44g	48g
Line C	*ad libitum*	36g	42g	43g
		Trial 2		
Line A	*ad libitum*	32g	38g	40g
Line B	*ad libitum*	36g	42g	46g
Line C	*ad libitum*	39g	43g	44g

All pullets were housed in a dark-out grower facility and subjected to an industry specific schedule of time and light intensity as measured by a photometer. For the first three days chicks were exposed to 24 hours of light at an intensity of 30 lux. From day 4 through 7 light was reduced to 18 hours per day at an intensity of 20 lux. From day 8

through day 21 lights were again reduced to 12 hours per day at the same intensity. From day 22 through the remainder of each trial light was reduced to 8 hours per day at an intensity of 10 lux.

Vaccination and Amprolium Administration

The commercially available coccidiosis vaccine Coccivac®-D (Intervet/Schering-Plough Animal Health; Summit, NJ) was used in these trials. This vaccine, a non-attenuated live oocyst coccidiosis vaccine for replacement broiler breeders and laying stock, was administered at the manufacturer recommended dose of 0.25 mL/bird on day of hatch using commercial spray cabinet in the commercial hatchery providing each genetic line of replacement breeders.

In both trials, each genetic line of pullets received the synthetic anticoccidial compound amprolium (Amprol® 9.6% Oral Solution; Huvepharma; Sofia, Bulgaria) according to one of four administration protocols. One group of each genetic line was maintained as a negative control while the three amprolium administrations consisted of a 0.006% concentration on days 10 through 12, a 0.006% concentration on days 16 through 18, and a 0.012% concentration on days 16 through 18. On the specified day birds were switched from main line water sources and given access to an unlimited water supply containing the specified concentration of medication. The medicated water supply was refreshed and replenished every 24 hours for the duration of each administration period.

Statistical Analysis

The experimental parameters from this trial were subject to a one way ANOVA (SPSS v. 11). Means were separated using Duncan's Multiple Range test and deemed statistically different at $p \leq 0.05$ (SYSTAT, 2001).

Results

Trial 1

With regard to flock uniformity, no significant differences were observed for lines A or B on any of the data collection days (Tables 3-3 and 3-4). However, trends were observed on day 21 ($p=0.11$), day 28 ($p=0.10$), and day 35 ($p=0.08$) which indicated potential increases in uniformity relevant to the control and 0.006% amprolium on days 16 through 18. A significant ($p \leq 0.05$) improvement in Line C flock uniformity was observed on days 21, 28, and 35 in the control group and both 0.006% amprolium concentration groups when compared with the 0.012% concentration administered from day 16 through 18 (Table 3-5). With regard to body weights at all data collection days, no significant differences were observed between treatment groups among any of the genetic lines.

All peaks occurring in oocyst shedding for Line A pullets were observed following day 15. The first set of peaks in oocyst shedding, which occurred between days 19 and 21 showed a decreased level of shedding in the 0.006% amprolium concentration on both days 10 to 12 and 16 to 18. In addition to this decrease in oocyst shedding, further depression was observed in the 0.012% concentration treatment group (Figure 3-1). For the second set of peaks, which occurred between days 24 and 26,

Table [3-3] Trial 1—Body weights and flock uniformity of Line A replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	40.6 ± 0.0	175.3 ± 1.6	306.9 ± 1.9	341.8 ± 0.8	444.1 ± 6.1	583.4 ± 6.8
0.006%@d10-12			301.4 ± 3.8	334.3 ± 3.3	434.2 ± 1.9	578.3 ± 9.8
0.006%@d16-18				330.1 ± 10.8	441.2 ± 7.0	599.7 ± 10.4
0.012%@d16-18				337.4 ± 6.4	440.1 ± 9.4	586.8 ± 31.0
			Flock Uniformity (Coefficient of Variation)			
Control	5.4 ± 0.1	17.3 ± 1.0	14.6 ± 0.6	14.5 ± 0.9	13.3 ± 1.2	13.9 ± 1.2
0.006%@d10-12			14.9 ± 1.8	13.5 ± 1.9	12.7 ± 1.4	12.9 ± 1.1
0.006%@d16-18				12.5 ± 0.6	13.0 ± 0.6	13.7 ± 0.9
0.012%@d16-18				13.5 ± 1.4	11.8 ± 1.7	12.3 ± 1.5

Table [3-4] Trial 1—Body weights and flock uniformity of Line B replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	39.4 ± 0.1	204.6 ± 3.5	340.8 ± 2.7	370.9 ± 5.0	501.7 ± 3.7	713.4 ± 11.7
0.006%@d10-12			334.4 ± 13.3	372.8 ± 8.7	503.2 ± 11.7	716.6 ± 45.3
0.006%@d16-18				368.5 ± 10.0	498.7 ± 4.7	688.5 ± 9.1
0.012%@d16-18				366.7 ± 9.3	506.4 ± 4.3	701.6 ± 5.4
			Flock Uniformity (Coefficient of Variation)			
Control	5.9 ± 0.1	12.1 ± 0.7	11.3 ± 0.5	9.2 ± 0.9	9.1 ± 0.8	9.1 ± 0.7
0.006%@d10-12			12.5 ± 0.9	10.9 ± 0.9	10.8 ± 0.6	11.2 ± 0.7
0.006%@d16-18				8.9 ± 0.2	9.2 ± 0.4	10.6 ± 0.8
0.012%@d16-18				11.5 ± 1.0	10.7 ± 0.5	12.1 ± 0.8

Table [3-5] Trial 1—Body weights and flock uniformity of Line C replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	39.8 ± 0.0	189.6 ± 3.8	303.0 ± 3.9	354.7 ± 2.7	479.4 ± 6.0	648.0 ± 14.8
0.006%@d10-12			309.9 ± 10.5	363.5 ± 6.4	491.8 ± 6.7	659.3 ± 7.8
0.006%@d16-18				360.7 ± 3.5	484.5 ± 3.8	657.0 ± 6.3
0.012%@d16-18				333.7 ± 15.7	471.0 ± 9.8	631.5 ± 15.4
			Flock Uniformity (Coefficient of Variation)			
Control	5.4 ± 0.1	12.5 ± 0.9	13.9 ± 0.8	10.7^{b} ± 0.4	10.3^{b} ± 0.4	10.3^{b} ± 0.3
0.006%@d10-12			12.0 ± 0.6	11.2^{b} ± 0.5	10.6^{b} ± 0.6	10.1^{b} ± 0.7
0.006%@d16-18				9.3^{b} ± 0.4	9.8^{b} ± 0.5	10.8^{b} ± 0.7
0.012%@d16-18				14.2^{a} ± 1.8	13.3^{a} ± 1.2	13.2^{a} ± 1.0

[a,b]Indicates significant difference between treatments at $p \leq 0.05$.

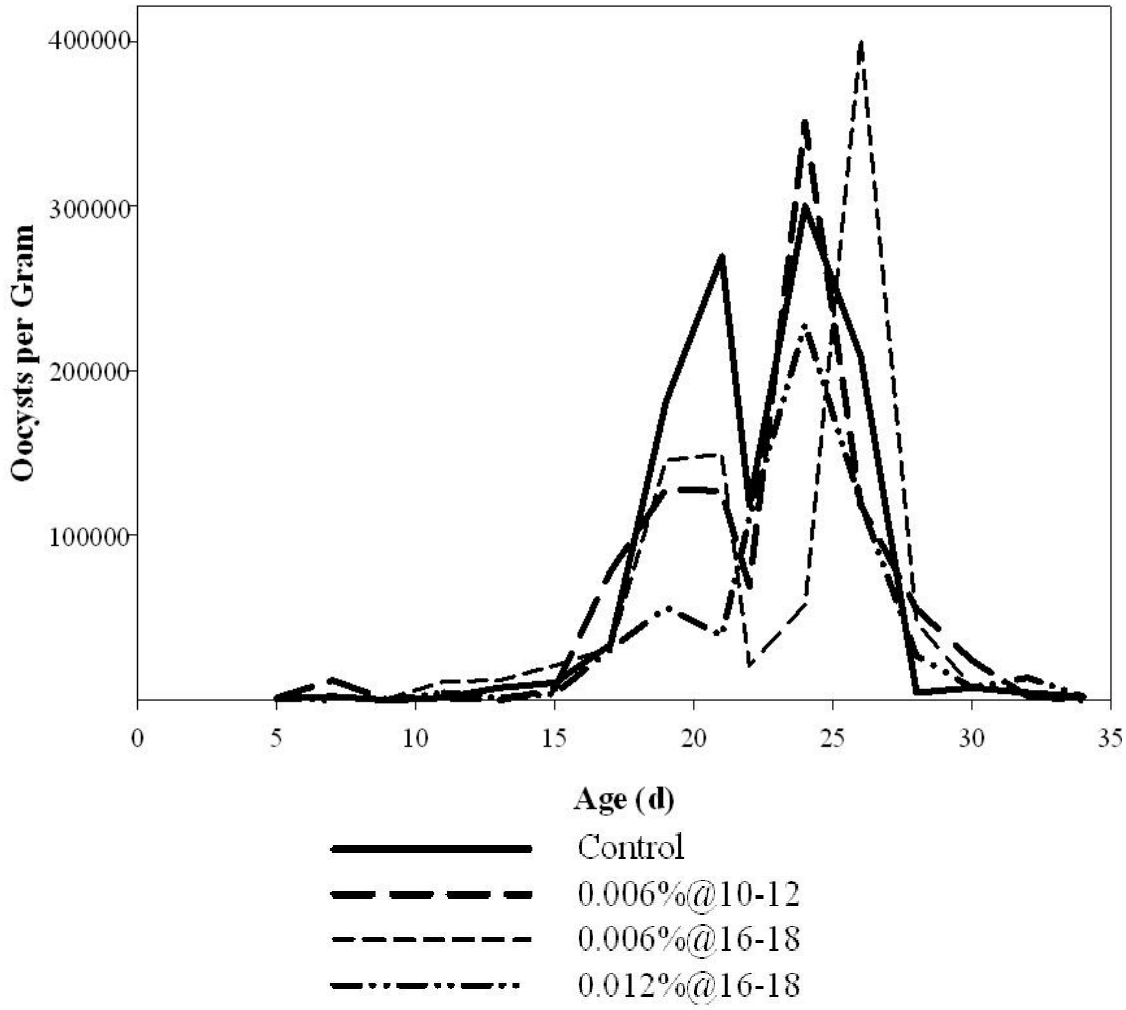

Figure [3-1] Trial 1—Oocyst shedding patterns (oocyst per gram (OPG) of feces) of Line A replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings beginning at d5 and continuing on an every other day basis through d35.

oocyst shedding of the 0.012% amprolium concentration group was depressed when compared to the group receiving no amprolium. In addition, the peaks for both low level amprolium administrations showed higher peaks when compared to the control group.

The first set of peaks in Line B pullets was shown to have occurred beginning at day 10 and continuing through day 13 with additional peaks varying according to treatment group. The greatest magnitude of first peak shedding was observed in the control group followed by the higher concentration group administered at day 16. The 0.006% administration of amprolium beginning at day 10 yielded the lowest first peak level (Figure 3-2). The group receiving 0.006% on days 10 through 12 had the highest second peak which occurred at day 19. Following this peak, the shedding for this group decreased and eventually discontinued without further notable peaks. In both groups receiving amprolium from day 16 to 18 second peaks were seen at similar magnitudes between days 17 and 19 followed by a decrease in shedding, and a relatively late peak again around day 26, again with similar magnitudes which greatly exceeded those of the control group.

The Line C control group exhibited an initial peak in shedding at day eleven with a subsequent peak at day 17 of a greater magnitude than the first (Figure 3-3). The group receiving amprolium beginning at day 10 showed peaks at the same intervals as the control group; however the magnitudes of both peaks were reduced relative to the control birds. The first peak in shedding for the group receiving the highest level of amprolium was approximately three times greater than that of the other three groups with a subsequent peak at day 17 of a magnitude similar to that of the control group.

Late peaking was observed in groups receiving 0.006% amprolium on days 16 through 18 with a peak of notable magnitude occurring around day 27 when shedding by all other treatment groups was negligible.

Trial 2

No significant differences were observed, with regard to body weight data, between any treatment groups in any of the genetic lines of replacement pullets for the second trial of this experiment (Tables 3-6, 3-7, and 3-8). In addition, no significant differences were shown among any of the three genetic lines when comparing uniformity among treatments for the duration of the trial.

The oocyst shedding observed in Line A pullets for trial two yielded a far greater magnitude than that which was observed in the first trial, particularly in the group receiving amprolium on days 10 through 12 (Figure 3-4) . One peak in shedding was observed in control birds around day 15 post-vaccination. In the early administration group, that first peak was suppressed—occurring on day 19, and showed more than a three-fold increase in magnitude compared to the control. The second, higher peak occurred around day 27. Peaking in the low level, late administration group was relatively non-existent when compared to all other groups. High level, late administration yielded a very low magnitude peak at day 19 followed by an increased level four days following.

The shedding patterns for Line B pullets in trial two differed slightly from that observed in trial one (Figure 3-5). Initial peaks in shedding were seen at day 13, primarily in the high level administration group. For the control group, the first peak

was observed at day 17, followed by a second peak of lesser magnitude at day 21, and again four days later. The low level, early administration resulted in a minor peak at day 13 followed by a second peak, approximately three folds higher, at day 17. This peak was about half the level of the control peak that occurred on the same day. After day 17, shedding decreased and no further peaks were observed. The low level, late administration yielded the highest level of shedding at day 17 followed by a second, lesser peak at day 23. High level administration exhibited the lowest, overall, amount of shedding in Line B. Low magnitude peaks were observed at days 13, 17, and 23 post vaccination.

Oocyst shedding in Line C for trial two varied slightly from what was observed in trial one (Figure 3-6). The birds which did not receive amprolium showed the lowest overall shedding of all treatment groups. Minor peaks were observed at days 11 and 19. Low level, early administration resulted in peak shedding at days 15 and 19, with the day 19 peak having the highest magnitude of all treatment groups. The low level, late administration group had one peak at day 17. Birds receiving the high concentration of amprolium exhibited two peaks—the first was observed at day 13 with the second, which was approximately two-fold higher, not occurring until 23 days post vaccination.

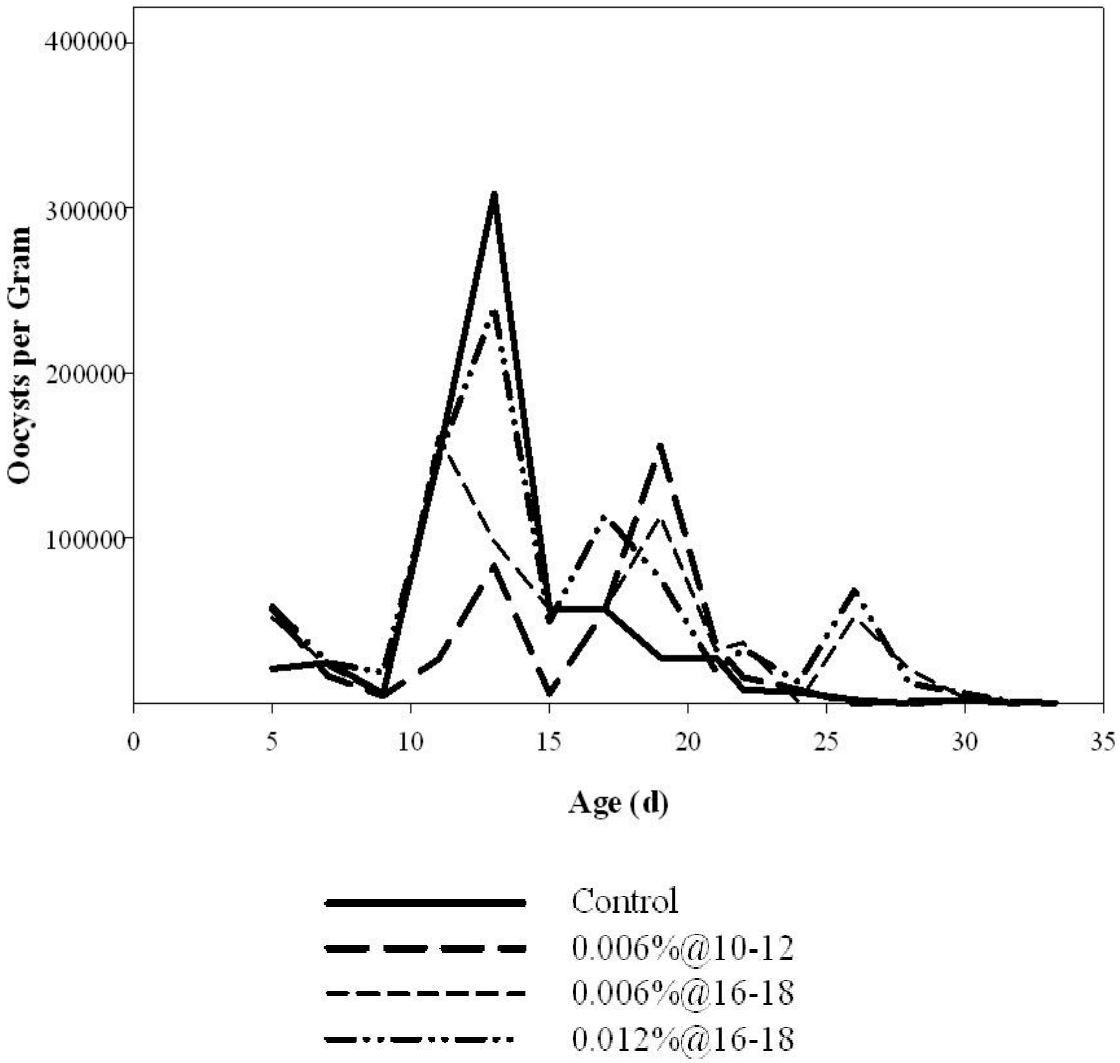

Figure [3-2] Trial 1—Oocyst shedding patterns (OPG) of Line B replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings beginning at d5 and continuing on an every other day basis through d35.

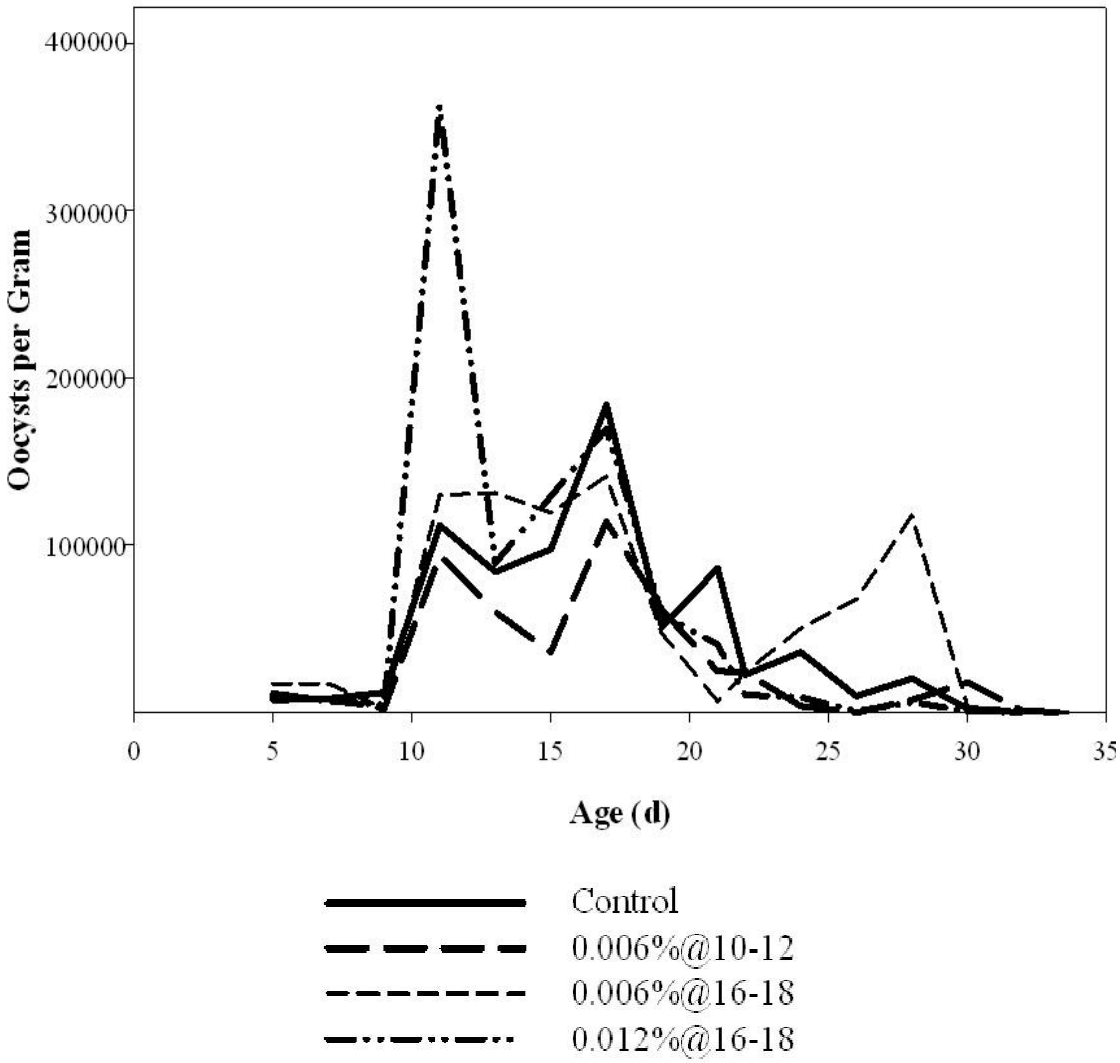

Figure [3-3] Trial 1—Oocyst shedding patterns (OPG) of Line C replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on fresh pine shavings beginning at d5 and continuing on an every other day basis through d35.

Table [3-6] Trial 2—Body weights and flock uniformity of Line A replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	39.4 ± 0.0	135.2 ± 4.4	224.8 ± 8.7	297.7 ± 5.7	406.4 ± 7.7	540.1 ± 12.4
0.006%@d10-12	39.4 ± 0.0	144.4 ± 2.2	232.7 ± 5.0	294.0 ± 3.8	405.0 ± 7.0	551.6 ± 9.9
0.006%@d16-18	39.4 ± 0.0	137.4 ± 2.8	229.4 ± 6.7	307.1 ± 4.7	407.8 ± 11.7	537.5 ± 16.1
0.012%@d16-18	39.4 ± 0.0	134.5 ± 3.3	225.7 ± 6.1	296.6 ± 8.3	404.0 ± 6.3	542.1 ± 13.4
			Flock Uniformity (Coefficient of Variation)			
Control	6.0 ± 0.2	19.6 ± 0.9	19.4 ± 1.5	17.3 ± 0.7	17.0 ± 1.1	17.2 ± 1.1
0.006%@d10-12	6.0 ± 0.2	19.6 ± 0.3	17.9 ± 0.6	17.3 ± 0.6	16.1 ± 0.5	15.9 ± 0.7
0.006%@d16-18	6.0 ± 0.2	20.2 ± 1.3	18.3 ± 1.5	16.2 ± 0.8	15.4 ± 0.8	15.8 ± 0.8
0.012%@d16-18	6.1 ± 0.1	20.7 ± 0.8	20.4 ± 0.4	17.4 ± 0.9	15.9 ± 0.5	15.3 ± 0.4

Table [3-7] Trial 2—Body weights and flock uniformity of Line B replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	45.8 ± 0.0	194.6 ± 2.4	325.6 ± 4.8	399.1 ± 5.6	513.3 ± 5.7	662.3 ± 12.1
0.006%@d10-12	45.8 ± 0.0	188.4 ± 4.0	317.1 ± 13.4	385.0 ± 15.0	496.7 ± 13.2	652.7 ± 12.8
0.006%@d16-18	45.8 ± 0.0	191.1 ± 2.2	331.3 ± 1.2	404.7 ± 4.5	513.4 ± 5.1	669.6 ± 8.5
0.012%@d16-18	45.8 ± 0.0	189.5 ± 2.4	317.3 ± 9.1	388.5 ± 8.0	505.1 ± 7.3	561.1 ± 83.4
			Flock Uniformity (Coefficient of Variation)			
Control	6.2 ± 0.2	10.6 ± 0.7	11.7 ± 1.0	10.8 ± 0.4	10.9 ± 0.3	11.1 ± 0.6
0.006%@d10-12	6.4 ± 0.2	11.3 ± 0.9	9.5 ± 1.1	10.0 ± 0.8	9.6 ± 0.4	9.0 ± 0.4
0.006%@d16-18	6.2 ± 0.2	11.7 ± 0.9	9.3 ± 0.5	9.6 ± 0.4	10.0 ± 0.4	10.2 ± 0.4
0.012%@d16-18	6.0 ± 0.2	12.3 ± 0.9	10.7 ± 1.1	10.0 ± 0.7	10.01 ± 0.5	10.0 ± 0.6

Table [3-8] Trial 2—Body weights and flock uniformity of Line C replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter.

Treatment	Age (d) 0	10	16	21	28	35
			Body Weight (g)			
Control	35.3 ± 0.0	143.5 ± 2.9	251.3 ± 3.2	344.9 ± 6.0	466.1 ± 5.7	613.3 ± 7.3
0.006%@d10-12	35.3 ± 0.0	137.1 ± 3.7	252.2 ± 4.4	358.6 ± 7.5	478.3 ± 6.4	622.7 ± 10.8
0.006%@d16-18	35.3 ± 0.0	141.7 ± 3.0	249.4 ± 5.8	347.0 ± 5.5	465.3 ± 6.5	610.8 ± 14.9
0.012%@d16-18	35.3 ± 0.0	145.1 ± 3.0	254.2 ± 8.7	354.2 ± 5.4	473.6 ± 4.5	615.8 ± 21.5
			Flock Uniformity (Coefficient of Variation)			
Control	5.9 ± 0.2	14.1 ± 1.0	13.7 ± 1.3	11.8 ± 0.7	12.1 ± 0.5	12.7 ± 0.5
0.006%@d10-12	6.0 ± 0.2	14.9 ± 1.2	15.1 ± 1.3	12.3 ± 0.7	12.5 ± 0.6	12.7 ± 0.8
0.006%@d16-18	6.0 ± 0.2	16.6 ± 1.6	17.7 ± 1.8	14.3 ± 1.4	14.0 ± 1.2	14.0 ± 1.4
0.012%@d16-18	6.0 ± 0.2	13.7 ± 1.5	14.1 ± 0.8	11.4 ± 1.1	11.6 ± 0.9	11.9 ± 0.9

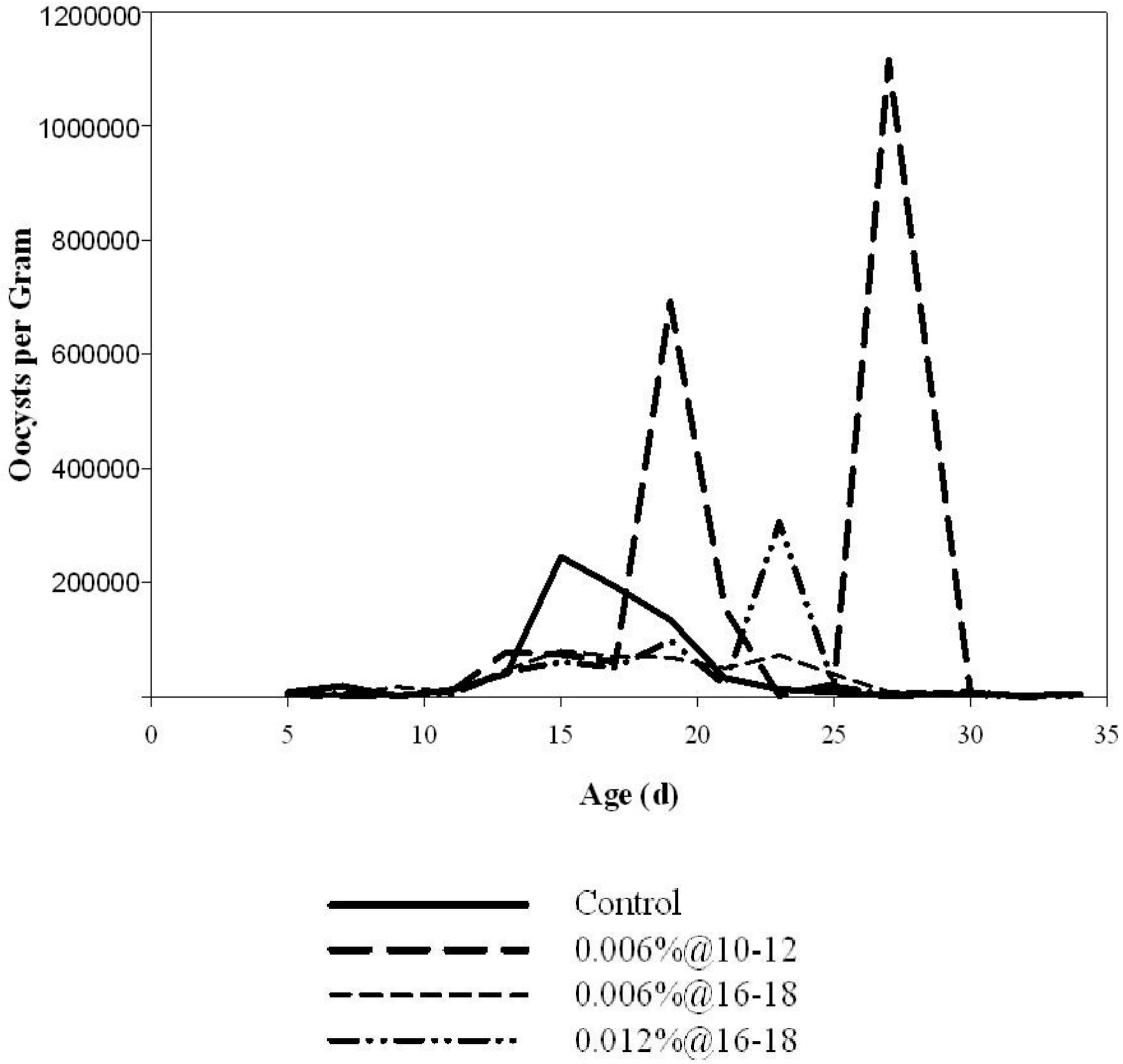

Figure [3-4] Trial 2—Oocyst shedding patterns (OPG) of Line A replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter beginning at d5 and continuing on an every other day basis through d35.

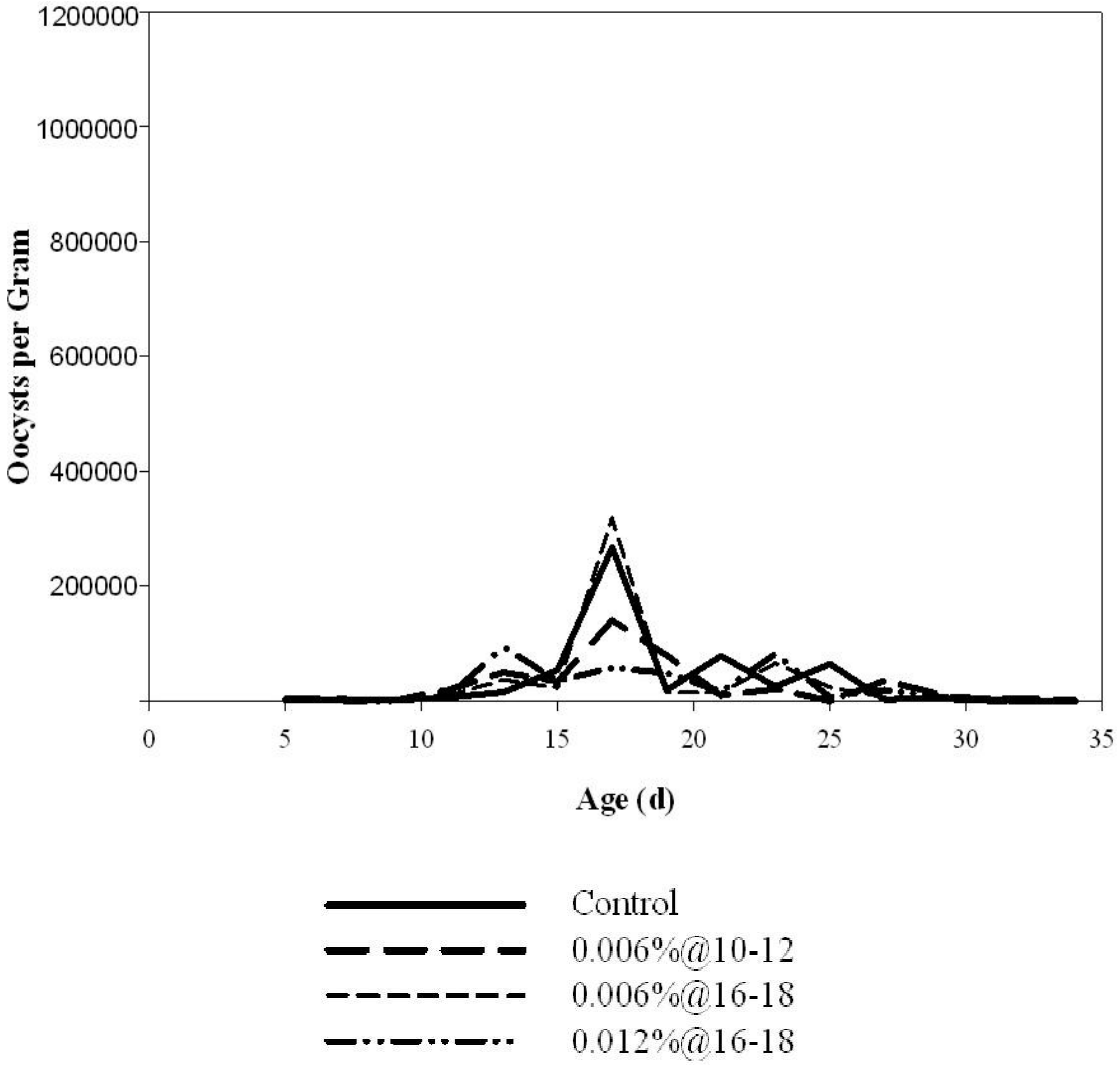

Figure [3-5] Trial 2—Oocyst shedding patterns (OPG) of Line B replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter beginning at d5 and continuing on an every other day basis through d35.

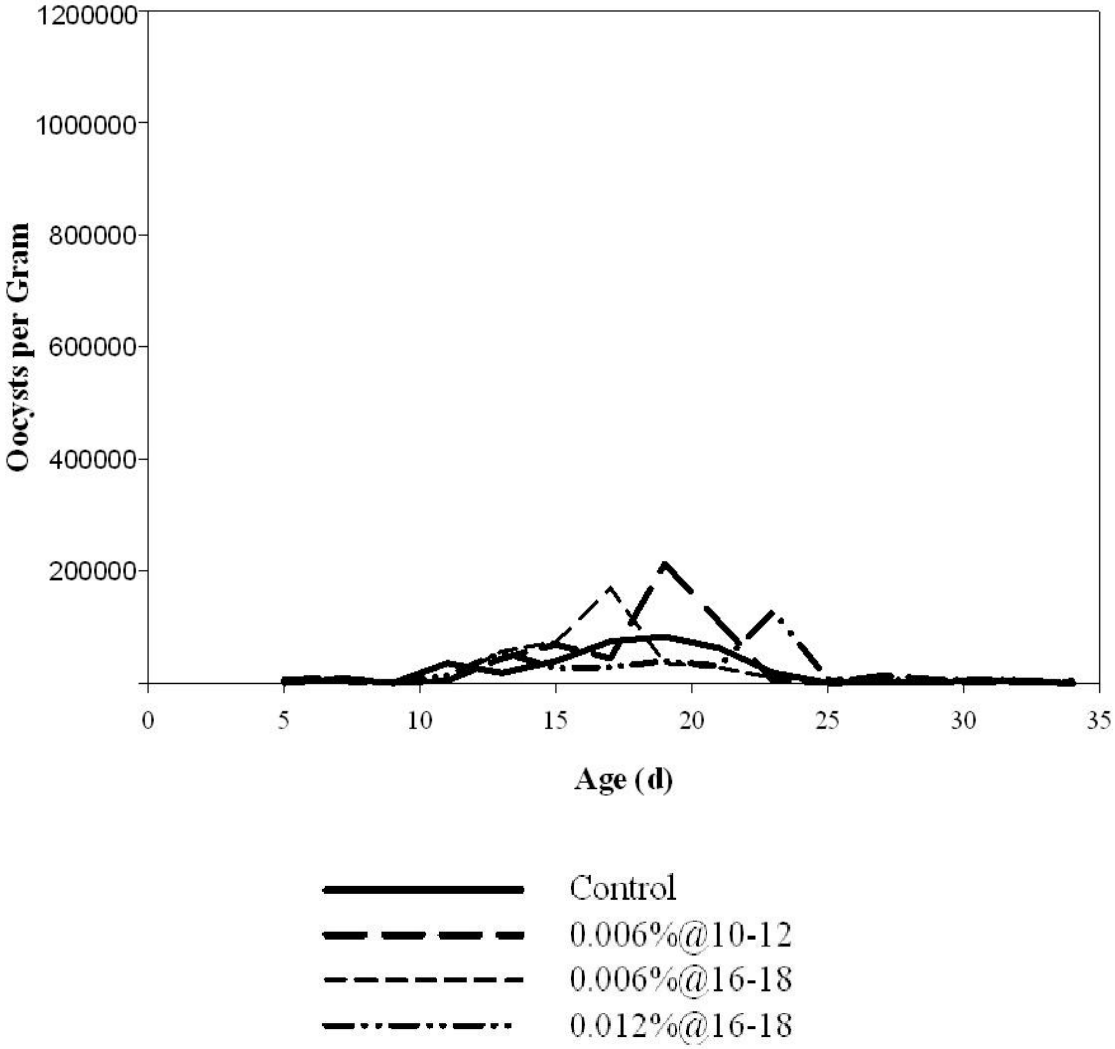

Figure [3-6] Oocyst shedding patterns (OPG) of Line C replacement pullets vaccinated with a live oocyst vaccine administered different concentrations of amprolium at different time periods through 35 days of age reared on used litter beginning at d5 and continuing on an every other day basis through d35.

Discussion

There exists a limited amount of research addressing the topic of amprolium administration in coccidiosis vaccinated pullets. Because coccidiosis vaccination is the primary method for control of this disease and amprolium is often administered in the field in conjunction with vaccination, it is felt that further investigation of the effects of the medication should be performed. One of the primary industry-based concerns addressed in this project was the expected decrease in performance of these replacement breeders during the early growth phases of the bird's life following coccidiosis vaccination. Previous research regarding amprolium administration has indicated that feeding programs can have a significant impact on the effects of amprolium particularly with regard to BWG. Often, due to the fact that replacement pullet flocks are subjected to restricted feeding and skip-a-day feeding programs which are put into place specifically to hinder BWG; it is difficult to ascertain the effects of coccidial infection in these birds. Ruff and Chute (1980) confirmed this after they compared a restricted feeding model and an ad libitum feeding model in conjunction with amprolium treatment. Results from their experiment showed that weight gain was a valid parameter of analysis only in *ad libitum* fed birds, as the restricted feeding program contributed to the depressed weight gain of pullets. This correlates with the data represented by this experiment in that no significant weight depression was observed at any time throughout the trial, although oocysts were actively being shed as was shown by the oocyst shedding pattern data.

In addition to issues regarding body weight and growth performance, personal communication with industry representatives has led to reports of inconsistent uniformity in vaccinated broiler breeder pullet flocks. Results of the current experiment indicate that the effects of amprolium on flock uniformity are highly variable according to genetic line and litter conditions. For example, in Line A, no indication was made to favor amprolium administration when birds were reared on new litter, whereas, when birds were reared on used litter in the second trial, trends indicated favorable outcomes when the later amprolium administrations were implemented. In addition, Line B pullets revealed trends indicating an increased level of uniformity associated with amprolium administration when birds were reared on used litter. However, in Line C pullets reared on new litter, there was an actual negative impact associated with amprolium, particularly with regard to the highest concentration when compared to all other treatment groups. Further, when reared on used litter, trends indicate the greatest level of uniformity to reside in the groups receiving no amprolium in Line C pullets.

Previous research has shown various indicators can be used in the evaluation of drug efficacy in poultry trials. One of the considerations that is made in relation to the degree of efficacy of anticoccidial drugs, and thereby the degree of infection, is the counts of oocysts excreted by treated animals when compared to control groups (Gard & Tonkinson, 1970). This trial implemented the use of oocyst counts to determine the effects of amprolium on post-vaccinal oocyst shedding with results indicating that the amprolium administered at different concentrations and time periods had varying effects on oocyst shedding between lines—taking into consideration both genetic line and litter

environment. For example, when comparing Line A with both Line B and Line C, it is obvious that Line A pullets began shedding oocysts until slightly later than the other lines. This could have significant influence on the effects of amprolium when taking into consideration the time of administration for the medication. Another difference between lines is the suggested litter effect that exists in Lines B and C. While in the first trial, both lines began shedding around day nine, it was apparent in the second trial, where used litter was present, that there was a delay in the onset of shedding. This delay was not observed in Line A. Taken together, these observations suggest that the effects of amprolium on coccidiosis vaccinated replacement broiler breeders with regard to growth performance and oocyst shedding are dependent on both the genetic makeup of the bird, as well as the environmental conditions in which they are reared—particularly in relation to oocyst shedding patterns. It is therefore important that the type of bird be taken into account prior to the administration of amprolium in replacement breeding stock.

CHAPTER IV

EFFECT OF AMPROLIUM ADMINISTRATION ON BODY WEIGHT GAIN AND IMMUNITY DEVELOPMENT BASED ON INTESTINAL LESION DEVELOPMENT AND TOTAL OOCYST OUTPUT IN COCCIDIOSIS VACCINATED REPLACEMENT BROILER BREEDERS

Introduction

Coccidiosis is an enteric disease caused by the protozoan parasite of the genus *Eimeria*. This is one of the most significant diseases currently afflicting commercial poultry producers. To date, there are nine species of *Eimeria* known to parasitize the intestine of the chicken, resulting in various complications such as intestinal lesion development, blood loss, mucoidal and hemorrhagic diarrhea, and in severe cases, death (McDougald and Fitz-Coy, 2008).

Various control measures exist in today's industry with regard to control and prevention of this disease. These consist, primarily, of an array of vaccination options as well as a variety of both synthetic and ionophorous chemical anticoccidial. The more heavily relied upon method of control over the past few decades within the replacement broiler breeder industry is live oocyst vaccination (Williams et al., 2000). This is due to the long term protective immunity to subsequent *Eimeria* infections that is provided by the immunological stimulation resulting from vaccination (Williams, 1994). Both attenuated and non-attenuated vaccines have been successful in stimulating immunity to coccidial infection (Shirley and Millard, 1986; Long et al., 1986; Bedrnik et al., 1989; Shirley, 1989).

In order for complete development of immunity to be achieved, it is necessary for complete and successful cycling of the parasite within the host to occur. If parasites are, in any way, incapacitated prior to this happening, the immunity development can be deficient or in some cases completely negated. The complete cycling begins with the initial peak in shedding which occurs approximately one week following vaccination wherein viable oocysts are excreted onto litter for sporulation and re-ingestion by the host (Chapman, 2000). Although the concentration of oocysts administered for the purpose of vaccination is minimal, it is still possible to cause infection, and there are often negative effects associated with its employment including depressed flock uniformity and growth performance. For this reason, it is common to see the concomitant administration of anticoccidials in vaccination protocols.

Various programs have been implemented within the replacement breeder industry including anticoccidial administration which allows for the shedding of enough oocysts to impart immunity while still preventing detrimental outbreaks, using drugs that have lost effectiveness, using drugs intermittently, or only in therapeutic instances (Reid et al., 1968; Long, 1979). The use of anticoccidial programs which still allow for the development of immunity is vital in classes of birds such as these in which floor-rearing allows for access to feces, and where anticoccidial drugs will be removed prior to birds entering the laying period (Chapman, 1999). Amprolium is one drug that has been suggested as a suitable option in the rearing of replacement breeders because it fits these criteria and has proven to be effective when high build up of infection is available in pens (Chapman, 1999).

Various research has shown that amprolium can be successfully administered in effort to alleviate post-vaccinal reactions to coccidiosis vaccine without interfering appreciably with immunity development (Hu et al., 2000; Ruff and Chute, 1991; Ruff and Chute, 1980). Although this information sheds a positive light on amprolium use, there still remains a certain degree of dispute over the proper timing of liquid amprolium administration for the use of controlling possible pathogenic effects of non-attenuated vaccines. While certain sources state that amprolium should be used beginning ten days post-vaccination (Chapman, 1999; 2000; Chapman and Cherry, 1997) others believe that amprolium administration should commence 16-17 days following vaccination for the reduction of reaction to infective oocysts while not disrupting the cycling of the parasite, and thusly the development of immunity (Chapman et al., 2002).

The objective of this research trial was to determine and compare the effects of amprolium administration at different times and concentrations in vaccinated replacement broiler breeders on the development of immunity as measured by gross and microscopic lesion development and total oocyst output following clinical *Eimeria* challenge.

Materials and Methods

Experimental Design

This experiment was conducted in an environmentally controlled dark-out battery cage growing facility at the Texas A&M University Poultry Science Teaching, Research, and Extension Center in College Station, TX. Animal care and husbandry were provided in accordance with an approved Texas A&M Institutional Animal Care and Use

(IACUC) protocol. Both trials of this experiment implemented an experimental design consisting of four treatment groups present in each of three genetic lines of birds. Five replicate pens were placed for each treatment group for a final total of 60 pens. The three genetic lines were denoted as Lines A, B, and C. Of the four treatment groups, one group was administered amprolium from days 10 through 12 at a concentration of 0.006%, another was administered from day 16 through 18 at the same concentration, while the other was administered from day 16 through 18 at a concentration of 0.012%. A negative control was maintained for each genetic line in which access to the main line water source was maintained for the duration of the trial. Following a 35 day grow-out period, eight pullets were removed from each of the floor pens, weighed, and randomly placed according to their strain and experimental treatment into 48 battery cages containing ten birds each for a total of 480 birds. Following a brief acclimation period of three to six days, a mixed species field strain inoculum was administered to all birds via oral gavage. Experimental parameters evaluated included body weight gain (BWG), total oocyst output associated with experimental challenge (oocysts shed per gram (OPG) of feces), gross lesion development, and microscopic lesion development.

Body Weight Gain

Initial body weights were taken prior to challenge administration as well as seven days post challenge in order to determine BWG for the challenge period.

Total Oocyst Output Determination

For ten days following the initial challenge, fecal contents for each pen were collected and weighed daily to determine total fecal output for the 24 hour period. Fecal

matter for each pen was homogenized and, from this, a sample obtained in a labeled bag to be used for oocyst per gram calculations. Each sample was weighed, diluted at a 3:1 ratio of water to feces, and 10 µL of fecal suspension were extracted and loaded into a hemacytometer using a 200µL pipette to be observed microscopically for oocyst presence. A standard light microscope and a 20× objective were used to determine the number of non-sporulated oocysts present in each milliliter of sample. This number was then used to calculate the total number of oocysts excreted by the corresponding pen of birds and adjusted on to a per bird, per day basis.

Experimental Animals and Rearing

Pullets of each line were randomly selected by genetic line at 35 days of age according to average body weights, and placed into battery brooders in an environmentally controlled, dark-out rearing facility. Birds were placed into cages randomly according to strain and treatment groups to achieve a final count of ten birds per cage. Breed specific grower rations were provided in a 3/4 skip-a-day feeding regime (Table 4-1) and fresh water was available *ad libitum* for the duration of the trial. An industry standard lighting schedule of eight hours per day of light was followed for the duration of the trial.

Pullets were allowed to acclimate to the new environment for three days in trial one and six days in trial two, after which time they received an oral challenge inoculum containing multiple species of *Eimeria*.

[Table 4-1] Feed allocation on a per bird, per day basis for each genetic line fed throughout the challenge period.

	Trial 1	Trial 2
Line A	38g	41g
Line B	45g	47g
Line C	43g	45g

Vaccination and Amprolium Administration

The commercially available coccidiosis vaccine (Coccivac®-D; Intervet/Schering-Plough Animal Health; Summit, NJ), a non-attenuated live oocyst coccidiosis vaccine for replacement broiler breeders was administered at the manufacturer recommended dose of 0.25 mL/bird on day of hatch via commercial spray cabinet in the commercial hatchery providing each genetic line of replacement breeders.

In both trials, each genetic line of pullets received amprolium (Amprol® 9.6% Oral Solution; Huvepharma; Sofia, Bulgaria) according to one of four administration protocols. One group of each genetic line was a negative control and provided an unmedicated water supply for the duration of the trial while the three amprolium administrations consisted of a 0.006% concentration on days 10 through 12, a 0.006% concentration on days 16 through 18, and a 0.012% concentration on days 16 through 18.

Eimeria *Challenge*

Species included in the challenge inoculum for trial one included *E. acervulina* (80,000 oocysts/bird), *E. maxima* (20,000 oocysts/bird), *E. tenella* (20,000 oocysts/bird),

and *E. necatrix* (15,000 oocysts/bird) for a total of 135,000 oocysts administered to each bird. Species included in the challenge inoculum for trial two included *E. acervulina* (139,500 oocysts/bird), *E. mivati* (98,000 oocysts/bird), *E. necatrix* (28,000 oocysts/bird), *E. maxima* (42,000 oocysts/bird), *E. tenella* (35,000 oocysts/bird), and *E. brunetti* (42,000 oocysts/bird) for a total of 384,500 oocysts being administered to each bird. In both trials challenge was administered to each bird via oral crop gavage.

Indices of* Eimeria *Challenge

Seven days post-challenge, body weights were collected from all challenged subjects, half of each pen (N=240) was euthanized, and the duodenum, jejunum, ileum, and ceca removed for gross and microscopic lesion assessment. Gross lesion development and severity were assessed on a scale of 0 to 4 using the methods described by Johnson and Reid (1970).

Microscopic lesion development and oocyst presence were evaluated by removing intestinal contents and taking a scraping of each individual section of gastrointestinal tract—duodenum, ileum, and cecum. Each scraping was placed on a microscope slide, covered, and observed under 200× magnification to determine the severity of infection on a scale of 0 to 4 according to the level of oocyst presence.

Statistical Analysis

The experimental parameters from this trial were subject to a one way ANOVA (SPSS v. 11). Means were separated using Duncan's Multiple Range test and deemed statistically different at $p \leq 0.05$ (SYSTAT, 2001).

Results

Trial 1

When comparing pre- and post-challenge body weights, as well as BWG that occurred following challenge, no significant differences were observed between treatment groups for Line A pullets. In addition, gross and microscopic lesion development between groups did not exhibit significant differences in any of the regions examined. However, total oocyst excretion in Line A pullets administered the 0.006% amprolium on days 10 through 12 was significantly lower than either of the other groups receiving amprolium, but was not different than that of the control group (Table 4-2). Additionally, the 0.006% amprolium administered on days 16 through 18 yielded total oocyst output significantly higher than that of the control group.

While no differences were observed in pre- or post-challenge body weights between treatment groups, Line B pullets receiving 0.006% amprolium administration at days 16 through 18 did exhibit significantly ($p \leq 0.05$) higher BWG when compared with the group receiving no amprolium (Table 4-3). Additionally, significantly ($p \leq 0.05$) higher gross lesion development was observed in the mid-gut of the birds receiving the 0.012% amprolium concentration at days 16 through 18 when compared to that of the other groups receiving amprolium (Table 4-3). However, no differences were seen in either the gross duodenal or cecal lesion development. Additionally, no differences were observed with regard to microscopic lesion development in any of the three regions of the intestine or in the total oocyst output data.

Table [4-2] Trial 1—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line A replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	586.2 ± 18.65	627.8 ± 19.77	41.7 ± 7.23
0.006%@d10-12	578.7 ± 9.46	623.5 ± 11.20	44.8 ± 12.90
0.006%@d16-18	604.4 ± 10.03	634.5 ± 14.38	30.1 ± 14.40
0.012%@d16-18	586.2 ± 20.38	628.7 ± 17.28	42.5 ± 8.83
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.45 ± 0.15	0.85 ± 0.24
0.006%@d10-12	0 ± 0.00	0.55 ± 0.27	0.95 ± 0.21
0.006%@d16-18	0 ± 0.00	0.45 ± 0.15	0.75 ± 0.21
0.012%@d16-18	0 ± 0.00	0.25 ± 0.13	0.85 ± 0.10
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0.11 ± 0.11	0.56 ± 0.18	0.89 ± 0.20
0.006%@d10-12	0 ± 0.00	0.50 ± 0.17	0.70 ± 0.21
0.006%@d16-18	0.20 ± 0.13	0.90 ± 0.18	0.90 ± 0.23
0.012%@d16-18	0.10 ± 0.10	0.90 ± 0.18	1.00 ± 0.21
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	$6{,}092{,}586^{bc}$ ± 1,128,225		
0.006%@d10-12	$4{,}539{,}235^{c}$ ± 180,748		
0.006%@d16-18	$10{,}250{,}949^{a}$ ± 1,273,102		
0.012%@d16-18	$8{,}609{,}484^{ab}$ ± 1,041,040		

[a-c]Indicates significant differences at $p \leq 0.05$.

Table [4-3] Trial 1—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line B replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	715.3 ± 13.67	760.8 ± 12.67	45.6^{b} ± 5.09
0.006%@d10-12	711.2 ± 15.35	764.4 ± 17.32	53.2^{ab} ± 5.81
0.006%@d16-18	695.1 ± 15.54	759.9 ± 15.26	64.8^{a} ± 7.27
0.012%@d16-18	709.5 ± 11.13	760.6 ± 12.60	51.1^{ab} ± 9.21
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.32^{ab} ± 0.15	0.95 ± 0.22
0.006%@d10-12	0 ± 0.00	0.20^{b} ± 0.13	0.60 ± 0.15
0.006%@d16-18	0 ± 0.00	0.11^{b} ± 0.10	0.74 ± 0.21
0.012%@d16-18	0 ± 0.00	0.55^{a} ± 0.13	0.65 ± 0.21
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0.11 ± 0.11	0.67 ± 0.17	0.67 ± 0.17
0.006%@d10-12	0 ± 0.00	0.80 ± 0.20	0.70 ± 0.15
0.006%@d16-18	0 ± 0.00	0.30 ± 0.15	0.60 ± 0.22
0.012%@d16-18	0 ± 0.00	0.70 ± 0.21	0.90 ± 0.23
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	10,613,380 ± 2,539,737		
0.006%@d10-12	10,678,137 ± 2,727,683		
0.006%@d16-18	8,308,315 ± 1,634,414		
0.012%@d16-18	5,298,748 ± 999,439		

[a-b]Indicates significant differences at $p \leq 0.05$.

Table [4-4] Trial 1—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line C replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	651.0 ± 16.31	710.4 ± 16.02	59.4 ± 14.23
0.006%@d10-12	656.5 ± 13.33	711.3 ± 11.35	54.8 ± 12.54
0.006%@d16-18	649.1 ± 7.30	708.2 ± 9.82	59.1 ± 9.00
0.012%@d16-18	640.1 ± 6.96	693.4 ± 6.85	53.3 ± 6.30
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.30 ± 0.15	0.85 ± 0.20
0.006%@d10-12	0 ± 0.00	0.40 ± 0.15	0.75 ± 0.23
0.006%@d16-18	0 ± 0.00	0.25 ± 0.10	1.05 ± 0.22
0.012%@d16-18	0 ± 0.00	0.45 ± 0.10	0.90 ± 0.20
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0^{b} ± 0.00	1.10 ± 0.10	0.70 ± 0.15
0.006%@d10-12	0^{b} ± 0.00	1.00 ± 0.15	0.50 ± 0.17
0.006%@d16-18	0^{b} ± 0.00	0.90 ± 0.18	1.00 ± 0.15
0.012%@d16-18	0.40^{a} ± 0.16	0.90 ± 0.23	0.90 ± 0.23
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	17,041,719 ± 1,908,207		
0.006%@d10-12	14,299,320 ± 1,360,287		
0.006%@d16-18	14,840,499 ± 3,510,965		
0.012%@d16-18	16,833,332 ± 2,076,113		

[a-b]Indicates significant differences at $p \leq 0.05$.

In the Line C pullets, no differences were observed when comparing pre- and post-challenge body weights or BWG. Also, no significant differences were observed with regard to gross lesion development in any region examined. And while significantly ($p \leq 0.05$) higher microscopic lesion development was observed in the duodenal loop of those receiving the 0.012% amprolium administration at days 16 through 18 when compared to all other treatment groups (Table 4-4), no differences were seen in either the ileum or the ceca. No differences were observed in the total oocyst shedding between treatments.

Trial 2

No significant differences were observed in Line A replacement pullets with regard to pre- or post-challenge body weights, nor were they observed between treatments when comparing BWG for the challenge period (Table 4-5). Additionally, no differences were observed in total oocyst output for the duration of the challenge.

In Line B pullets, no differences were shown in pre- or post-challenge body weights, or in BWG. With regard to gross lesion development in the duodenum and ileum, no differences were observed; however, those pullets subjected to the 0.006% amprolium concentrations at days 10 through 12 and 16 through 18 exhibited a significantly higher degree of gross lesion development in the ceca when compared to the pullets which did not receive any amprolium (Table 4-6). No differences were shown by the microscopic lesion data for all gut regions. Total oocyst output data for the challenge in Line B, also did not show significant differences between treatments.

Table [4-5] Trial 2—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line A replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	496.0 ± 20.53	607.7 ± 20.49	111.7 ± 4.34
0.006%@d10-12	502.3 ± 13.16	620.7 ± 14.48	118.3 ± 5.35
0.006%@d16-18	493.0 ± 12.66	602.3 ± 13.80	109.4 ± 4.69
0.012%@d16-18	499.0 ± 13.41	604.3 ± 20.81	105.4 ± 11.64
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.75 ± 0.22	0.65 ± 0.13
0.006%@d10-12	0 ± 0.00	0.50 ± 0.15	0.45 ± 0.17
0.006%@d16-18	0 ± 0.00	0.95 ± 0.22	0.60 ± 0.24
0.012%@d16-18	0 ± 0.00	0.80 ± 0.16	0.50 ± 0.17
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.10 ± 0.10	1.10 ± 0.23
0.006%@d10-12	0.11 ± 0.11	0.10 ± 0.10	1.50 ± 0.17
0.006%@d16-18	0 ± 0.00	0 ± 0.00	0.89 ± 0.20
0.012%@d16-18	0 ± 0.00	0.30 ± 0.15	1.10 ± 0.18
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	106,985 ± 65,426		
0.006%@d10-12	138,838 ± 90,874		
0.006%@d16-18	737,701± 277,378		
0.012%@d16-18	504,989± 296,448		

Table [4-6] Trial 2—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line B replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	621.4 ± 9.62	756.7 ± 14.32	135.3 ± 9.01
0.006%@d10-12	619.0 ± 7.16	755.4 ± 12.99	136.4 ± 9.06
0.006%@d16-18	627.0 ± 7.57	763.6 ± 11.91	136.6 ± 6.56
0.012%@d16-18	631.2 ± 12.49	767.3 ± 12.86	137.3 ± 4.48
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.80 ± 0.13	0.95^{a} ± 0.28
0.006%@d10-12	0 ± 0.00	0.79 ± 0.19	0.47^{b} ± 0.21
0.006%@d16-18	0 ± 0.00	0.70 ± 0.15	0.35^{b} ± 0.17
0.012%@d16-18	0 ± 0.00	0.65 ± 0.18	0.60^{ab} ± 0.17
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0.10 ± 0.10	0.30 ± 0.15	0.70 ± 0.21
0.006%@d10-12	0.36 ± 0.15	0.36 ± 0.20	0.91 ± 0.28
0.006%@d16-18	0.30 ± 0.15	0.20 ± 0.13	1.30 ± 0.21
0.012%@d16-18	0 ± 0.00	0 ± 0.00	0.56 ± 0.34
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	1,205,774 ± 421,133		
0.006%@d10-12	494,845 ± 367,413		
0.006%@d16-18	1,224,777 ± 1,126,239		
0.012%@d16-18	558,795 ± 243,232		

[a-b]Indicates significant differences at $p \leq 0.05$.

Table [4-7] Trial 2—Post-challenge BWG, gross and microscopic lesion development, and total oocyst output (per bird) over a seven day period of vaccinated Line C replacement pullets administered a clinical *Eimeria* challenge.

Post-Challenge BWG (g)			
Treatment	Pre-Challenge BW	Post-Challenge BW	Weight Gained
Control	574.9 ± 12.19	692.5[a] ± 15.61	117.6 ± 6.68
0.006%@d10-12	580.0 ± 10.56	694.8[a] ± 11.75	114.6 ± 4.83
0.006%@d16-18	573.9 ± 11.05	687.0[ab] ± 15.23	113.1 ± 5.84
0.012%@d16-18	563.2 ± 9.98	669.1[b] ± 11.71	105.9 ± 5.91
Gross Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0.85 ± 0.15	0.50 ± 0.22
0.006%@d10-12	0 ± 0.00	0.95 ± 0.13	0.35 ± 0.15
0.006%@d16-18	0 ± 0.00	0.95 ± 0.15	0.75 ± 0.21
0.012%@d16-18	0 ± 0.00	0.80 ± 0.10	0.50 ± 0.16
Microscopic Lesion Development			
	Duodenum	Ileum	Ceca
Control	0 ± 0.00	0 ± 0.00	0.70 ± 0.21
0.006%@d10-12	0.20 ± 0.13	0.20 ± 0.13	0.60 ± 0.22
0.006%@d16-18	0.10 ± 0.10	0.40 ± 0.16	0.60 ± 0.22
0.012%@d16-18	0.30 ± 0.15	0.20 ± 0.13	0.90 ± 0.28
Total Oocyst Output			
	Oocysts Excreted (per bird)		
Control	10,363,825 ± 8,507,180		
0.006%@d10-12	718,629 ± 680,587		
0.006%@d16-18	597,793 ± 288,788		
0.012%@d16-18	493,366 ± 265,856		

[a-b]Indicates significant differences at $p \leq 0.05$.

While differences were not shown in pre-challenge body weights or BWG in Line C pullets, a higher final body weight following the challenge period was observed in both the control group and the group receiving the 0.006% amprolium at days 10 through 12 than those receiving the highest concentration of amprolium (Table 4-7). When comparing both gross and microscopic lesion development between treatments for all areas of the intestine, no significant differences were observed. No differences were shown between treatments with regard to total oocyst output.

Discussion

Previous research has shown that BWG is often not a sufficient means of determining the significance of the effect of *Eimeria* infection on growth inhibition in birds which are restrict-fed due to the already present reduction in weight gain which results from the limited intake (Ruff and Chute, 1980). This helps explain the lack of differences between treatment groups within genetic lines, with the exception of Line B pullets in the first trial. Due to the suppression that already exists as a result of the restricted feeding program, it is difficult to attribute a lack of BWG directly to challenge in these trials.

Because weight gain is not deemed a sufficient determinant of challenge effects, lesion score assessment in challenged birds is considered to be a more sensitive measure of the level of infection in challenged birds (Karlsson and Reid, 1978; Ruff and Chute, 1980; Ruff et al., 1991). This clarifies the increased BWG observed in Line B pullets in trial one, as differences in lesion development did not exist between the groups exhibiting differences in BWG.

Another indicator of the level of immunity development that exists in birds is the number of oocysts excreted following infection (Lillehoj and Ruff, 1987). The differences that were observed in the Line A pullets in trial one indicate an improvement in immunity development when amprolium was administered beginning on day 10; however the decrease in oocyst output was not found in coordination with decreases in lesion development or post-challenge BWG.

Comprehensively, taking into account all parameters of measurement including post-challenge BWG as well as gross and microscopic lesion development and total oocyst output by all treatment groups, although trends were indicative, no direct indication of an inhibition of immunity development was shown to be associated with amprolium administration in replacement broiler breeders of the three genetic lines examined. This is in accordance with previous research evaluating amprolium use in broiler breeders in which pullets previously exposed to coccidia, and administered amprolium while on a restricted feeding program were resistant to subsequent challenge infection which supports the premise that vaccination (previous exposure) in accordance with amprolium administration does not inhibit immunity development (Ruff and Chute, 1980; Ruff et al., 1991).

CHAPTER V

CONCLUSION

Currently in the poultry industry, nearly 100 percent of replacement breeding stock are vaccinated against coccidiosis using a live oocyst vaccine. However, reports from the field have indicated negative effects on weight gain and uniformity relating to the inherent infection associated with vaccination. In effort to combat these negative reactions, integrators have implemented an amprolium medication program to control the infection with further concerns of inhibited immunity development. Various experts in the field disagree on the proper administration protocol to implement in order to minimize this inhibition. The main objectives of this experiment were to determine and compare the effects of amprolium administration at specific times and concentrations on performance and immunity development in separate genetic lines of coccidiosis vaccinated replacement broiler breeders.

The results of this research indicate that effects of amprolium at different concentrations administered at different time points on both flock uniformity and oocyst cycling vary according to genetic line and litter conditions. While no apparent advantage was associated with amprolium administration with respect to body weight in any of the genetic lines, effects on uniformity were variable by line. While one line may have reacted positively to the amprolium administration when raised on used litter, the amprolium could have had little to no effect when that same line was raised on fresh litter. Additionally, one line was negatively affected by certain concentrations on new litter yet reacted positively to the medication when reared on used litter. Litter condition

also appears to alter the pattern and magnitude of oocyst output in certain genetic lines. On fresh pine shavings, the first identifiable peak was observed on days 9 to 11 in certain lines while not appearing until days 17 to 20 in others, whereas on built up litter, the peak of oocyst output is delayed in lines exhibiting early peaking on fresh litter but occurs at similar times in the later peaking line. The magnitude of oocyst output and number of identifiable peaks are influenced by genetic line.

Parameters used to evaluate immunity generation included post-challenge BWG, lesion development following challenge, and total number of oocysts shed per bird. Line B exhibited a significantly higher degree of weight gain in the group receiving the low concentration at days 16 through 18 as compared to the control, which indicates better immunity development, while all other groups were the same. The same treatment in Line B appeared to decrease lesion development in both the mid-gut and ceca in challenged pullets, although there were no notable effects on total oocyst output. In Line C, significantly higher microscopic lesion scores were associated with the highest concentration administration compared to all other groups. Here again, results varied with genetic line.

These data indicate that genetic line and litter environment can have significant impacts on flock uniformity as well as post-vaccination oocyst cycling in vaccinated replacement pullets that receive amprolium. These criteria should be taken into account when considering the appropriate coccidiosis treatment of replacement breeding stock.

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