EFFECTS OF PROBIOTIC ADMINISTRATION DURING COCCIDIOSIS VACCINATION ON PERFORMANCE AND LESION DEVELOPMENT IN BROILERS

A Thesis

by

Anthony Emil Klein, Jr.

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

August 2009

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	David J. Caldwell
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ABSTRACT

Effects of Probiotic Administration during Coccidiosis Vaccination on Performance and Lesion Development in Broilers. (August 2009)

Anthony Emil Klein, Jr., B.S., Texas A&M University

Chair of Advisory Committee: Dr. David J. Caldwell

The principal objective of this investigation was to evaluate coccidiosis vaccination, with or without probiotic administration, for effects on broiler performance and clinical indices of infection due to field strain *Eimeria* challenge during pen trials of commercially applicable durations. During trials 1 and 2, body weights of vaccinated broilers were reduced ($P<0.05$) compared to other experimental groups during rearing through the grower phase. Final body weights, however, were not different among experimental groups at the termination of each trial. Similarly, feed conversion in trials 1 and 2 was increased ($P<0.05$) in vaccinated broilers during rearing through the grower phase when compared to non-vaccinated broilers. Significant improvements ($P<0.05$) in feed conversion were measured in trials 1 and 2 in vaccinated broilers during the withdrawal phase of grow-out. Probiotic administration significantly reduced ($P<0.05$) feed conversion during the withdrawal phase of trial 2. During trial 3, body weights of broilers in the vaccine with probiotic (water) group were higher ($P<0.05$) at termination (d 44) than all other experimental groups and equivalent to the ionophore alone and ionophore with probiotic groups. Similarly, cumulative mortality corrected feed conversion ratio (FCR) was lower ($P<0.05$) in broilers from the vaccine with probiotic

(water) group compared to negative controls, and not different from FCR in ionophore administered broilers.

Trial 2 observations revealed body weight gains among vaccinated broilers that were significantly increased ($P<0.05$) during a seven day clinical field strain *Eimeria* challenge period compared to non-vaccinated broilers. Both probiotic and vaccine significantly decreased ($P<0.05$) gross lesion scores in upper and mid-intestinal regions. A significant reduction ($P<0.05$) in gross lower intestinal lesion score was also observed in the vaccine alone group. In Trial 3 general observations showed, broilers in the ionophore alone group were associated with higher ($P<0.05$) microscopic mid and lower intestine lesion scores when compared to broilers receiving vaccine or vaccine + probiotic. These data suggest that co-administration of probiotic during coccidiosis vaccination results in performance parameters that are improved when compared to vaccination alone and indistinguishable from protection conferred by feeding an ionophore in the presence of field strain *Eimeria*.

DEDICATION

I would like to dedicate this work to my parents and my siblings. To my parents, Tony and Debbie Klein, I would like to thank you for all the support and love you have provided me throughout the years. You have instilled a good work ethic as well as excellent morals into the character that I demonstrate daily as a young man. To Beth and Victoria thank you so much for being there all the time when I needed you even if it was just to make me laugh and relax for a while. To my best friend and little brother Joe, thank you for all of the support and help that you have provided me especially on those late nights when you would help me up at the barn with these projects. I love all of you very much and appreciate everything you have done for me throughout my life.

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CHAPTER I

INTRODUCTION

Avian coccidiosis is an enteric disease condition of commercial poultry caused by host-specific intracellular protozoa of the genus *Eimeria.* Eight *Eimeria* species have been recognized to infect chickens which include *Eimeria acervulina, E. maxima, E. mivati, E. tenella, E. brunetti, E. necatrix, E. mitis,* and *E. praecox* (Conway and McKenzie, 2007). All species parasitize the epithelial cells of the intestine causing pathological changes ranging from local destruction of the mucosal barrier and underlying tissues, to systemic effects such as blood loss, shock syndrome, and even death (Vermeulen et al., 2001). Coccidiosis continues to be one of the most economically critical diseases of the industry exacting economic losses of around 800 million dollars a year (Allen and Fetterer, 2002). The bulk of these costs come from performance losses related to infection and the cost of anticoccidial drugs to control the disease. As stated previously *Eimeria* is extremely host specific. Consequently *Eimeria* species that infect chickens will not infect turkeys, other birds, or mammals, and vice versa (McDougald, 1998). Coccidian parasites replicate via a precisely programmed life cycle that includes three phases: sporogony, merogony, and gametogony (Lillehoj et al., 2000). Once the oocyst is shed into the environment in the feces of the bird, it undergoes sporogony and

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This thesis follows the style and format of Poultry Science.

becomes the infected state know as a sporozoite. Following sporogony, four sporozoites are contained within a single sporulated oocyst. The oocyst is then ingested by the bird from the environment to repeat the life cycle in the intestine of the bird. The rigid life cycle of coccidian parasites is a valuable diagnostic tool for identifying the species of *Eimeria* causing an infection (McDougald, 1998).

There are different control measures for coccidiosis available for use in the U.S. poultry industry today. Two principal types of control measures are anticoccidial drugs and vaccination. The use of anticoccidial drugs is not a new concept. Growers in the industry have been using anticoccidials since the 1950's (Danforth, 1998). These drugs can be classified as either synthetic anticoccidials that are produced by chemical synthesis (chemicals) or ionophorous antibiotics (ionophores) that are produced by fermentation (Chapman, 1999). These types of drugs are still the most commonly used control method for coccidiosis used by today's grower. Earlier studies have shown that many of the compounds that are currently available are no longer as effective against coccidiosis due to emerging drug resistance (Chapman, 1997). Some anticoccidials also require a one, two, or three week withdrawal period from feed prior to slaughter. Although this withdrawal may result in savings with the cost of medication, it must be balanced against increased risk of losses due to coccidiosis if birds become infected (McDougald and Reid, 1997). Many growers today use a shuttle system for administration of these anticoccidial drugs which entails feeding one medication in the diet for a certain period of time and then switching to another type of drug or vaccination, and then continuing this practice in cycles. More recent studies have shown

that even with rotational "shuttle" programs for feeding anticoccidial drugs to minimize the development of drug-resistance in field strain *Eimeria*, resistance to once effective anticoccidials continues to develop on a widespread basis throughout the U.S. and world (Williams, 2005). The potential for residues of anticoccidial drugs to be present in broiler meat is promoting a change in consumer preference towards more natural alternative treatments. Consequently, vaccination is gaining interest among integrators or growers both from an economical and a consumer preference standpoint.

Vaccines, like anticoccidial drugs, have been used as a coccidiosis control measure by the industry for decades, but improved vaccine efficacy seems to be a requirement before broad-based acceptance will be realized. Different types of vaccines are available for use in the industry, including attenuated and non-attenuated variations as well as live and killed vaccines. Killed vaccines usually elicit a poor immune response and provide only limited protection (Du et al., 2005). Although the appropriate antigens are present in killed vaccines required to stimulate the immune system, the immune response is not protective because the vaccine is not invasive and does not replicate within the host. Live vaccines, comprised of oocyst of various *Eimeria* species, are the only practical alternative to anticoccidial drugs for the control of coccidiosis in poultry (Chapman et al., 2002). Live oocyst vaccines usually consist of live oocyst from a "cocktail" of *Eimeria* species. These vaccines work by introducing a low-level infection aimed at stimulating mucosal immunity early in life, thus allowing protective immunity during a subsequent field strain challenge. Live oocyst vaccination has been shown to generate increased weight gain, improved feed conversion, and reduce clinical

lesions associated with coccidial challenge in broilers (Danforth, 1998; Crouch et al., 2003; Li et al., 2005).

Vaccines are usually administered in the first week of rearing. Administration of vaccine can be performed in several ways, which include feed and water applications, gel administration, and the most commonly used method, spray application using a vaccination (spray) cabinet at the hatchery (Williams, 2002). All reports regarding vaccination have not been positive however, and those of reduced performance with vaccination as compared to anticoccidial usage have likely led to the present incomplete acceptance of vaccination as a means of coccidiosis control throughout integrated broiler production (Allen and Fetterer, 2002; Williams, 2002).

An emerging area of interest related to broiler enteric health involves feeding probiotics to stimulate mucosal immunity and improve intestinal health (Dalloul et al., 2003; Farnell et al., 2006). Probiotics are naturally occurring bacteria or a combination of different types of bacteria that possess the potential to improve intestinal or gut health. Probiotic therapy or prophylaxis is a natural approach to improve intestinal health because these bacteria can be found, in some quantity, in the normal microflora of the host. As stated earlier, coccidiosis can have major implications on enteric health in commercial poultry. So given its vital role in animal health, modulation of gut mucosal immunity would seem an appropriate way to affect the economic impact of infectious enteric diseases (Dalloul et al., 2005). Gut mucosal surfaces play a key role in the exclusion and elimination of potentially harmful dietary antigens and enteric microorganisms. Feeding probiotics to poultry has been shown to maintain beneficial

intestinal microflora and may modulate the mucosal immune system enhancing the host's resistance to enteric pathogens (Dalloul et al., 2003; Farnell et al., 2006). Several studies have shown disease prevention or immune enhancement resulting from oral administration of probiotics in poultry (La Ragione, et al., 2004; Koenen et al., 2004). Several papers have also been published demonstrating protection against *Eimeria acervulina* infections in chickens when given a preventative treatment of probiotic bacteria (Dalloul et al., 2003; Dalloul et al., 2005). The probiotic approach would also be considered more natural then medication control measures, and with consumer trends shifting in this direction, probiotics could be a viable alternative to anticoccidial drugs. Probiotics alone have been shown to benefit the host, but to date investigation into the effects of probiotics on the efficacy of coccidiosis vaccination has not been performed. The principal objective of this body of research was to investigate whether a *Lactobacillus*-based probiotic could improve live oocyst coccidiosis vaccination efficacy when administered to broilers under simulated commercial rearing conditions.

CHAPTER II

LITERATURE REVIEW

History and Life Cycle

Coccidia consist of a wide variety of single celled, parasitic animals in the subkingdom *Protozoa* of the phylum *Apicomplexa* (Conway and McKenzie, 2007). This phylum consist of all protozoa that possess an apical complex, which by definition is an assembly of organelles located at the anterior end of certain life cycles stages that facilitate attachment and or entry into the host cell (Current et al., 1990). Within this phylum are two suborders known as *Adeleorina* and *Eimeriorina*. *Eimeria,* which is a pathogen of concern to commercial poultry, belongs to the suborder *Eimeriorina* which consists of 10 families, 37 genera, and around 1500 named species (Levine, 1982). Records show that the first coccidia were studied in 1674 when Leeuwenhoek found oocysts of *Eimeria stiedai* in rabbit bile. Coccidia were not actually described until 1839 by Hake when he thought he found a new form of puss globule (Levine, 1982). Later in 1865, Lindemann named them *Monocystis stiedae.* Finally, after many changes to the name, in 1913 Poche called it Eimeriorina which is accepted today (Levine, 1973).

As described above, there are many genera of parasitic protozoa belonging to the phylum *Apicomplexa* and among those is the genus *Eimeria*. The first avian *Eimeria* life cycle was described in 1910 and the organism was named *Eimeria avium* (Yabsley, 2008). Avian coccidiosis is an enteric disease condition of commercial poultry caused by host specific intracellular parasitic protozoa of the genus *Eimeria.* To date, around

200 species of *Eimeria* have been described to infect avian species (Yabsley, 2008). Eight *Eimeria* species have been recognized to infect chickens, including *Eimeria acervulina, E. maxima, E. mivati, E. tenella, E. brunetti, E. necatrix, E. mitis,* and *E. praecox* (Conway and McKenzie, 2007).

Coccidian parasites, including *Eimeria* species, replicate via a precisely programmed life cycle that includes three phases: sporogony, merogony, and gametogony (Lillehoj et al., 2000). This life cycle also includes parasitic and non-parasitic stages. The life cycle stages consist of two asexual (sporogony,merogony) stages and one sexual (gametogony) stage of reproduction. The parasitic stage begins with the sporulated oocyst which exists in the environment (McDougald and Reid, 1997). The life cycle begins when the infective stage or sporulated oocyst is ingested by the bird. Once ingested, mechanical and chemical (trypsin and bile) actions in the gut break down the oocyst wall and release sporocysts, then sporozoites into the duodenal lumen of the gastrointestinal tract (McDougald and Reid, 1997). Sporozoites invade the intestinal lining of epithelial cells and then proceed into merogony. In the merogony stage of the life cycle, the sporozoites become what are known as first generation meronts. Each meront then, through multiple fission, forms around 900 first generation merozoites (Levine, 1982). Merozoites then enter the lumen of the intestine about two to three days after first inoculation. Form this point some merozoites begin to reinvade the intestinal epithelium to continue the life cycle, while others continue with the further stages of asexual reproduction. After four or five days, the merozoites form around 250-300 second generation merozoites, while some enter new intestinal cells in order to form

third generation meronts which produce anywhere from four to 30 third generation merozoites (Levine, 1982). After repeated cycles of asexual merogony, other merozoites enter the host cell in order to begin the sexual phase of the lifecycle known as gametogony. Gametocytes in this stage develop intracellularly into macrogametocytes (female) and microgametocytes (male). From here, microgametocytes produce many microgametes which are flagellated and motile so that they can migrate to the macrogametocytes for fertilization (Vetterling and Doran, 1966). Macrogametocytes developed into a macrogamete and can then be fertilized by the motile microgametes. Upon fertilization, the macrogamete develops into a zygote and finally to an oocyst. The oocyst is very durable and resistant mostly because of its durable outer wall. This is developed during the entire life cycle by intracytoplasmic granules located peripherally that eventually unite to form the outer wall (Levine, 1982).

An important feature of the life cycle of avian *Eimeria* is that the prepatent period is extremely rapid. From the time of ingestion to the time the oocyst is back into the environment is only 5-7 days, depending upon species. The multiplication of oocysts can occur at a rate of anywhere from 1,000 to 1 million oocysts from one ingested oocyst (Chapman, 1993). The rate of oocyst production from one ingested oocyst varies from species to species. Once the oocyst is in the environment, sporulation can occur through a process called sporogony. In order for sporulation to occur the proper environmental conditions must be present including sufficient oxygen, temperature (72 to 90F), and moisture content (Relative Humidity around 30%). Once the oocyst is passed through the feces into the environment it contains a single celled, diploid sporont. In the

presence of oxygen, the cell undergoes reduction division and a polar body is thrown off (Levine, 1982). Now the cell is haploid and it divides into four sporoblasts. Each sporoblast then turns into a sporocyst containing two sporozoites (Levine, 1982). When the oocyst reaches this stage of the life cycle it becomes a sporulated oocyst. This process usually takes one to two days in the environment under ideal conditions. After sporulation, the oocyst can then be ingested by the animal to repeat the life cycle once again. The characteristic cell wall of an *Eimeria* oocyst makes it extremely hard to eliminate during sanitation. It takes extreme heat to kill a sporulated oocyst in the environment and oocysts are inherently resistant to chemical treatments.

Site Specificity and Immunity

As mentioned previously, *Eimeria* species are extremely host specific enteric pathogens. Chicken *Eimeria* are also site or location specific within the gut, causing damage to different sections of the intestine depending on which species has invaded the host. This characteristic of *Eimeria* infection in commercial poultry represents a valuable tool for field diagnosis of coccidiosis cases. The most commonly diagnosed infections in poultry are those consistent with *Eimeria acervulina*, *E. maxima*, and *E. tenella* derived from heavily populated commercial rearing farms (McDougald et al., 1997). *Eimeria acervulina* and *E. mivati* are site specific to the duodenum or most cranial part of the gastro intestinal tract (Witlock and Ruff, 1977). The mid gut is parasitized and lesions are formed by *E. maxima* and *E. necatrix,* while the highly pathogenic *E. tenella* and *E. brunetti* cause lesions in the ceca and large intestine (Kogut,

1990). *E. acervulina* is possibly the most common species found in domesticated fowl. The life cycle of this species contains 4 asexual generations of meronts. The parasite usually attacks the epithelial cells of the villi, but in some cases it can be found in glandular cells of the upper intestine (Joyner, 1982). This species is usually considered mild to moderately pathogenic. Meronts of *E. maxima* usually develop in the epithelial cells of the villi of the small intestine, and is considered the most immunogenic species of *Eimeria* that is known to infect chickens (Rose and Long, 1962; Joyner, 1982; Chapman et al., 2005). Two asexual generations of meronts are common with *E. maxima* (Joyner, 1982). Perhaps the most pathogenic strain of *Eimeria* known to infect chickens is *E. tenella*. This species parasitizes the villar epithelial cells and the submucosa of the ceca. *Eimeria tenella* is also recognized to have three asexual generations of meronts and is associated with hemorrhagic enteritis and even death in young chicks (Joyner, 1982). All species of chicken *Eimeria* parasitize the epithelial cells of the intestinal lining causing pathological changes ranging from local destruction of the mucosal barrier and underlying tissues, to systemic effects such as blood loss, shock syndrome, and even death (Vermeulen et al., 2001).

Eimeria infections are generally associated with varying degrees of malaise, nutrient malabsorption, reduction of growth rate, and reduced intestinal viscosity depending on the species infecting the host (Allen and Fetterer, 2002). Common signs of infection include bloody or watery diarrhea, morbidity, poor digestion, bloody or soiled vent, and mortality. When the oocyst wall is broken down following ingestion, sporozoites begin to parasitize mucosal and submucosal epithelial cells of the intestine.

The immune response of the chicken to *Eimeria* species is very complex and involves innate and adaptive mechanisms. Primary and secondary infections of *Eimeria* species will stimulate an acquired response that involves both cell mediated and humoral immune components (Lillehoj and Trout, 1996). Since *Eimeria* during an infection parasitize the intestinal mucosa, the first line of defense is the gut associated lymphoid tissue or GALT. Birds lack the classic well defined lymph nodes found in mammals, therefore the GALT has to represent a principal site of coordination for adaptive and innate immune response (Caldwell et al., 2004). The GALT is located above the basement membrane and contains many cells including epithelial, lymphoid, Natural Killer (NK) cells, and other antigen presenting cells which can be found in cecal tonsils, Peyers patches, and the bursa of Fabricius (Lillehoj and Trout, 1996). The lamina propria is located below the basement membrane and contains the submucosa and lymphoid tissue (Yun et al., 2000). The GALT is generally associated with three functions involved in host defense against coccidiosis. These include antigen processing and presentation, production of intestinal antibodies, and activation of cell mediated response (Brandtzaeg et al., 1987).

Peyer's patches are specialized lymphoid organs found in the GALT of the domestic fowl. They consist of lymphoid tissues that provide a microenvironment for lymphocytes and antigen to interact (Burns, 1982). Peyer's patches contain B-cells, T-cells, and antigen presenting cells as well as Microfold cells (M-cells) that allow for antigen uptake from the lumen for inducing immune responses (Yun et al., 2000). Cecal tonsils are structures located at the ileo-cecal junction of the chicken and serve as a B-

cell and T-cell storage site to allow for transport to areas of the body when signaled for immune response (Befus et al., 1980; Glick et al., 1981). Stimulation from contact with continuous new foreign antigen can be achieved here due to the continuous backflow from the urodaeum of the cloaca to the cecal tonsils. Birds have a unique structure known as the bursa of Fabricius that is the central organ of B-cell maturation, B-cell lymphoiesis, and antibody diversity generation (Ratcliffe, 1989). All three of these structures are associated with the GALT and play a key role in immune response to the invading parasite.

Epithelial cells of the various intestinal regions, described above, are the host cells for parasite invasion and replication. These cells obviously also serve as the principle absorptive cells for nutrients obtained from the digesta of the bird. When pathogens are present in the intestinal tract, these cells come in contact with the pathogen and then generate cell death after ingestion of the parasite to try and rid the body of the invading parasites (Yun et al., 2000). More recently epithelial cells have been described as sources of cytokines and other chemicals involved in immunity to invading pathogens (McGee et al., 1993; Reinecker et al., 1996). Once an epithelial cell is invaded by a pathogen several documented cytokines including IL-1, IL-6, IL-8, TNFα are produced (Keelan et al., 1998). These cytokines and other chemicals play an important role in innate and adaptive immunity by contributing to inflammation during the immune response. Although not generally classified as professional antigen presenting cells, intestinal epithelial cells have been shown to express MHC class I and II molecules. This allows them to present antigen to not only CD8+ T-cells but also

CD4+ T-cells (Kaiserlian et al., 1989). The overall function of epithelial cells in resisting coccidiosis infection is not completely understood, but the basis to responses to other invading intestinal pathogens suggests that they are important in immune responses to coccidia.

Once the parasite has been recognized in the host, the innate immune system begins the initial response. Innate immunity is responsible for destruction of parasite during the early phases of the primary infection (Lillehoj and Trout, 1994). Among the first responders are macrophages which possess the proper MHC complex on their surface for presentation to B and T lymphocytes during active immunity (Dalloul and Lillehoj, 2006). They also express co-stimulatory molecules (B7-1 and B7-2) which amplify T-cell activation (Yun et al., 2000). Macrophages also have the capacity to secret a broad array of cytokines which up regulate immune responses (Dalloul and Lillehoj, 2006). Vainio and Lassila (1989) found that chickens that ingested *E. acervulina* oocysts also had significant numbers of sporozoites within macrophages that responded to the infection. Macrophages were also shown to secret large amounts of TNFα upon stimulation by ingested sporozoites (Zhang et al., 1995). These cells thereby induce inflammation at the site of infection which contributes to the severity of the infection. Macrophages play an important role in both innate and active immune response to invading pathogen of the intestine (Dalloul et al., 2007).

Since these parasites are intracellular by nature and are generally ingested by responding cells, NK cells also play an important role in innate immune response. NK cells are phenotypically similar to CD8+ T cells but lack the ability to be antigen

specific. These cells of the innate system are derived from lymphocyte lineage and are usually characterized by a large granular morphology in the cytoplasm. NK cells kill target cells in similar fashion to CD8+ T cells but are not restricted to MHC antigen recognition (Bancroft, 1993). The effects of the NK cells on the invading parasite are controlled by several mechanisms. These include secretion of cytokines (IFNγ), lysis of infected host cells, and direct inhibition of growth of microorganisms through interactions with T-cells (Bancroft, 1993). Besides being able to kill invading cells, the production of IFNγ by NK cells plays an important role in macrophage stimulation (Perussia, 1991). NK cell presence is also associated with an increase in lymphocytes that express CD8+ receptors within the infected epithelial cells (Lillehoj and Bacon, 1991). Therefore, they are believed to be involved in cell mediated as well as innate immune mechanisms directed against *Eimeria*.

Other cells likely involved in innate immunity to coccidial infection are mast cells. Mast cells have very important characteristics that aid in destruction of parasitized cells. These include secretion of pro inflammatory and anaphylactic mediators as well as the ability to release these mediators in multiple cycles to provide response over a long period of infection (Abraham and Arock, 1998). The exact role of mast cells in the innate immune response is not well known however they do seem to play a role. Caldwell and colleagues (2004) have shown the mucosal mast cell population has increased during periods of *Eimeria* challenge. Avian mast cells contain mediators such as histamine and serotonin that stimulate degranulation and hypersensitivity response (Rose et al., 1980). Degranulation of mast cells in response to infection could be

associated with the diarrhea that often accompanies some *Eimeria* infections. Morris and colleagues (2004) noted an increase in mast cell presence during infection with *E. acervulina* as well as an increase in anaphylactic secretary action that can be associated with mast cell response and degranulation. Mast cells are also thought to be involved in the immune response to *Eimeria* however; more research needs to be done to identify the exact role of these cells in protective immune responses.

The adaptive immune response is the second phase of a complete immune response to a foreign antigen. Coccidiosis infections induce a response from both humoral and cell mediated systems in order to protect the chicken's intestinal tract from the infection. B and T lymphocytes are, as described earlier, located within intestinal epithelial cells and in the lamina propria of the GALT of the chicken. Although both cell mediated and humoral responses are present, it has been well documented that the cell mediated responses seems to be the primary mechanism for resolving coccidiosis infections in chickens (Lillehoj and Trout, 1996; Yun et al., 2000; Lillehoj and Lillehoj, 2000).

The role of T-cells in response to coccidial infection in chickens has been investigated thoroughly throughout the years. The presence of *Eimeria* infection has been associated with an increase in CD4+, CD8+, and γδ TCR in the intestine of the bird (Bessay et al., 1996). T-cells are separated into CD4+ (helper/inducer/regulatory) T-cells and CD8+ (cytotoxic) T-cell sub-types. These classes can also be separated by the types of T-cell antigen receptor (TCR) expressed, which include αβ or γδ TCR. CD8+ T cells recognize antigen from MHC class one molecules while CD4+ T-cells recognize

antigen from MHC class two molecules. CD8+ T-cells seem to be the most common responding cell in the presence of *Eimeria* parasites. One study revealed during a characterization of the peripheral blood that 75-80% of the circulating lymphocytes were CD8+ T-cells while 5-15% were CD4+ T-cells during *Eimeria* infection, with the majority of the CD8+ T-cells expressing γδ TCR (Selby et al., 1984). Since *Eimeria* parasites are ingested, γδ TCR CD8+ T-cells are poised for immediate contact as the vast majority of this phenotype of T-cell is found within IECs of the chicken. During coccidial infection, CD4+ T-helper cells likely aid in antibody synthesis during humoral responses and assist in delayed type hypersensitivity reactions.

Eimeria infection in chickens has been shown to result in the production of antigen specific IgA, IgM, and IgY in mucosal secretions. The intestine is the largest immunological organ of the chicken's body, containing 70 to 80% of the total immunoglobulin producing cells, and more secretory IgA than the total production of IgG (Yun et al., 2000). Secretory IgA (sIgA) has been shown to stimulate immunity and provide protection to various enteric pathogens (Ganguly and Waldman, 1980). During coccidiosis, *Eimeria*-specific antibodies may actually have more of an indirect role on parasitic infection. Suggested roles in protective immunity include parasite agglutination, neutralization, causing changes in parasite host cell receptor molecules, and by inhibition of intracellular parasite growth and development (Lillehoj and Lillehoj, 2000).

Cytokines are one of the most important components of a complete immune response in the chicken to coccidial infection. Cytokines are released by various cells

throughout the immune response and are important in regulating both innate and adaptive responses. IFNγ is secreted by T_h1 CD4+ T-cells and NK cells and participates in innate immunity through stimulation of proliferation and differentiation of hematopoietic cells (Lillehoj and Lillehoj, 2000). Macrophage activation is also stimulated by IFNγ which improves the cytolytic activity of the macrophage. Other noted cytokines that are secreted during coccidiosis infection include TNFα, TGFβ, IL-1, and IL-15. TNFα is secreted by macrophages and is associated with reduction in parasite pathogenesis and the development of protective immunity (Byrnes et al., 1993). TGFβ may induce growth of intestinal epithelial cells and villi which can aid in recovery and subsequent protection from the infection (Byrnes et al., 1993). IL-15 promotes γδ TCR expression on the CD8+ T-cell, which assists in cell mediated responses, and is known to promote NK cell proliferation and activation (Choi et al., 1999). Despite significant advances in recent years, complete immunity to *Eimeria* infection is not completely understood. A better understanding could aid in the efforts to develop alternative and more effective methods of control.

Coccidiosis Control Measures-Anticoccidials

The most commonly used method of controlling *Eimeria* infections in commercial poultry is prophylactic chemotherapy through the use of dietary anticoccidial drugs. The first true anticocidials, known as sulphonamides, were introduced to the poultry industry during the 1940's (Chapman, 1999). Over the past 60 years, anticoccidial drugs have made a major contribution to the growth of the poultry

industry due to the effectiveness of the drugs in controlling coccidiosis (Allen and Fetterer, 2002). The two most commonly used types of anticoccidials are synthetic anticoccidials produced by chemical synthesis and ionophorous antibiotics (ionophores) that are produced by fermentation. Synthetic or chemical anticocidials usually have a specific mode of action on parasite metabolism, while ionophores act through a general mechanism of altering membrane ion transport causing a disruption of osmotic balance (Chapman, 1999; Allen and Fetterer, 2002). Examples of chemical or synthetic anticoccidials are amprolium, clopidol decoquinate, nicarbizin, and halofuginone, while some commonly used ionophores include monensin, salinomycin, narasin, and maduramycin. Of the two classifications of anticoccidials, ionophores tend to be the most frequently used by the U.S. broiler industry due to the fact that they have a broad spectrum of activity and are coccidiocidal in action (McDougald, 1990). In general most drugs used for control of *Eimeria* are extremely effective against some species but not very effective against others. As a result, combinations of different drugs may be used to generate better control of the parasite within commercial operations.

Chemical anticoccidial drugs work by acting on specific stages of the *Eimeria* life cycle. Most drugs act upon the asexual stages of the *Eimeria* lifecycle (Chapman, 1993). Ionophores used in coccidiosis control typically target sporozoites, but merozoites can be affected if they come in contact with the drug. Ionophores do not work as well during *in vitro* laboratory tests, but *in vivo* they seem to be the most effective form of control causing significant damage to the parasite (McDougald, 1990). The ionophorous drug acts on the sporozoites by facilitating the actions of inorganic

cations across the cell membrane interrupting important physiological functions such as balance of sodium and potassium (McDougald, 1990). Chemical anticoccidials in general effect the latter stages of the life cycle of *Eimeria* species. One example is the mode of action of amprolium, which disrupts normal function of the parasite by blocking the transport of thymine across the cell membrane (Chapman, 1993). Though not deemed as successful as ionophores, chemical anticoccidial drugs are still used regularly in the industry.

Despite the success of anticoccidials for controlling coccidiosis in commercial environments, certain drawbacks have been associated with continued drug usage throughout the years. The primary concern is drug resistance of field strain *Eimeria* due to repeated exposure to anticoccidials. Drug resistance has been a concern ever since the introduction of anticoccidial drugs in the 1940's. It has been well documented that growing resistance of *Eimeria* species to anticoccidial drugs has occurred over the past several decades (Jeffers, 1974; Chapman,1986; McDougald, 1990) including more recent studies showing resistance in Nicarbazin-containing anticoccidials(Bafundo et al., 2008). Resistance at some level has been identified in all forms of anticoccidials that have been used for control of coccidiosis in the poultry industry (Chapman, 1994). Resistance has been associated with the *Eimeria* species changing levels of sensitivity to continuously used drugs including the extensively used ionophorous drugs (McDougald, 1981). Another downside to anticoccidial use is that some require a mandatory withdrawal period from poultry feed prior to slaughter due to the possibility of residual drug being passed to carcasses entering the retail market. Other concerns stem from

animal welfare-related issues. An example includes the use of nicarbazine and products containing nicarbizine. Nicarbizine is known to have an extremely broad spectrum of anticoccidial activity and can potentiate the activity of ionophorous anticoccidial drugs (Bafundo et al., 2008). It also causes heat stress in commercial poultry when used in broilers that are reared in climates with higher temperatures, as are common in the southern United States during the summer months (McDougald, 1990).

There have been studies conducted which have tested the effect of the withdrawal period on disease outbreak. One thought is that reduced sensitivity to drugs may actually help birds obtain immunity. Using a drug that is known to be effective against certain species of *Eimeria* but not others could allow for mild exposure to the pathogen allowing for immune stimulation even in the presence of anticoccidial drugs, which may allow for a long term withdrawal period (Chapman, 1999). However other studies have shown that obtaining immunity in the presence of anticoccidial drugs could take as long as seven weeks (Chapman, 1999; Chapman et al., 2004). If true, such observations present an obvious problem to broiler integrators since most grow-out programs for commercial broilers only last at most between six and eight weeks. Most regulations on anticoccidial withdrawal periods call for the drug to be pulled at least one week before slaughter. If immunity is not established by this point and the birds are exposed to *Eimeria* during late-phase production, they will become infected and possibly experience performance loss from the infection. Ideally, the most effective method of control while using anticoccidial drugs would be achieved through medication throughout the entire rearing process. As described above, this is not possible for most

anticoccidials used in the U.S. due to mandatory withdrawal regulations in place. Another complication of continual usage would be the likely acceleration of the emerging pattern of drug-resistance among field strain *Eimeria*.

The most commonly recommended control measures for coccidiosis are strategies involving anticoccidial usage with shuttle or rotation programs (McDougald et al., 1986; Vermeulen et al., 2001; Peek and Landman, 2006). Shuttle programs call for the use of one drug for the first 21-28 days of grow-out before switching to another drug for the remainder of rearing. A rotation program consists of one drug or combination of drugs for a certain period of time throughout the year, followed by a switch to vaccination and then continuing this cycle. The rotation program can be applied on a timeline of anywhere from every six months to as long as two years (Levine, 1982). The purpose of these two types of programs is to allow for control while trying to avoid or counteract drug resistance. Studies have shown that resistance is stable and almost impossible to avoid, but the loss of resistance may occur when a drug is not used for a period of time (Chapman, 1993). This phenomenon is thought to be the result of the presence of drug resistant and drug sensitive strains co-populating the rearing environment, where as the drug resistant strain becomes the predominate strain in the population (Long et al., 1985). It has also been stated that since many of the anticoccidials have a similar mode of action, they could also share common resistance when used in the field (Chapman, 1993). The mechanisms involved in the spread of drug resistance are not completely understood and there have not been many studies investigating the length of time it takes for an *Eimeria* species to become resistant to

drugs (Williams, 2006). Increased consumer awareness to the use of medication in poultry feed will presumably demand that broilers be reared using minimal feed additives and medication in the near future (Vermeulen et al., 2001). Eventually poultry producers will have to find alternative control measures for coccidiosis to comply with these restrictions.

Control Measures-Vaccination

As a result of the continued pattern of drug resistance developing in field-strain *Eimeria* species, there is increasing interest in developing alternative control measures to combat coccidiosis infection. Vaccination with live *Eimeria* oocysts is a viable alternative to prophylactic anticoccidial control and vaccination has been proven successful in commercial settings (Danforth, 1998). The use of vaccines as a control measure for avian coccidiosis is not a new concept and has been used since the 1950's (Edgar, 1958; Shirley and Bellatti, 1988). The ability for chickens to become resistant to parasite infection after previously being exposed to the same parasite has been well documented (Gilbert et al., 1988; Augustine et al., 1991; Stiff and Bufando, 1993; Lillehoj and Trout, 1994; Williams, 1998). The purpose of vaccination is to expose the host to low numbers of the parasite in order to stimulate protective immunity against parasitic infection later on in life (Lillehoj and Trout, 1994). Live vaccines used in the poultry industry typically include several species and strains of *Eimeria* typically isolated from commercial poultry production facilities that can either be attenuated (egg-adapted and/or precocious lines) or non-attenuated (Danforth, 1998).

Attenuated vaccines in the past have been more appealing to growers because the strains used for vaccination are not as virulent as those used in non-attenuated vaccines. Attenuation generally involves repeated passage *in vivo* of a virulent strain of the parasite and the subsequent selection of early maturing, less pathogenic, or precocious strains (McDonald and Ballingall, 1983). Once a precocious strain as been selected, the prepatent period of the parasite is decreased due to the reduced generations of merogony, resulting in a reduction in virulence of the parasite (Lillehoj et al., 2000). Another more recently explored method for attenuating *Eimeria* species is irradiation. Sporozoites from sporulated *Eimeria* oocysts exposed to gamma irradiation are apparently still capable of infecting the host, but asexual development of the parasite in the host seems to be reduced (Jenkins et al., 1997), thus reducing the virulence of the parasite. Egg passage or *in vivo* embryo passage of *Eimeria* has also been used for attenuation. While some species showed a reduction in virulence after passage, other species were not amenable to this process (Innes and Vermeulen, 2006). Other methods of attenuation, including inoculating isolated and *in vitro* cultured parasites from different regions of the intestine in order to stimulate immunity, have been studied (Lillehoj et al., 2000) but success has not been common. In theory, attenuated vaccines would be successful in stimulating immunity without the risk of performance loss associated with more virulent non-attenuated vaccines. Aside from fewer reports of efficacy, other negative aspects of attenuated vaccines include the higher production cost associated with the vaccine (Dalloul and Lillihoj, 2005). Furthermore, a study

conducted by Crouch and colleagues (2003) noted that the broilers receiving an attenuated vaccine did not perform as well as broilers receiving anticoccidial medication.

The second classification of live oocyst vaccines available for use in the U.S. poultry industry is the non-attenuated type. Vaccines categorized as non-attenuated are comprised of live *Eimeria* species obtained from the field or laboratory which have not been changed in any way, and typically possess drug sensitivity to commonly used anticoccidials (Dalloul and Lillihoj, 2005). Live non-attenuated vaccines usually contain various species of *Eimeria* depending on the vaccine. For example, broilers are reared for short duration grow-out periods and usually don't need protection against the less common species, so vaccines designed for broilers may only include field strains of *E. maxima, E. acervulina*, and *E. tenella* (Chapman et al., 2005). Live non-attenuated vaccines have been used regularly, but raise some concerns among growers because of the risk of early performance loss. Importantly, vaccines of this type typically stimulate protective immunity to various *Eimeria* species (Shirley and Millard, 1986; Long et al., 1986; Bedrnik et al, 1989; Shirley, 1989).

Live oocyst vaccines have been used for many years, but a common complication to vaccination exists with methods for applying the vaccine to large numbers of animals in a cost-effective, management-feasible, fashion. In order to vaccinate with live oocyst vaccines, growers need an efficient and economically feasible method that will inoculate all birds with a low number of oocysts, while assuring that every chick is receiving the correct dose (Danforth, 1998). Some of the earlier methods explored for live oocyst vaccine administration included spraying vaccine directly on the feed or delivering

orally through a medication tank connected to the watering system (Dalloul and Lillehoj, 2005). Another method that was explored involved inoculating chicks through a spraying mechanism directly into the eye. The idea was the oocyst would travel down the nasolacrimal duct and pass to the intestine through the oropharanx. While deemed effective, this method required highly trained personnel and was not considered feasible by the U.S. poultry industry (Dalloul and Lillihoj, 2005). Immucox® is a live oocyst vaccine used by the industry that is administered as a colored gel placed in chick trays or in feeders at day of hatch for the chicks to ingest. During an investigation of four methods of vaccine delivery, gel administration in this fashion was determined to be the most effective route of administration (Danforth, 1998). While not feasible due to intense labor requirements, the most effective route of administration of a vaccine for achieving uniform exposure would be through oral gavage of each individual chick with live oocysts (Chapman et al., 2002). This method would not be economically possible with the number of birds reared in the United States each year. Administration of live oocyst coccidiosis vaccines in broiler hatcheries is most commonly performed using a spray cabinet delivery system (Augustine et al., 2001). This method, used most extensively for viral vaccination, has been modified for live oocyst coccidiosis vaccines (Chapman et al., 2002). Spray cabinet vaccination can be done quickly and is an economically plausible method of vaccination, but there are efficacy issues associated with this method, such as proper dosage uptake for each individual bird receiving vaccination.

Even though vaccines have been available for a number of years, they have not been widely accepted in terms of use when compared to anticoccidial drug usage. Broilers receiving a vaccine have not always performed, with regards to weight gain and feed conversion, as well as broilers receiving prophylactic medication (Danforth, 1998). Coccidiosis vaccines have been shown to reduce performance parameters early on followed by a period of compensatory growth during the last half of the trial after protective immunity has been established (Danforth et al., 1997a; Mathis, 1999). The period of early performance loss is associated with the mild infection brought on by vaccination in order to stimulate immunity (Danforth et al., 1997a). Studies comparing vaccinated broiler performance to the performance of medicated broilers have shown varying results. Some reports demonstrated that vaccinated broilers did not perform as well as broilers fed medicated diets (Danforth, 1998; Allen and Fetterer, 2002; Williams, 2002), while others suggest that vaccinated broilers can perform just as well if not better than broilers receiving medication (Mathis, 1999; Suo et al., 2006). Despite these different reports, it has been well documented that vaccination can generate increased weight gain, improved feed conversion, and reduce clinical lesions in broilers that have been challenged with *Eimeria* (Danforth, 1998, Crouch et al., 2003, Li et al., 2005). There is continuing research for the advancement of live vaccine use in the industry and vaccine efficacy must be improved to gain broader acceptance. Many different ideas on improvement of vaccine efficacy are beginning to emerge as the industry seems to be moving away, to some degree, from anticoccidial drug use.

Probiotic Use in Commercial Poultry

As the concern of drug resistance to anticoccidials and the threat of restricting certain drugs for use commercially begins to receive attention here in the U.S., the need for alternative methods of coccidiosis control is vital (Lee et al., 2007). One alternative that has been explored is using various dietary and microbial supplements in feed to influence the host's immune system against disease (Dalloul et al., 2003; Duffy et al., 2005). Probiotics are live microbial supplements that improve microbial balance within the intestine resulting in benefits to the host (Fuller, 1989). These bacteria, classified as probiotics, typically include organisms from the genera *Lactobacillus, Saccharomyces, Bacillus,* and *Streptococcus* (Tannock, 2001). The most commonly found probiotic strains used in the poultry industry are *Bacillus* and *Lactobacillus* species (Jin et al., 1996; Zhang et al., 2005). In commercial poultry, maintaining a healthy gut mucosa represents an essential first-step in protection against invading pathogens and dietary antigens (Dalloul et al., 2003). The goal in feeding probiotics to any animal is to ensure that a healthy microbial population exists in the gut microflora. Probiotics are beneficial to the host by creating a beneficial microflora through competitive exclusion, improving digestion, and by changing bacterial metabolism (Jin et al., 1997). If a balanced microbial population is achieved, intestinal immunological defense mechanisms are allowed to achieve optimum performance resulting in a better control of intestinal pathogens (Pollmann et al., 2005). Studies have shown improvement in growth parameters and feed conversion ratio of broilers feed diets containing probiotic supplements when compared to broilers not receiving probiotic supplements (Cavazzoni

et al., 1998; Zulkifli et al., 2000; Samli et al., 2007; Gao et al., 2008; Awad et al., 2009). More recent studies have demonstrated the effects of probiotic usage as an effective tool against various enteric diseases associated with chickens (La Ragione et al., 2004 ; Koenen et al., 2004). This benefit of feeding probiotics that may allow for improved resistance to enteric pathogens might be related to the demonstrated ability of probiotics to stimulate immunity. Farnell and colleagues (2006) reported innate immune response stimulation for broilers administered probiotics through stimulation of heterophil oxidative burst and degranulation. Another study revealed broilers fed *Lactobacillus* based probiotics were positively immunostimulated in the presence of a field strain *E. acervulina* infection when higher levels of IFNγ and IL-2 were observed in broilers receiving probiotics (Dalloul et al., 2005). These reports suggest that broilers receiving probiotics will potentially have an enhanced immune stimulation. Broilers are reared to a young age ranging from six to nine weeks in duration. As such, there typically is insufficient time to develop an extremely effective immune response to many classifications of pathogens. Through early immunostimulation, feeding probiotics to broilers could be an alternative method of enhancing the host resistance to enteric pathogens by creating a more responsive and developed immune system (Dalloul et al., 2003). The only potential negative to feeding probiotics is the possibility of over stimulation of the immune system, which can have a negative effect on performance parameters such as feed conversion and body weight gain. To our knowledge, there has not been a study to date evaluating the effects of probiotic administration on broilers receiving a non-attenuated, live oocyst coccidiosis vaccine. If probiotics stimulate early

immunity in broilers, a reduction in the well documented early production losses associated with the low-level infection introduced by the vaccine might be achievable. Probiotics and other natural feed additives are likely going to be integrally involved in the future with regard to natural supplementation programs for control of diseases and maintenance of a healthy digestive tract in commercial poultry.

Conclusion

Coccidiosis will continue to be a concern in both the world and the U.S. poultry industry. The purpose of the current research project was to evaluate the effects of a commercially available probiotic on live oocyst coccidiosis vaccinate efficacy in broilers. Experiments were conducted measuring performance parameters and intestinal health parameters of broilers administered probiotics and a non-attenuated live oocyst vaccine, as well as a combination of the two. The working hypothesis was that by stimulating mucosal immunity through probiotic administration, while simultaneously vaccinating to build immunity against *Eimeria*, allowance for an improvement in vaccine efficacy throughout the course of broiler grow-out would be achievable.

CHAPTER III

BROILER PERFORMANCE DURING LIVE OOCYST COCCIDIOSIS VACCINATION: INFLUENCE OF PROBIOTIC ADMINISTRATION

Introduction

Coccidiosis is an enteric disease that the poultry industry has battled ever since the beginning of commercial poultry production. The disease is caused by a parasitic protozoan of the genus *Eimeria*. Several species exist that are known to parasitize the epithelial cells of the intestine of chickens causing moderate to severe damage to the intestinal lining or mucosa of the bird (Conway and McKenzie, 2007). Subclinical and clinical cases of coccidiosis are responsible for severe economic and performance losses every year in the industry (Lee et al., 2009). The high density rearing conditions of modern commercial broiler production represent an ideal environment for survival and propagation of *Eimeria* oocysts. Historically, the primary control method for poultry producers to combat this disease has been prophylactic treatment through the use of anticoccidial drugs or "coccidastats". The most commonly used anticoccidial drugs are classified into two categories, synthetic anticoccidials and ionophores. Synthetic anticoccidials (chemicals) are produced by chemical synthesis and affect parasite metabolism at some point in the reproductive life cycle. Ionophorous antibiotics (ionophores) are produced by fermentation and function using a mechanism that affects

ion transport across the parasite membrane to disrupt osmotic balance (Chapman, 1999; Allen and Fetterer, 2002). Through the years, these drugs have played a significant role in the success of the industry by suppressing the negative influence of *Eimeria* infection on broiler performance (Allen and Fetterer, 2002). One concern that has burdened the industry ever since the introduction of anticoccidial drugs is the development of drug resistance to anticoccidials resulting from continued use. Extensive use of anticoccidial drugs, including the ionophores, has generated resistance among *Eimeria* species through reduction of sensitivity to these drugs in the field (McDougald, 1981). Many studies have shown resistance is emerging in the field to all types of anticoccidials currently being used (Jeffers, 1974; Chapman, 1986; McDougald, 1990; Williams, 2006; Bafundo et al., 2008). Alternative methods must be considered in order to assure continued control of this disease.

At present, the only viable and proven alternative approach to controlling coccidiosis in commercial poultry involves immunological stimulation, which can provide for long term protective immunity to subsequent *Eimeria* infection (Williams, 1994). Vaccines have been available for use by the industry for coccidiosis control for many decades, with most common usage being in replacement breeder or layer flocks (Williams et al., 2000). The most common types of vaccines currently available are live oocyst vaccines which are either attenuated or non-attenuated. Non-attenuated vaccines have been shown to be successful in stimulating long term protective immunity in poultry (Shirley and Millard, 1986; Long et al., 1986; Bedrnik et al, 1989; Shirley, 1989). They are not always widely accepted by broiler integrators since the *Eimeria*

present in the vaccine are more virulent than oocysts found in attenuated vaccines, and often are associated with some degree of performance loss (Danforth et al., 1997b; Mathis, 1999; Chapman, 2002). Since these non-attenuated oocysts are unaltered compared to attenuated vaccines, they likely provide for a more natural immune response increasing magnitude and longevity of protective immunity (Lillehoj and Lillehoj, 2000). Attenuation of oocysts is also associated with high production costs for a low yield of product, making this type of vaccine more costly to the grower (Dalloul and Lillehoj, 2005). Attenuated and non-attenuated vaccines have both been successful with regard to stimulating protective immunity to coccidiosis (Shirley and Millard, 1986; Long et al., 1986; Bedrnik et al, 1989; Shirley, 1989). Broiler performance during vaccination, however, when compared to birds receiving prophylactic anticocidials in the diet, has not been always been positive (Danforth, 1998). The current shift in consumer preference regarding poultry rearing in a direction away from traditional medicated control strategies suggests that vaccination should continue to become more of a primary method of coccidiosis control in future years. For vaccines to become a more relied upon control measure for traditional integrated broiler production, several inconsistencies associated with efficacy and administration require improvement.

Probiotic bacteria have been suggested by several investigators to be a natural control method for coccidiosis (Dalloul et al., 2003; Duffy et al., 2005). The commensal bacteria present in probiotic cultures colonize epithelial surfaces of the gut in the intestinal tract of the host (Fuller, 1989). When introduced to the host, probiotics assist in the development of a beneficial microflora through competitive exclusion, which in

turn improves digestion and changes bacterial metabolism (Jin et al., 1997). This colonization has been shown to stimulate mucosal immunity during times of enteric pathogen invasion, thereby increasing the host's resistance to the invading pathogen (Dalloul et al., 2003). Intestinal pathogens can then be detected and destroyed by the host immune system more effectively because a balanced and healthy microbial population could allow for the intestinal immunological defense mechanisms to achieve optimum performance, resulting in a more functional and timely immune system (Pollmann et al., 2005). The objective of this investigation was to determine if the drinking water administration of the commercially available probiotic for poultry, Biomin PoultryStar®, would improve broiler performance and efficacy of the live oocyst coccidiosis vaccine, Coccivac-B®, during a 48 day pen trial. We hypothesize that administration of probiotic during live coccidiosis vaccination will improve broiler performance and vaccine efficacy during this simulated commercial grow-out.

Materials and Methods

Experimental Design

This experimental trial was conducted in a broiler rearing barn 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. The experimental design was a 2X2 factorial (vaccine and probiotic) based ANOVA design with four experimental groups. Groups evaluated were control, probiotic alone, vaccine alone, and

combination of probiotic and vaccine (Vaccine + Probiotic). Each experimental group had 10 replicate pens randomly distributed throughout the barn bringing the total number of pens to 40. Experimental parameters evaluated included body weight, mortality corrected feed conversion, and oocyst output (oocysts shed per gram (OPG) of feces).

Body Weight and Feed Conversion Ratio (FCR)

Body weights were determined on day of placement (d zero), on the day of each feed change (d 15, 30, 40), and again at termination (d 48). Body weights were collected as bulk pen weights and the number of animals present in each pen at the time of weighing allowed for calculation of average broiler weight within each pen. Mortality corrected feed conversion ratio (FCR) was calculated on d 15, 30, 40, and 48.

Oocyst Output (OPG Determination)

Feces were collected from four pens per experimental group beginning on d six post-placement and continuing on an every other day basis until termination of the trial for calculation of oocyst output per gram of feces. After collection of the feces, five grams of fecal material from each sample collected was weighed and diluted in 15 ml of water. Samples were then homogenized and an appropriate volume was loaded onto a McMasters counting chamber using a 200 ul pipette. Using a standard light microscope and a 20X objective, non-sporulated oocysts were counted to determine the oocyst output per gram of feces (OPG).

Experimental Animals and Rearing

The experimental animals used in this trial were straight-run Cobb x Ross broilers obtained at day of hatch from a local commercial hatchery on day-of-hatch.

Each chick was weighed and received a wing-band for identification prior to placement in rearing pens. To ensure uniform body weights at placement (d 0) for all experimental rearing pens or groups, all chicks were randomized according to d 0 body weight. This was performed by separating the heaviest and the lightest 5 % of all d zero body weights and then randomly distributing the remaining chicks to allow for an evenly distributed starting pen weight for each pen. Broilers were distributed to rearing pens at placement density of 0.8 ft^2 per bird to simulate local commercial broiler rearing conditions. This required 45 chicks to be placed per pen achieving a total placement of 1,800 straight-run broilers for the entire trial. Each pen contained a 30 pound tube feeder and commercial-style nipple drinkers. Food and water were provided *ad libitum* to all broilers throughout the trial. The litter or bedding material within each pen during this trial was comprised of 50% fresh pine shavings and 50% used litter removed from a local commercial broiler rearing barn. Birds were fed a non-medicated, corn-soy based diet formulated to approximate the diets of a local broiler integrator. Diets were fed to broilers during this trial according to a four-phase program. This called for the feeding of a crumbled starter diet (d one to 15), followed by pelleted grower (d 15 to 30), pelleted finisher (d 30 to 40), and pelleted withdrawal (d 40 to 48) diets.

Vaccination and Probiotic Administration

Birds designated for vaccination were spray vaccinated using a commercial style (Spraycox® II) vaccination cabinet. The vaccine used was Coccivac®-B (Intervet/Schering-Plough Animal Health, Millsboro, DE), a non-attenuated commercially available live oocyst coccidiosis vaccine for broiler chickens. After

vaccination, chicks were allowed to preen for at least one hour before placement in rearing pens. Probiotic was administered intermittently through the drinking water using a commercial-style water medication system present in the rearing barn. The probiotic administered was Biomin® Poultry Star (Biomin GmbH, Herzogenburg, Austria), a commercially available viable probiotic culture containing beneficial bacterial microflora, including *Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis,* and *Lactobacillus reuteri,* in addition to the carrier inulin. Probiotic was applied at a concentration 20 g per 1000 broilers according to manufacturer's dosage recommendations. Broilers designated for probiotic administration received intermittent drinking water application on d zero through four, d eight through ten, and then again one day prior through one day following feed change (d 14 through 16, 29 through 31, and 39 through 41). Probiotic containing drinking water was colored with a red and green food coloring dye in a pattern that alternated colors on consecutive days. The presence of a dye in the drinking water allowed water lines to be completely drained and re-filled each day of probiotic administration.

Statistical Analysis

Data were analyzed using a factorial ANOVA for vaccine and probiotic administration in the GLM Procedure (SPSS v 11.0). Means were deemed statistically different at $P \leq 0.05$. A significant interaction was present with regard to body weight on d 40. Thus, these data were subjected to a one-way ANOVA. Means were separated using a Duncan's Multiple Range test. Mortality was subjected to an arcsine transformation prior to analysis.

Results

Experimental parameters investigated during this trial included body weight, mortality corrected feed conversion ratio (FCR), cumulative mortality, and oocyst output or shedding measurements (oocyst shed per gram (OPG) of feces). Vaccinated broilers were observed to have a significant ($P<0.05$) reduction in body weight on d 30 of grow-out compared to non-vaccinated broilers (Table 3-1).

Table 3-1. Average body weights (kg) for broilers in all experimental groups on the day of each feed change and termination of the trial.

	Day 15				Day 30		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	0.52 ± 0.00	0.51 ± 0.01	0.51 ± 0.00	Non-Vacc	1.73 ± 0.02	1.70 ± 0.01	1.71 ± 0.01[a]
Vacc	0.52 ± 0.01	0.52 ± 0.00	0.52 ± 0.00	Vacc	1.64 ± 0.01	1.66 ± 0.02	1.65 ± 0.01[b]
Mean	0.52 ± 0.00	0.51± 0.00		Mean	1.68 ± 0.02	1.68 ± 0.01	
	Day 40*				Day 48		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	2.55 ± 0.02[a]	2.51 ± 0.01[ab]	2.53 ± 0.01	Non-Vacc	3.18 ± 0.02	3.08 ± 0.02	3.13 ± 0.02
Vacc	2.45 ± 0.03[b]	2.50 ± 0.03[ab]	2.48 ± 0.02	Vacc	3.10 ± 0.04	3.13 ± 0.04	3.12 ± 0.03
Mean	2.50 ± 0.02	2.50 ± 0.01		Mean	3.14 ± 0.02	3.11 ± 0.02	

[a,b] Means of main effects on different sampling days with different subscripts differ significantly at P<0.05.

*[a,b] Means of individual experimental groups with different subscripts differ significantly at P<0.05 due to a significant interaction observed between vaccine and probiotic.

At termination of the trial on d 48, however, differences in body weight among broilers in all experimental groups were not observed. A probiotic associated interaction ($P<0.05$) was observed among vaccinated broilers with respect to body weights on d 48 (Table 3-1).

A significant ($P<0.05$) increase in FCR in vaccinated broilers was noted during the grower (d 15 to 30) phase of grow-out, similar to observed trends of reduced body weight (Table 3-2). Improvements ($P<0.05$) in FCR were observed in vaccinated broilers during the withdrawal (d 40 to 48) phase of this trial, suggesting improved performance and efficiency during the later phases of grow-out. Probiotic administration did not effect FCR during any of the dietary phases of this trial (Table 3-2). Differences among broilers in all experimental groups were not observed at termination of the trial for cumulative FCR (Table 3-2). Cumulative mortality was significantly ($P<0.05$) reduced in broilers receiving probiotic (Table 3-3).

Table 3-2. Average mortality corrected feed conversion ratio (FCR) of all experimental groups during each dietary phase and at termination of the trial.

	Starter (FCR)				Grower (FCR)		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	1.17 ± 0.01	1.20 ± 0.02	1.19 ± 0.01	Non-Vacc	1.58 ± 0.02	1.57 ± 0.01	1.57 ± 0.01[b]
Vacc	1.16 ± 0.01	1.17 ± 0.01	1.17 ± 0.01	Vacc	1.66 ± 0.01	1.66 ± 0.02	1.66 ± 0.01[a]
Mean	1.17 ± 0.01	1.19 ± 0.01		Mean	1.62 ± 0.02	1.62 ± 0.02	
	Finisher (FCR)				Withdrawal (FCR)		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	2.27 ± 0.02	2.25 ± 0.02	2.26 ± 0.01	Non-Vacc	2.59 ± 0.04	2.83 ± 0.10	2.72 ± 0.06[a]
Vacc	2.28 ± 0.04	2.25 ± 0.02	2.26 ± 0.02	Vacc	2.53 ± 0.05	2.50 ± 0.06	2.52 ± 0.04[b]
Mean	2.28 ± 0.02	2.25 ± 0.01		Mean	2.56 ± 0.03	2.67 ± 0.07	
	Cumulative (FCR) Day 1-48						
	Control	Probiotic	Mean				
Non-Vacc	1.92 ± 0.01	1.95 ± 0.01	1.93 ± 0.01				
Vacc	1.96 ± 0.02	1.96 ± 0.02	1.96 ± 0.01				
Mean	1.94 ± 0.01	1.95 ± 0.01					

[a,b] Means of main effects during different sampling phases with different subscripts differ significantly at $P<0.05$.

Table 3-3. Cumulative mortality of broilers from each experimental group throughout the entire trial.

	Average Mortality %		
	Control	Probiotic	Mean
Non-Vacc	4.09 ± 0.76	2.27 ± 0.97	3.18 ± 0.56[b]
Vacc	7.95 ± 1.30	4.08 ± 2.00	6.02 ± 1.02[a]
Mean	6.02 ± 0.88[a]	3.18 ± 0.76[b]	

[a,b] Means of main effects with different subscripts differ significantly at $P<0.05$.

Oocyst output data supported the body weight and mortality corrected feed conversion ratio data that was measured in this trial. Vaccinated broilers had an increase in oocyst output during the first 26 days of the trial, which can be related to the reduction in body weight on day 30 and an increase in mortality corrected feed conversion ratio observed for vaccinated broilers during the grower (d 15 to 30) phase of rearing. After day 26 vaccinated broilers oocyst output diminished to virtually nothing which allowed for improved performance parameters during the final weeks of the trial. Non-vaccinated broilers showed an increase in oocyst output after d 26, which corresponds to the observed slight performance loss, which could be associated with mild infection, that allowed for vaccinated broilers to compensate for the earlier performance losses that these broilers experienced (Figure 3-1).

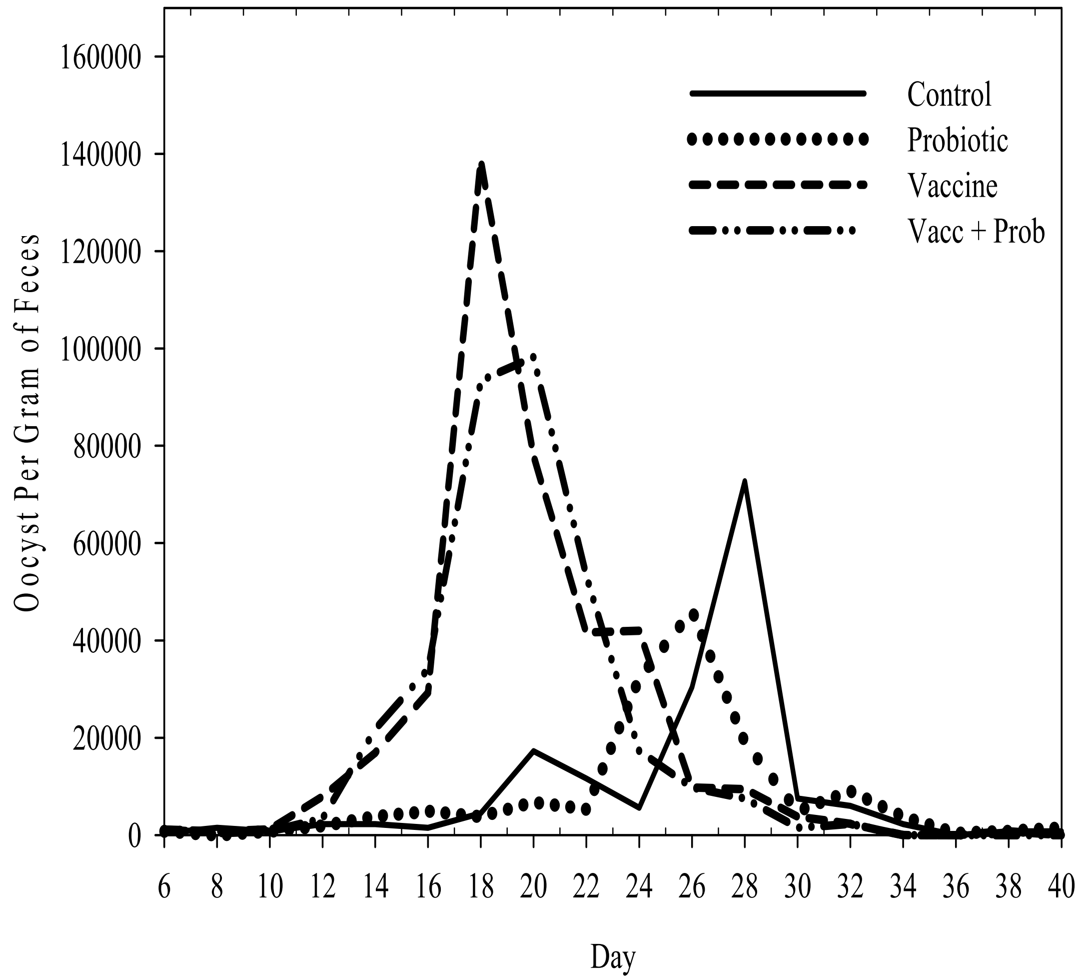

Figure 3-1. Oocyst output (oocyst per gram (OPG) of feces) from four randomly selected pens from each experimental group beginning on d six and continuing on an every other day basis until d 40.

Discussion

When a broiler receives a live oocyst coccidiosis vaccine, it is not uncommon to experience some degree of performance loss in the early phases of production due to the mild infection brought on by vaccination (Chapman et al., 2002). In order to stimulate protective immunity during later phases of rearing, exposure to the parasite must occur early in the animal's life. Body weights and FCR were negatively affected by vaccination during the grower phase of the current trial (Table 3-1, 3-2). Body weights on d 30 for vaccinated broilers were lower than non-vaccinated broilers, representing a performance loss during the period of vaccine-induced infection. Similarly, FCR in the grower phase reflected this pattern among vaccinated birds with a significant increase in FCR when compared to non-vaccinated broilers during period of grow-out (Table 3-2). These findings were expected and are supported by previously published work that yielded similar findings (Danforth et al., 1997a; Mathis, 1999). This observation suggests that the probiotic administration improved vaccine efficacy throughout the withdrawal phase of the trial.

During commercial grow-out, broilers are exposed to and can be infected by field strain *Eimeria* present in the rearing environment. Throughout this trial OPG data was collected in order to determine the presence of wild type and vaccine strain *Eimeria* that existed in rearing environment of this pen study. Oocyst output was reduced after d 26 of the trial for vaccinated broilers (Figure 3-1). Although body weights were not significantly different at termination, the pattern of oocyst output for vaccinated broilers can be associated with a decrease of FCR during the withdrawal phase of this trial.

Mortality was recorded daily during the duration of the trial and birds receiving probiotic had a reduction in cumulative mortality at termination of the trial. To our knowledge, these or similar observations have not been reported in literature.

Earlier research has been reported which suggests vaccination decreased cumulative mortality (Danforth, 1998), however in this trial, our observations revealed that vaccination did not significantly (P<0.05) reduce cumulative mortality. Non-vaccinated broilers showed an increase in oocyst output after d 26 of this trial (Figure 3-1). However, output was still considerably lower during this time then the peaks seen early on associated with vaccinated broilers. Birds were raised on used litter, but oocyst output data suggest that, though *Eimeria* was present in the litter, the concentration of field-strain oocysts was likely not enough to induce a significant infection in non-vaccinated broilers. Mild infection during the last 14 to16 days of the trial in non-vaccinated broilers could be associated with the vaccinated birds gaining more weight in the final phases of the trial allowing these birds to complete the trial with similar performance numbers. Taken together, these observations suggest that the administration of a probiotic in combination with a live oocyst vaccine may lead to improved performance during coccidiosis vaccination in broilers, potentially due to enhanced immunostimulation and vaccine efficacy that positively affect enteric health during rearing.

CHAPTER IV

EVALUATION OF PROBIOTIC ADMINISTRATION ON COCCIDIOSIS VACCINATION IN BROILERS: EFFECTS ON PERFORMANCE PARAMETERS AND OOCYST OUTPUT

Introduction

Avian coccidiosis, caused by the protozoan pathogen of the genus *Eimeria*, is one of the most economically important health problems within the commercial poultry industry. Eight species of *Eimeria* are known to parasitize the intestinal epithelium of the chicken, causing pathophysiological complications that include morbidity, watery or mucoidal diarrhea, and even hemorrhagic diarrhea or death (Kogut, 1990). Concerns over drug resistance associated with the continuous feeding of anticoccidial drugs in poultry diets have existed since the industry began using anticoccidials as a primary control method for avian coccidiosis decades ago (McDougald, 1981). More recently, the industry has shown a keen interest in developing alternative methods for controlling coccidiosis in commercial poultry flocks. Alternative methods that have been researched to date include live oocyst vaccination and drug-free additives such as probiotics or direct fed microbial cultures.

Vaccination has been available for use in the industry for approximately 60 or more years (Edgar, 1958; Shirley and Bellatti, 1988). There are two types of vaccines commonly used in the industry, attenuated and non-attenuated live oocyst vaccines. Attenuated vaccines have been more appealing to growers in the past because they are

less virulent then their non-attenuated counterparts (Williams, 1994). Non-attenuated vaccines have been proven affective and may have advantages in stimulating protective immunity over attenuated vaccines (Lillehoj and Lillehoj, 2000). Non-attenuated live oocyst vaccines likely provide added protection, when compared to attenuated vaccines, during subsequent *Eimeria* challenge since they are unaltered oocysts that are more similar to field strains encountered by poultry during commercial grow-out. Both types of vaccines have been shown to stimulate some degree of long term protective immunity to coccidiosis (Shirley and Millard, 1986; Long et al., 1986; Bedrnik et al, 1989; Shirley, 1989; Allen et al., 1997). There are concerns, however, regarding the efficacy of vaccines in the minds of many producers and tech service personnel, as many feel anticoccidials have been shown to be more effective under field applications. As such, additional research into vaccine efficacy and usage is needed.

Another alternative method of control that has been explored recently involves the use of microbial feed additives, probiotics or direct fed microbials, which have potential properties of stimulating mucosal immunity in poultry. Probiotics are naturally occurring live strains of bacteria that can be beneficial to the host gut microflora either in terms of intestinal development, nutrient utilization, or defense against enteric pathogen challenge (Tannock, 2001; Dalloul et al., 2003; Duffy et al., 2005). The inclusion of probiotics in feed has been shown to stimulate mucosal immunity and perhaps aid in the host defense against invading pathogens (La Ragione et al., 2004; Koenen et al., 2004). To date, very little research, if any, has focused upon the effects of probiotic administration on coccidiosis vaccination in commercial poultry. Given the effects

observed to date, which suggest probiotics improve gut maturation, nutrient utilization, and intestinal immunity in commercial poultry, we feel investigation into the potential positive effects probiotics may have on enteric vaccination, including coccidiosis vaccination in commercial strain poultry, is warranted. The purpose of the following research was to evaluate coccidiosis vaccination (Coccivac®-B), with or without drinking water probiotic (Biomin® PoultryStar) administration, in commercial strain broiler chickens for protection against field strain *Eimeria* challenge during a 42 day pen trial. Our hypothesis was that feeding probiotics to broilers during live coccidiosis vaccination could improve vaccine efficacy and resultantly improve protection against field-strain *Eimeria* challenge during a 42 day pen trial.

Materials and Methods

Experimental Design

This experimental trial was conducted in a broiler rearing barn at the Texas A&M University Poultry Science Teaching, Research, and Extension Center facility 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. The experimental design was a 2X2 factorial (vaccine and probiotic) based ANOVA design with four experimental groups. Groups evaluated were control, probiotic alone, vaccine alone, and combination of probiotic and vaccine (Vacc + Prob). Each experimental group had 10 replicate pens randomly distributed throughout the barn bringing the total number of pens to 40. Birds were reared to day 42 under industry simulated rearing

conditions. A field strain *Eimeria* challenge was spray applied to the litter for all pens in the study on d 14 of the trial. On d 35, four birds from each pen were removed, weighed, and challenged by oral gavage before being placed in four separate pens according to respective experimental group. These birds were reared for the last seven days of the trial in these pens to evaluate performance and intestinal parameters during a clinical challenge. Experimental parameters measured for all birds in the main performance trial included body weight, mortality corrected feed conversion (FCR), and oocyst output associated with vaccination or challenge (oocysts shed per gram (OPG) of feces). Intestinal and performance parameters for the clinically challenged birds included gross and microscopic intestinal lesion score, intestinal weights, and weight gain over the seven day challenge period.

Body Weight and Feed Conversion Ratio (FCR)

Body weights were determined on day of placement (d zero), on the day of each feed change (d 14, 28, 35), and again at termination (d 42). Body weights were collected as bulk pen weights and the number of animals present in each pen at the time of weighing allowed for calculation of average broiler weight within each pen. Mortality corrected feed conversion ratio (FCR) was calculated on d 14, 28, 35, and 42.

Oocyst Output (OPG Determination)

Feces were collected from four randomly selected pens per experimental group beginning on d six post-placement and continuing on an every other day basis until termination of the trial for calculation of oocyst output per gram of feces. After collection of the feces, five grams of fecal material from each sample collected was

weighed and diluted in 15 ml of water. Samples were then homogenized and an appropriate volume was loaded onto a McMasters counting chamber using a 200 ul pipette. Using a standard light microscope and a 20X objective, non-sporulated oocysts were counted to determine oocyst output per gram of feces (OPG).

Experimental Animals and Rearing

The experimental animals used in this trial were straight-run Cobb x Ross broilers obtained on day-of- hatch from a local commercial hatchery. Each chick was weighed and received a wing-band for identification prior to placement in rearing pens. To ensure uniform body weights at placement for all experimental rearing pens or groups, all chicks were randomized according to day-of-hatch body weight. This was performed by separating the heaviest and the lightest 5 % of all d zero body weights and then randomly distributing the remaining chicks to allow for an evenly distributed starting pen weight for each pen. Broilers were distributed to rearing pens at placement density of 0.8 ft^2 per bird to simulate local commercial broiler rearing conditions. This required 45 chicks to be placed per pen achieving a total placement of 1,800 straight-run broilers for the entire trial. Each pen contained a 30 pound tube feeder and commercial-style nipple drinkers. Food and water were provided *ad libitum* to all broilers throughout the trial. The litter or bedding material within each pen during this trial was comprised of litter used to rear broilers in a previous trial (Chapter III), top dressed with an equivalent amount per pen of fresh pine shavings. Birds were fed a non-medicated, corn-soy based diet formulated to approximate the diets of a local broiler integrator. Diets were fed to broilers during this trial according to a four-phase program. This

called for the feeding of a crumbled starter diet (d zero to 14), followed by pelleted grower (d 14 to 28), pelleted finisher (d 28 to 35), and pelleted withdrawal (d 35 to 42) diets.

Vaccination and Probiotic Administration

Birds designated for vaccination were spray vaccinated using a commercial style (Spraycox® II) vaccination cabinet. The vaccine used was Coccivac®-B (Intervet/Schering-Plough Animal Health, Millsboro, DE), a non-attenuated commercially available live oocyst coccidiosis vaccine for broiler chickens. After vaccination, chicks were allowed to preen for at least one hour before placement in rearing pens. Probiotic was administered intermittently through the drinking water using a commercial-style water medication system present in the rearing barn. The probiotic administered was Biomin® Poultry Star (Biomin GmbH, Herzogenburg, Austria), a commercially available probiotic culture containing beneficial bacterial microflora, including *Enterococcus faecium, Pediococcus acidilactici, Bifidobacterium animalis*, and *Lactobacillus reuteri* in addition to the carrier inulin. Probiotic was applied at a concentration 20 g per 1000 broilers according to manufacturer's dosage recommendations. Broilers designated for probiotic administration received intermittent drinking water application on d zero through three, d eight to ten, and then again one day prior through one day following feed change (d 13 through 15, 27 through 29, and 34 through 36). Probiotic containing drinking water was colored with a red or green food coloring dye in a pattern that alternated colors on consecutive days. The presence of a

dye in the drinking water allowed water lines to be completely drained and re-filled each day of probiotic administration.

Field Strain* Eimeria *Challenge

Eimeria oocysts used for both the d 14 litter spray challenge and the d 35 oral challenge were field strain species derived from a local commercial broiler production facility. *Eimeria* species in the challenge inoculum included *E. acervulina*, *E. tenella*, *E. maxima*, and *E. mivati.* The d 14 challenge involved spray applying 35,000 oocyst per bird to the litter in each rearing pen. Broilers removed from grow-out pens that were orally challenged on d 35 received approximately 550,000 oocyst per bird.

Indices of Clinical* Eimeria *Challenge

Broilers designated for clinical challenge were removed on d 35 from performance rearing pens and placed in 4 separate pens according to experimental group. The body weight of each individual bird was recorded before placement in designated pens. After the seven day challenge period, each broiler was weighed in order to calculate weight gain during challenge. Once the weight was recorded, the bird was killed and necropsy was performed. Gross upper (duodenal loop), mid (jejunum and ileum), and lower (cecum) intestine lesion scores were determined following methods published by Johnson and Reid (1970). After gross lesion scores were determined, a scrapping of each intestinal section was taken and placed on a microscope slide with a coverslip. Each slide was examined using a standard light microscope with a 20X objective to determine the upper, mid, and lower microscopic intestinal lesion score. In total, 10 samples from each group were subjected to this method of lesion scoring. The

final intestinal parameter measured was upper (small intestine) and lower (large intestine) intestinal weight. Ingesta or fecal material was removed with water and physical action before intestinal sections were weighed.

Statistical Analysis

All data were analyzed using a factorial ANOVA for vaccine and probiotic administration in the GLM Procedure (SPSS v 11.0). Means were deemed statistically different at $P \leq .05$. A significant interaction was present with regard to mid gross and microscopic lower-intestinal lesion score. Thus, data were subjected to a one-way ANOVA. Means were separated using a Duncan's Multiple Range test.

Results

At termination of this trial body weight, body weight gain, and mortality corrected feed conversion ratio in broilers in all experimental groups were compared. Average body weight was significantly ($P<0.05$) decreased on d 28 in vaccinated broilers when compared to non-vaccinated broilers. However at termination of the trial on d 42, there were no significant differences in body weight among broilers in all experimental groups (Table 4-1).

Table 4-1. Average body weights (kg) for broilers in all experimental groups on the day of each feed change and termination of the trial on d 42.

	Day 14				Day 28		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	0.44 ± 0.00	0.43 ± 0.01	0.43 ± 0.01	Non-Vacc	1.52 ± 0.01	1.53 ± 0.02	1.52 ± 0.01[a]
Vacc	0.43 ± 0.01	0.44 ± 0.00	0.44 ± 0.00	Vacc	1.49 ± 0.01	1.47 ± 0.02	1.48 ± 0.01[b]
Mean	0.44 ± 0.00	0.43± 0.00		Mean	1.50 ± 0.01	1.50 ± 0.01	
	Day 35				Day 42		
	Control	Probiotic	Mean		Control	Probiotic	Mean
Non-Vacc	2.21 ± 0.02	2.21 ± 0.03	2.21 ± 0.02	Non-Vacc	2.83 ± 0.02	2.85 ± 0.03	2.84 ± 0.02
Vacc	2.21 ± 0.02	2.20 ± 0.01	2.20 ± 0.01	Vacc	2.85 ± 0.02	2.83 ± 0.02	2.84 ± 0.02
Mean	2.21 ± 0.01	2.20 ± 0.02		Mean	2.84 ± 0.02	2.84 ± 0.02	

[a,b] Means of main effects on day 28 differ significantly at $P<0.05$.

Mortality corrected feed conversion (FCR) comparisons among experimental groups revealed a significant (P<0.05) increase among the vaccinated broilers during the grower phase (d 14 to 28) of the trial, which correlates to the reduction in body weight observed on day 28 for vaccinated broilers. During the finisher (d 28 to 35) and withdrawal (d 35 to 42) phases, a significant decrease in FCR was observed in vaccinated broilers, indicating improved performance in the second half of grow-out in vaccinated groups. Probiotic administration was associated with a significant (P<0.05) reduction of FCR during the withdrawal phase of the trial. Probiotic administration did not affect FCR during any other dietary phase throughout the trial (Table 4-2). Cumulative mortality was calculated at termination of the trial for all experimental groups. However there were no differences (P>0.05) among experimental groups (Table 4-3).

Table 4-2. Average mortality corrected feed conversion ratio (FCR) of all experimental groups by dietary phase and at termination of the trial.

Starter (FCR)	Control	Probiotic	Mean
Non-Vacc	1.18 ± 0.01	1.21 ± 0.02	1.19 ± 0.01
Vacc	1.22 ± 0.02	1.21 ± 0.02	1.21 ± 0.01
Mean	1.20 ± 0.01	1.21 ± 0.01	

Grower (FCR)	Control	Probiotic	Mean
Non-Vacc	1.57 ± 0.01	1.58 ± 0.01	1.57 ± 0.01^{b}
Vacc	1.62 ± 0.02	1.63 ± 0.02	1.62 ± 0.01^{a}
Mean	1.60± 0.01	1.60 ± 0.01	

Finisher (FCR)	Control	Probiotic	Mean
Non-Vacc	1.90 ± 0.02	1.94 ± 0.03	1.92 ± 0.02^{a}
Vacc	1.86 ± 0.01	1.82 ± 0.04	1.84 ± 0.02^{b}
Mean	1.88 ± 0.01	1.88 ± 0.03	

Withdrawal (FCR)	Control	Probiotic	Mean
Non-Vacc	2.23 ± 0.06	2.08 ± 0.04	2.16 ± 0.04^{a}
Vacc	2.05 ± 0.01	2.03 ± 0.04	2.04 ± 0.02^{b}
Mean	2.14 ± 0.04^{a}	2.05 ± 0.03^{b}	

Cumulative (FCR) Day 1-48	Control	Probiotic	Mean
Non-Vacc	1.74 ± 0.01	1.72 ± 0.01	1.73 ± 0.01
Vacc	1.72 ± 0.01	1.71 ± 0.01	1.71 ± 0.01
Mean	1.73 ± 0.01	1.71 ± 0.01	

^{a,b} Means of main effects during different sampling phases with different subscripts differ significantly at $P<0.05$.

Table 4-3. Cumulative mortality of broilers from each experimental group during the entire trial.

	Cumulative Mortality %		
	Control	Probiotic	Mean
Non-Vacc	7.07 ± 0.76	3.41 ± 0.97	5.24 ± 0.73
Vacc	5.61 ± 1.30	8.29 ± 2.00	6.95 ± 1.22
Mean	6.34 ± 0.77	5.85 ± 1.23	

Oocyst output supported the observed performance data in this trial. Increased output peaks during the grower phase (d 14 to 28) for vaccinated broilers were associated with the increase in mortality corrected feed conversion ratio during this phase and the decrease in average body weight that was observed on d 28 of grow-out (Figure 4-1). Vaccinated broilers also had a reduction of output during the last 16 days of the trial that correspond with the observed reduction of FCR and increase in body weight gain during these phases of grow-out. Non vaccinated broilers in this trial were associated with an increase in oocyst output during the finisher and withdrawal phases of the diet, suggesting oocyst exposure later in production was associated with poorer performance in these animals when compared to vaccinated broilers (Figure 4-1). The oocyst output data in this trial followed similar trends that were observed in the previous trial that is discussed in Chapter III of this thesis.

Broilers that were removed from the original performance trial and challenged orally with field strain *Eimeria* were subject to performance and intestinal parameter evaluation during the challenge phase of this trial. Gross lesion scoring revealed a significant ($P<0.05$) decrease in lesion score in vaccinated broilers in the upper, mid, and lower intestinal regions (Table 4-4).

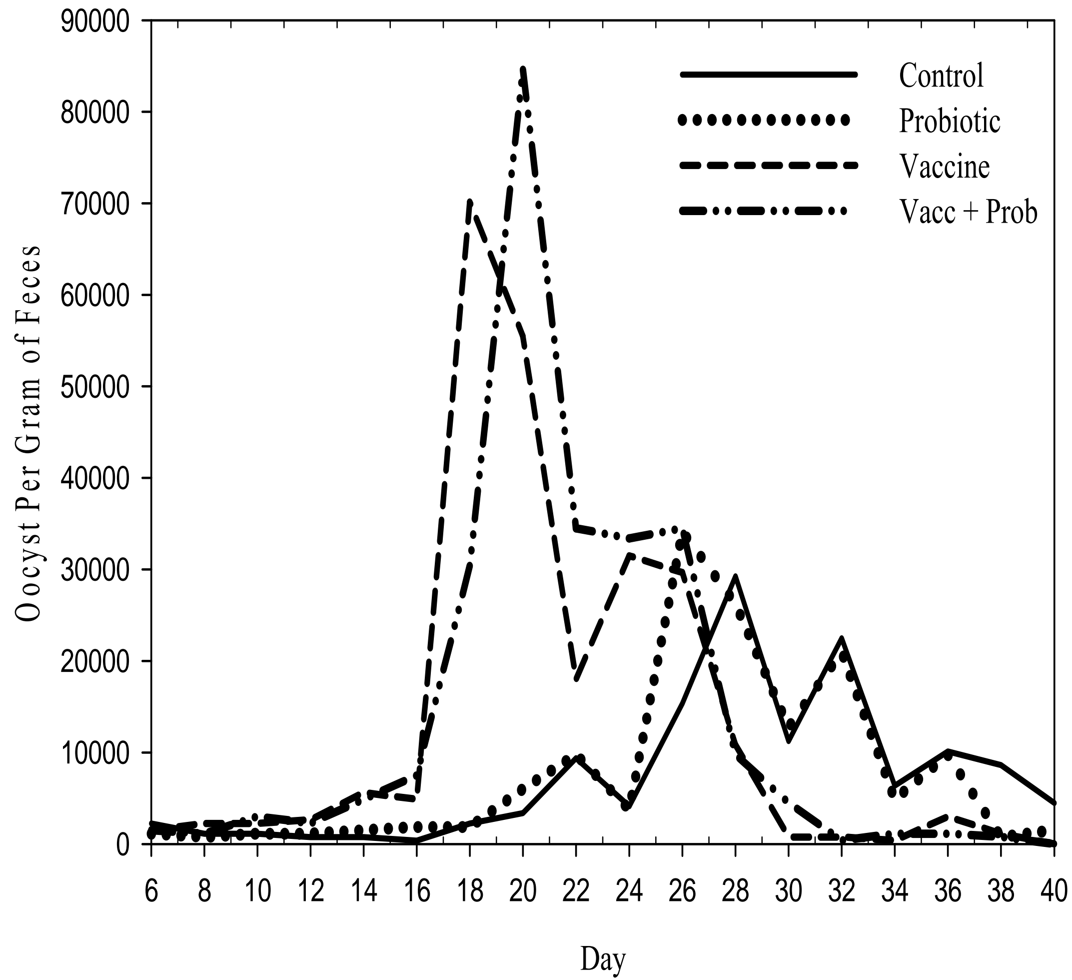

Figure 4-1. Oocyst output (oocyst per gram (OPG) of feces) from four randomly selected pens from each experimental group starting on d six and continuing on an every other day basis until d 40.

Table 4-4. Gross intestinal lesion score, by region of intestine, for each experimental group of broilers included in the d 35 clinical *Eimeria* challenge.

Gross Upper Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	0.33 ± 0.09	0.14 ± 0.07	0.24 ± 0.06[a]
Vacc	0.10 ± 0.06	0.07 ± 0.05	0.08 ± 0.04[b]
Mean	0.22 ± 0.05	0.10 ± 0.04	

*Gross Mid Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	0.93 ± 0.14[a]	0.34 ± 0.09[b]	0.64 ± 0.09
Vacc	0.43 ± 0.09[b]	0.53 ± 0.10[b]	0.48 ± 0.07
Mean	0.68± 0.09	0.44 ± 0.07	

Gross Lower Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	1.13 ± 0.17	0.90 ± 0.18	1.02 ± 0.12[a]
Vacc	0.43 ± 0.09	0.53 ± 0.09	0.38 ± 0.06[b]
Mean	0.78 ± 0.11	0.61 ± 0.11	

[a,b] Means of main effects for different regions of the intestine with different subscripts differ significantly at P<0.05.

*[a,b] Means of individual experimental groups with different subscripts differ significantly at P<0.05 due to a significant interaction observed between vaccine and probiotic.

Probiotic administration was linked to a decrease ($P<0.05$) in gross lesion score in the mid intestinal region (Table 4-4). Microscopic lesion scores were supportive of gross lesion scores, as vaccination reduced ($P<0.05$) upper, mid, and lower intestinal microscopic scores. Probiotic administration reduced ($P<0.05$) microscopic lesion score in the lower intestine (Table 4-5).

A significant interaction was observed between probiotic and vaccine in the gross mid intestinal region and the microscopic lower intestinal region, suggesting that all groups receiving treatment had a significant reduction in lesion development in that specific region of the intestine. Body weights at the initiation and end of challenge (seven days post challenge) were used to calculate weight gain during challenge. Vaccinated broilers were associated with a significant ($P<0.05$) increase in body weight gain during the seven day challenge period when compared to weight gain of non vaccinated broilers. Vaccination had an apparent effect on relative upper intestinal weights, but these findings were not shown to be significantly ($P=0.07$) different (Table 4-6).

Table 4-5. Microscopic intestinal lesion score, by region of intestine, for each experimental group of broilers included in the d 35 clinical *Eimeria* challenge.

Micro Upper Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	0.64 ± 0.15	0.44 ± 0.18	0.55 ± 0.11^{a}
Vacc	0.00 ± 0.00	0.20 ± 0.13	0.10 ± 0.07^{b}
Mean	0.33 ± 0.11	0.32 ± 0.11	

Micro Mid Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	0.82 ± 0.18	0.56 ± 0.18	0.70 ± 0.13^{a}
Vacc	0.30 ± 0.15	0.40 ± 0.16	0.35 ± 0.11^{b}
Mean	0.57± 0.13	0.47 ± 0.12	

*Micro Lower Intestinal Legion Score	Control	Probiotic	Mean
Non-Vacc	2.00 ± 0.38^{a}	1.10 ± 0.11^{b}	1.60 ± 0.23
Vacc	0.60 ± 0.31^{b}	1.00 ± 0.26^{b}	0.80 ± 0.20
Mean	1.33 ± 0.29	1.05 ± 0.14	

[a,b] Means of main effects different regions of the intestine with different subscripts differ significantly at P<0.05.

*[a,b] Means of individual experimental groups with different subscripts differ significantly at P<0.05 due to a significant interaction observed between vaccine and probiotic.

Table 4-6. Average body weight gain (g) and relative intestinal weights (g) for each experimental group of broilers included in the d 35 clinical *Eimeria* challenge.

	Avg. Body Weight Gain (g)		
	Control	Probiotic	Mean
Non-Vacc	616.2 ± 43	628 ± 19	622 ± 23 [b]
Vacc	746.1 ± 35	691.7 ± 18	718.9 ± 20 [a]
Mean	681.2 ± 29	660.4 ± 14	

	Relative Upper Intestinal Wt. (g)		
	Control	Probiotic	Mean
Non-Vacc	3.14 ± .17	2.98 ± .16	3.06 ± 0.12
Vacc	2.84 ± .10	2.89 ± .11	2.86 ± 0.07
Mean	2.98 ± 0.10	2.93 ± 0.09	

	Relative Lower Intestinal Wt. (g)		
	Control	Probiotic	Mean
Non-Vacc	0.47 ± .03	0.48 ± .03	0.48 ± 0.02
Vacc	0.45 ± .02	0.41 ± .02	0.43 ± 0.02
Mean	0.46 ± 0.02	0.45 ± 0.02	

[a,b] Means of main effects for average body weight gain with different subscripts differ significantly at $P<0.05$.

Discussion

Body weight, weight gain, and mortality corrected feed conversion were affected by vaccination during this trial. Reduced body weight was observed on d 28 when vaccinated broilers were compared to non-vaccinated broilers (Table 4-1). Similarly, FCR was increased during the grower phase of the trial in vaccinated broilers, which can be directly linked to the reduction in body weight for the first 28 days of the trial. Vaccination causes a mild infection in broilers in order to stimulate long term protective immunity. Clearly, this would be associated with these early losses in performance (Danforth et al., 1997b). However during the final 14 days of this trial, vaccinated broilers showed a decrease in feed conversion when compared to non-vaccinated broilers (Tables 4-2). Average body weight at termination of the trial showed no significant differences among experimental groups (Table 4-1). These findings were not surprising as it has been previously published that vaccination will cause performance loses in broilers during the first half of rearing followed by a period of compensatory growth during the last half of grow-out (Danforth et al., 1997a; Mathis, 1999). Probiotic administration was shown to decrease FCR in the withdrawal phase of the diet (Table 4-2). Previous studies have shown similar results with different types of probiotic supplements improving broiler performance parameters including FCR (Gao et al., 2008; Awad et al., 2009). Oocyst output or shedding supports broiler performance data in this trial (Figure 4-1). Output among vaccinated broilers was associated with two significant peaks of oocyst shedding. The first peak occurred in both vaccinated groups between d 14 and 18 and was more intense than the second peak which occurred between d 22 and

26 (Figure 4-1). Following these peaks, vaccinated broilers were associated with essentially no oocyst output or shedding for the remainder of the trial. This pattern is predictive of the increase in feed conversion, decrease in body weight, and decrease in body weight gain during the first 28 days of the trial. The minimal output during the final 14 days of the trial supports these data showing a decrease in feed conversion and an increase in body weight. Non-vaccinated broilers demonstrated a similar pattern of output with two major peaks or cycles of shedding (Figure 4-1). These peaks, however, occurred during the last 14 days of the trial. Although these peaks were less intense, reflecting a lower number of oocysts causing infection, they probably were the cause of the decrease in body weight, as well as an increase in feed conversion, seen in the non-vaccinated broilers during the last 14 days of rearing. These observations describe how vaccinated broilers were able to compensate for the losses experienced early, within the last 14 days of the trial, thus allowing ending body weights and FCR to be equivalent among experimental groups.

Broilers that were removed from the performance trial and challenged with field strain *Eimeria* revealed similar trends respective to performance data. Vaccinated broilers had an increase in weight gain when compared to non-vaccinated broilers during the seven day clinical challenge period (Table 4-6). This can be associated with immunity to challenge obtained from previous exposure through vaccination. Intestinal parameters were also affected by vaccination. Gross and microscopic upper, mid, and lower lesion scores were reduced significantly in vaccinated broilers (Tables 4-4, 4-5). These results are similar to that of previously published trials where lesions were still

present in the vaccinated broilers, but they were not associated with clinical disease (Bushell et al., 1990; Bushell et al., 1992). Probiotic administration was associated with a gross lesion score reduction in the mid intestinal region and a microscopic lesion score reduction in the lower intestinal region (Tables 4-4, 4-5). It has been reported that feeding probiotics may reduce oocyst output during coccidiosis infection (Dalloul et al., 2005). This along with mucosal immune stimulation is suggestive of how broilers supplemented with probiotic were associated with a decrease in lesion development in these areas measured in our trial. The results from the clinical challenge phase of this trial demonstrate that broilers that received vaccine and vaccine combined with probiotic had improved clinical intestinal and performance parameters during field strain *Eimeria* challenge when compared to non vaccinated broilers. These data suggest that administration of a probiotic during a coccidiosis vaccination program may lead to improved vaccine efficacy and can potentially stimulate immunity and improve intestinal health in broilers receiving clinical field strain *Eimeria* challenge.

CHAPTER V

EFFECTS OF PROBIOTIC ADMINISTRATION DURING COCCIDIOSIS VACCINATION ON PERFORMANCE IN BROILERS EXPOSEDTO FIELD STRAIN *EIMERIA*: COMPARISON TO MONENSIN ADMINISTRATION

Introduction

Every year the poultry industry experiences losses in excess of 800 million dollars due to the enteric disease avian coccidiosis (Allen and Fetterer, 2002). The bulk of these losses exist in the form of costs of control measures and performance losses in infected birds. Coccidiosis is an enteric disease of commercial poultry caused by host-specific intracellular parasitic protozoa of the genus *Eimeria*. There are eight species known to infect chickens, including *E. acervulina*, *E. brunetti*, *E. mivati*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella* (Conway and McKenzie, 2007). Clinical signs of infection include mucoid or watery diarrhea, hemorrhagic diarrhea, reduction in body weight or weight gain, high morbidity, and sudden death (Kawahara et al., 2008). For the past 60 years growers have relied heavily on anticoccidial drugs, such as the ionophore monensin, for control of coccidiosis (Chapman, 1999). Environmental conditions of high density commercial rearing barns make commercial poultry houses ideal locations for the survival and propagation of *Eimeria* oocysts, the infective stage of this protozoan. As such, eradication from commercial rearing operations is virtually

impossible. While anticoccidial drugs have played a principal role in the degree of success experienced by the commercial poultry industry, this form of control is not without inherent problems due to constant emergence of drug resistance in field-strain *Eimeria* (Williams, 2006). It has been well documented that some degree of resistance exists for all available anticoccidial drugs currently used in the U.S. (Jeffers, 1974; Chapman, 1986; McDougald, 1990; Williams, 2006; Bafundo et al., 2008). This continuous development of resistance by field-strain coccidia has created interest in the search for alternative measures of control (Guzman et al., 2003).

Another widely researched method of control that has been around for many years is live oocyst coccidiosis vaccination. Live oocyst vaccines in general fall into one of two categories, attenuated or non-attenuated. Currently, vaccination with a live oocyst vaccine has been proposed to be the only viable alternative to anticoccidial usage in coccidiosis control programs (Danforth, 1998). Current vaccines have been shown to stimulate immunity, but concerns related to efficacy and administration of the vaccine must be alleviated before vaccination receives broad-scale acceptance as a primary method of control (Gilbert et al., 1988; Augustine et al., 1991; Stiff and Bufando, 1993; Lillehoj and Trout, 1994; Williams, 1998).

A new concept that has emerged through recent years in poultry production is the use of probiotic bacteria to nonspecifically stimulate the mucosal immune system of commercial poultry (Farnell et al., 2006). Feeding probiotics to birds has been shown to stimulate intestinal immunity and improve enteric health in commercial lines of broilers (Dalloul et al., 2003). Other studies have shown improvement in performance

parameters such as body weight and feed conversion ratio in broilers administered probiotics (Cavazzoni et al., 1998; Zulkifli et al., 2000; Samli et al., 2007). Such reports suggest the administration of probiotics may enhance efficacy of enteric vaccines, particularly live oocyst coccidiosis vaccines that are often associated with early performance losses during commercial rearing. The current research was conducted to evaluate broiler performance during coccidiosis vaccination (Coccivac®-B), with or without probiotic (Biomin® PoultryStar) administration during a 44 day pen study. Additionally, the potential interaction between vaccination and probiotic administration was compared during field strain *Eimeria* challenge. We hypothesized that probiotic administration would improve vaccine efficacy in the presence of a field strain *Eimeria* challenge. A medicated (monensin) diet was added to the experiment design for comparison of performance in birds fed a medicated industry type feed.

Materials and Methods

Experimental Design

This trial was conducted in a broiler rearing barn 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. The base experimental design consisted of eight different experimental groups including negative control, positive control (monensin medicated diet), probiotic (water applied), probiotic (feed applied), vaccine + probiotic (water applied), vaccine + probiotic (feed applied), probiotic

(medicated diet), and vaccine alone. Birds were reared in a simulated commercial-type environment for a period of 44 days. Each experimental group evaluated had eight replicate groups totaling to 64 pens in the trial. A field strain *Eimeria* challenge was applied to feed in all pens on d 14. A separate *Eimeria* feed challenge was administered to three birds per pen on d 36 of grow-out. Birds designated for challenge were randomly selected from each rearing pen of the main trial, weighed, and placed in separate pens according to their respective experimental groups. Rearing of challenged broilers occurred in these pens for seven days to evaluate the response of each group of broilers to clinical *Eimeria* challenge. Experimental parameters measured for all birds in the main 44 day performance trial included body weight, mortality corrected feed conversion (FCR), and oocyst output or shedding associated with vaccination or challenge. Intestinal and performance parameters for the clinically challenged broilers included gross and microscopic intestinal lesion score, intestinal weights, and weight gain over the seven day challenge period.

Body Weight and Feed Conversion Ratio (FCR)

Body weights were determined on day of placement (d zero), on the day of each feed change (d 14, 26, 34), and again at termination (d 44). Body weights were obtained as bulk pen weights and the number of animals present in each pen at the time of weighing allowed for calculation of average broiler weight within each pen. Mortality corrected feed conversion ratio (FCR) was calculated on d 14, 26, 34, and 44.

Oocyst Output (OPG Determination)

Fresh fecal samples were collected from three pens per experimental group beginning on d six post-placement and continuing on an every other day basis until termination of the trial for calculation of oocyst output per gram of feces. After collection of the feces, five grams of fecal material from each sample were weighed and diluted in 15 ml of water. Samples were then homogenized and an appropriate volume was loaded onto a McMasters counting chamber using a 200 ul pipette. Using a standard light microscope and a 20X objective, non-sporulated oocysts were counted to determine oocyst output per gram of feces (OPG).

Experimental Animals and Rearing

The experimental animals used in this trial were 2,880 Cobb by-product males off of the Cobb 500 line obtained on day-of-hatch from a local commercial hatchery. Each chick was weighed and received a wing-band for identification prior to placement in rearing pens. To ensure uniform body weights at placement (d zero) for all experimental rearing pens or groups, all chicks were randomized according to d zero body weight. This was performed by separating the heaviest and the lightest 5 % of all d zero body weights and then randomly distributing the remaining chicks to allow for an evenly distributed starting pen weight for each pen. Broilers were distributed to rearing pens at placement density of 0.8 ft^2 per bird to simulate local commercial broiler rearing conditions. This required 45 chicks to be placed per pen of 64 total pens achieving a total placement of 2,880 broilers for the trial. Each pen contained a 30 pound tube feeder and commercial-style nipple drinkers. Feed and water were provided *ad libitum*

to all broilers throughout the trial. The litter or bedding material within each pen during this trial was comprised of 50% fresh pine shavings and 50% used litter removed from another rearing facility where poultry had been housed. Broilers in non-medicated groups were fed a corn-soy based diet formulated to approximate the nutrient density and requirements of a local broiler integrator. Diets were fed to broilers during this trial according to a four-phase program. This called for the feeding of a crumbled starter diet (d zero to 14), followed by pelleted grower (d 15 to 26), pelleted finisher (d 27 to 34), and pelleted withdrawal (d 35 to 44) diets. Medicated diets contained the ionophore monensin at manufacturer's recommended inclusion rates in all rations except the withdrawal ration. Birds receiving probiotic in the feed were fed probiotic continuously throughout the entire trial to comply with the manufacturer's application recommendations.

Vaccination and Probiotic Administration

Birds designated for vaccination were spray vaccinated using a commercial style (Spraycox® II) vaccination cabinet. The vaccine used was Coccivac®-B (Intervet/Schering-Plough Animal Health, Millsboro, DE), a non-attenuated live oocyst coccidiosis vaccine for broiler chickens. After vaccination, chicks were allowed to preen for at least one hour before placement in rearing pens. Probiotic was administered intermittently through the drinking water using a commercial-style water medication system present in the rearing barn. The probiotic administered was Biomin® Poultry Star (Biomin GmbH, Herzogenburg, Austria), a commercially available probiotic containing beneficial bacterial microflora, including *Enterococcus faecium, Pediococcus*

acidilactici, Bifidobacterium animalis, and *Lactobacillus reuteri* in addition to the carrier inulin. Probiotic was applied at a concentration 20 g per 1,000 broilers according to manufacturer's dosage recommendations. Broilers designated for probiotic administration received intermittent drinking water application d zero through three, eight to ten, and then again one day prior through one day following feed changes (d 13 through 15, 25 through 27, and 33 through 35). Probiotic containing drinking water was colored with a red or green food coloring dye in a pattern that alternated colors on consecutive days. The presence of a dye in the drinking water allowed water lines to be completely drained and re-filled each day of probiotic administration. Probiotic administration in the feed consisted of applying one pound per ton of feed-grade Biomin® Poultry Star according to manufacturer's instructions.

Field Strain* Eimeria *Challenge

Eimeria oocysts used for both the d 14 and the d 36 feed challenge were field strain species derived from a local commercial broiler production facility. *Eimeria* species in the challenge inoculum included *E. acervulina*, *E. tenella*, *E. maxima*, and *E. mivati*. The d 14 challenge involved feed applying 55,000 oocyst per bird to the feed in each rearing pen. The birds removed from the grow-out trial were similarly challenged in the feed on d 36 and received approximately 750,000 oocyst per bird.

Indices of Clinical* Eimeria *Challenge

Broilers designated for clinical challenge were removed on d 36 from individual performance rearing pens and placed in separate challenge pens according to experimental group. The placement weight of each individual bird was recorded before

placement in a designated pen. After the seven day challenge period, each broiler was weighed in order to calculate weight gain during challenge. Once the weight was recorded, each broiler was killed and a necropsy was performed. Gross upper (duodenal loop), mid (jejunum and proximal ileum), and lower (cecum) intestine lesion scores were determined following methods published by Johnson and Reid (1970). After the gross lesion scores were determined, a mucosal scrapping of each intestinal region was obtained and placed on a microscope slide with a coverslip. All slides were then examined using a standard light microscope to determine the upper, mid, and lower microscopic intestinal lesion score. In total, 10 samples from each group were subjected to this method of lesion scoring. The final intestinal parameter measured was upper (small intestine) and lower (large intestine) intestinal weight. Ingesta or fecal material was removed with water and physical action before the intestinal sections were weighed and recorded.

Statistical Analysis

The experimental parameters from this trial were subject to a one way ANOVA (SPSS v 11.0). Means were separated using a Duncan's Multiple Range test. Means were deemed statistically different at $P \leq .05$.

Results

The effects of coccidiosis vaccination with or without probiotic administration in male broilers were evaluated according to several common performance parameters during grow-out in this trial. Body weights on d 14 were significantly ($P<0.05$) higher in broilers receiving medication, vaccine + probiotic (water), and medication + probiotic when compared to negative control broilers (Table 5-1). At termination of the trial on d 44, body weights for broilers receiving medication, vaccine + probiotic (water), and medication + probiotic were significantly ($P<0.05$) higher

Table 5-1. Average body weights (kg) for broilers in all experimental groups on the day of each feed change and at termination of the trial on d 44.

Experimental Group	Average Body Weight by Day (kg)			
	Day 14	Day 26	Day 33	Day 44
Neg. Control	0.41 ± 0.003^{d}	1.25 ± 0.01^{abc}	1.89 ± 0.02^{abc}	2.65 ± 0.02^{b}
Medicated Diet	0.45 ± 0.01^{ab}	1.32 ± 0.01^{a}	1.96 ± 0.02^{a}	2.74 ± 0.03^{a}
Probiotic (water)	0.42 ± 0.01^{d}	1.25 ± 0.01^{bc}	1.86 ± 0.03^{bc}	2.64 ± 0.02^{b}
Probiotic (feed)	0.43 ± 0.01^{cd}	1.25 ± 0.01^{c}	1.83 ± 0.03^{c}	2.62 ± 0.02^{b}
Vacc + Prob (water)	0.44 ± 0.004^{bc}	1.28 ± 0.01^{a}	1.93 ± 0.02^{a}	2.75 ± 0.02^{a}
Vacc + Prob (feed)	0.41 ± 0.01^{d}	1.31 ± 0.01^{a}	1.95 ± 0.02^{a}	2.67 ± 0.03^{b}
Med + Prob	0.46 ± 0.01^{a}	1.31 ± 0.01^{a}	1.96 ± 0.02^{a}	2.74 ± 0.02^{a}
Vaccine (alone)	0.41 ± 0.003^{d}	1.28 ± 0.01^{ab}	1.91 ± 0.02^{ab}	2.62 ± 0.04^{b}

$^{a-d}$ Means with different subscripts within columns differ significantly at $P<0.05$.

than weights obtained for broilers from all other experimental groups (Table 5-1). With respect to mortality corrected feed conversion (FCR) during the starter phase (d one to 14), broilers receiving probiotic + medication had the lowest measured FCR, but this value was not significantly ($P<0.05$) different than FCR measured in birds in the medication alone group. Broilers in the vaccine + probiotic (water group), although not statistically ($P<0.05$) different from the medication alone group and statistically ($P<0.05$) higher than the probiotic + medication group, had a significantly ($P<0.05$) lower FCR then all other groups present in the experiment. During the grower phase (d 14 to 26), broilers receiving vaccine + probiotic (water), probiotic alone (feed/water), medication, and medication + probiotic were associated with a significant ($P<0.05$) increase in FCR when compared to birds in the vaccine + probiotic (feed) group. Throughout the finisher phase (d 26 to 33), broilers receiving probiotic alone (feed/water) had a significantly ($P<0.05$) higher FCR than birds that in the vaccine, vaccine + probiotic (feed/water), and medication + probiotic groups. The withdrawal phase (d 33 to 44) was not associated with differences in FCR among all experimental groups (Table 5-2). Cumulative FCR (d one to 44) followed trends seen with body weight in that broilers receiving vaccine + probiotic (water) had a significantly ($P<0.05$) lower feed conversion compared to all other groups except the medication alone , vaccine + probiotic (feed), and medication + probiotic groups (Table 5-2). Cumulative mortality was calculated for all experimental groups in this trial, but no significant differences were observed (Table 5-3).

Table 5-2. Average mortality corrected feed conversion ratio (FCR) for each experimental group by dietary phase and at termination of the trial.

Experimental Group	Mortality Corrected Feed Conversion Ratio (FCR)				
	Starter	Grower	Finisher	Withdrawal	Cumulative (d 1-44)
Neg. Control	1.32 ± 0.02^{a}	1.58 ± 0.01^{bc}	1.92 ± 0.03^{bc}	2.57 ± 0.06	1.89 ± 0.01^{ab}
Medicated Diet	$1.16 \pm 0\ .01^{bc}$	1.61 ± 0.01^{ab}	$1.90 \pm 0\ .04^{bc}$	2.53 ± 0.09	1.85 ± 0.02^{bc}
Probiotic (water)	1.28 ± 0.02^{a}	1.60 ± 0.02^{ab}	2.01 ± 0.06^{ab}	2.53 ± 0.10	1.89 ± 0.02^{ab}
Probiotic (feed)	$1.27 \pm 0\ .02^{a}$	1.63 ± 0.01^{a}	$2.10 \pm 0\ .06^{a}$	2.43 ± 0.07	1.90 ± 0.02^{a}
Vacc + Prob (water)	1.21 ± 0.01^{b}	1.63 ± 0.01^{a}	1.88 ± 0.02^{c}	2.38 ± 0.04	1.83 ± 0.01^{c}
Vacc + Prob (feed)	1.30 ± 0.02^{a}	1.55 ± 0.03^{c}	1.85 ± 0.03^{c}	2.57 ± 0.07	1.85 ± 0.01^{bc}
Med + Prob	1.14 ± 0.01^{c}	1.62 ± 0.01^{ab}	$1.88 \pm 0\ .02^{c}$	2.56 ± 0.09	1.84 ± 0.02^{bc}
Vaccine (alone)	1.31 ± 0.03^{a}	1.59 ± 0.01^{abc}	1.88 ± 0.04^{c}	2.53 ± 0.11	1.88 ± 0.01^{ab}

^{a,b,c} Means with different subscripts within columns differ significantly at $P<0.05$.

Table 5-3. Cumulative mortality of broilers in each experimental group throughout the entire trial.

Experimental Group	Cumulative Mortality %
Neg. Control	3.04 ± 0.26
Medicated Diet	3.12 ± 0.43
Probiotic (water)	4.44 ± 0.46
Probiotic (feed)	4.44 ± 0.46
Vacc + Prob (water)	4.18 ± 0.61
Vacc + Prob (feed)	3.60 ± 0.50
Med + Prob	3.62 ± 0.38
Vaccine (alone)	2.78 ± 0.45

Oocyst output data revealed two peaks before d 24 for all vaccinated broilers. Non-vaccinated groups were associated with a small peak in shedding or output between days 18 and 24, followed by a larger peak after d 24 of rearing (Figure 5-1). These observations of oocyst output or shedding followed patterns similar to those measured in the previous two trials discussed in this thesis. The vaccinated groups apparent early peaks were linked to vaccination while the non-vaccinated groups, including medicated broilers, showed later phase peaks in shedding, likely attributed to field strain *Eimeria* challenge.

A subset of broilers from each experimental rearing pen of the main performance trial was removed and challenged on d 36 for evaluation of performance and intestinal parameters during clinical coccidiosis infection.

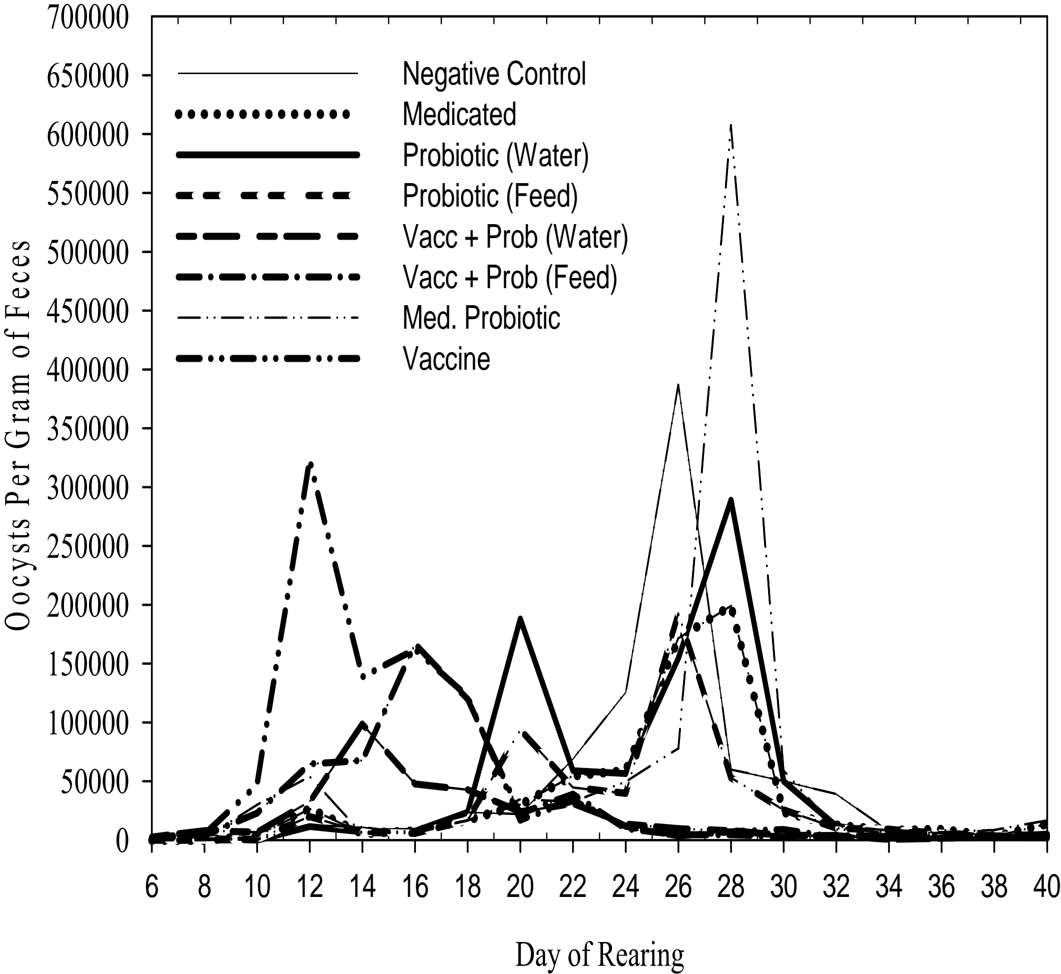

Figure 5-1. Oocyst output (oocyst per gram (OPG) of feces) from three randomly selected pens from each experimental group beginning on d six and continuing on an every other day basis until d 40.

During gross mid intestinal lesion scoring, a significant (P<0.05) reduction of lesion score among all experimental groups was observed when compared to the scores of the negative control group (Table 5-4). Broilers in the probiotic alone (water) and the vaccine alone groups were associated with a significant (P<0.05) reduction in gross lower intestinal lesion score when compared to all other groups, with exception of broilers receiving vaccine + probiotic (feed) (Table 5-4). Microscopic mid-intestinal lesion scoring revealed that broilers receiving a probiotic alone (feed/water), vaccine alone, and vaccine + probiotic (feed/water) had significantly (P<0.05) lower scores when compared to broilers administered medication alone. Similar reductions were seen for microscopic lower lesion scores with all groups showing a significant (P<0.05) reduction in score when compared to medication alone (Table 5-5).

Table 5-4. Gross intestinal lesion score, by region of intestine, for each experimental group of broilers included in the d 36 clinical *Eimeria* challenge.

Experimental Group	Gross Lesion Score		
	Upper	Mid	Lower
Neg. Control	0.08 ± .06	1.20 ± .13[a]	0.75 ± .12[a]
Medicated Diet	0.00 ± .00	0.83 ± .15[b]	0.83 ± .12[a]
Probiotic (water)	0.04 ± .04	0.74 ± .14[bc]	0.26 ± .01[b]
Probiotic (feed)	0.04 ± .04	0.29 ± .01[d]	0.79 ± .15[a]
Vacc + Prob (water)	0.08 ± .08	0.67 ± .12[bcd]	0.54 ± .12[a]
Vacc + Prob (feed)	0.00 ± .00	0.42 ± .12[cd]	0.50 ± .13[ab]
Med + Prob	0.00 ± .00	0.46 ± .12[bcd]	0.75 ± .11[a]
Vaccine (alone)	0.04 ± .04	0.43 ± .12[cd]	0.30 ± .01[b]

[a-d] Means with different subscripts within columns differ significantly at $P<0.05$.

Table 5-5. Microscopic intestinal lesion score, by region of intestine, for each experimental group of broilers included in the d 36 clinical *Eimeria* challenge.

Experimental Group	Microscopic Lesion Score		
	Upper	Mid	Lower
Neg. Control	0.10 ± 0.10	0.40 ± 0.16^{ab}	0.80 ± 0.25^{bc}
Medicated Diet	0.50 ± 0.17	0.80 ± 0.20^{a}	1.60 ± 0.22^{a}
Probiotic (water)	0.10 ± 0.10	0.20 ± 0.13^{b}	0.40 ± 0.16^{cd}
Probiotic (feed)	0.00 ± 0.00	0.20 ± 0.13^{b}	0.20 ± 0.13^{d}
Vacc + Prob (water)	0.27 ± 0.14	0.18 ± 0.12^{b}	0.09 ± 0.09^{d}
Vacc + Prob (feed)	0.11 ± 0.11	0.11 ± 0.11^{b}	0.00 ± 0.00^{d}
Med + Prob	0.20 ± 0.13	0.50 ± 0.17^{ab}	1.10 ± 0.18^{b}
Vaccine (alone)	0.20 ± 0.13	0.20 ± 0.13^{b}	0.50 ± 0.17^{cd}

$^{a-d}$ Means with different subscripts within columns differ significantly at P<0.05.

Furthermore, broilers receiving vaccine + probiotic (feed/water) and probiotic alone (feed) had a significantly ($P<0.05$) lower microscopic lower intestinal lesion score when compared to all other groups in the trial (Table 5-5).

Body weight gain was determined during the seven day challenge period and results revealed that the broilers in the medication + probiotic and the vaccine alone had a significantly ($P<0.05$) lower body weight gain during challenge compared to all other groups included in the trial (Table 5-6). Intestinal weights were taken for two regions that included upper (small intestine) and lower (large intestine and ceca) weights. Upper relative weights were ($P<0.05$) lower for broilers receiving vaccine + probiotic (feed or water) and vaccine alone when compared to broilers receiving medication alone. All groups were associated with a reduction ($P<0.05$) in relative lower intestinal weight when compared to broilers in the medication alone group (Table 5-6).

Table 5-6. Average body weight gain (g) and relative intestinal weights (g) for each experimental group of broilers included in the d 36 clinical *Eimeria* challenge.

Experimental Group	Body Weight Gain (g) / Relative Intestinal Weight (g)		
	Weight Gain	Relative Upper Wt.	Relative Lower Wt.
Neg. Control	644.25 ± 25[a]	3.16 ± 0.01[ab]	0.53 ± 0.01[b]
Medicated Diet	613.57 ± 31[a]	3.20 ± 0.11[a]	0.61 ± 0.03[a]
Probiotic (water)	568.17 ± 21[a]	2.93 ± 0.10[abc]	0.54 ± 0.02[b]
Probiotic (feed)	617.50 ± 34[a]	2.95 ± 0.11[abc]	0.49 ± 0.01[b]
Vacc + Prob (water)	576.02 ± 38[a]	2.72 ± 0.12[c]	0.52 ± 0.02[b]
Vacc + Prob (feed)	575.12 ± 35[a]	2.80 ± 0.15[bc]	0.52 ± 0.02[b]
Med + Prob	461.63 ± 35[b]	2.88 ± 0.12[abc]	0.54 ± 0.03[b]
Vaccine (alone)	463.97 ± 28[b]	2.72 ± 0.11[c]	0.49 ± 0.01[b]

[a,b,c] Means with different subscripts within columns differ significantly at $P<0.05$.

Discussion

Given the ubiquitous nature of *Eimeria* in modern commercial broiler rearing environments, coccidiosis treatment and control is important to the profitability of the U.S. industry. During this research trial, different combinations and permutations of control methods were evaluated to determine which method proved most efficacious in ameliorating performance losses during rearing and clinical parameters related to coccidial infection. At termination of the trial on d 44, broiler body weights were higher in groups receiving medication, medication + probiotic, and vaccine + probiotic (water) (Table 5-1). A d 14 field strain *Eimeria* challenge was administered to all rearing pens in this study to ensure that *Eimeria* was present in these pens in some capacity. The final body weights suggest that the vaccine + probiotic (water) group performed just as well as broilers that were receiving the ionophore monensin continuously in the diet, excluding the withdrawal phase of rearing. This was also supported by cumulative FCR data. Calculated FCR were significantly lower in broilers receiving vaccine + probiotic (feed/water) when compared to all groups except birds receiving ionophorous chemotherapy. Differences between the medicated groups and the vaccine + probiotic groups when looking at cumulative feed conversion were not observed, however broilers receiving vaccine alone did not perform as well as medicated broilers (Tables 5-2). This suggests that the probiotic may have improved vaccine efficacy during this trial allowing for equivalent performance when compared to medicated broilers. Feeding probiotics to broilers has been shown to improve intestinal health and stimulate mucosal immunity in previously published research (Dalloul et al., 2003). Mucosal immune system

stimulation by probiotics administered selectively during this trial could be responsible for the observed improved vaccine efficacy. Other articles published within the past decade report that vaccinated broilers perform just as well if not better then medicated broilers during periods of *Eimeria* challenge, however an influence of probiotic administration was not evaluated (Mathis, 1999; Suo et al., 2006). Late-phase performance differences between vaccinated and mediated broilers is likely due to infection in broilers receiving medication once the ionophore was removed from the feed during the withdrawal phase. Interference with development of immunity by ionophore administration in this trial probably occurred, since it has been suggested that it takes as long as seven weeks in some cases to obtain complete immunity to some *Eimeria* species (Chapman, 1999; Chapman et al., 2004). Performance parameters in broilers receiving vaccine + probiotic (water) with respect to body weight and FCR was equivalent if not improved compared to broilers receiving medication in the later phases of rearing during this trial. Oocyst output or shedding data followed trends seen in the previous two trials reported in this Thesis (Chapters III and IV). Vaccinated broilers were observed to have two major peaks in oocyst output prior to d 24, while non-vaccinated broilers had two peaks in output following d 24 of rearing (Figure 5-1).

When evaluating clinical parameters associated with d 36 field strain *Eimeria* challenge, broilers in the vaccine (alone) and medication + probiotic groups had the lowest weight gain throughout the challenge period (Table 5-6). These findings, referring to the vaccine (alone) group, contradicted previous findings by our lab that support a vaccination-associated increase in weight gain during challenge. Gross

intestinal lesion scoring was associated with a reduction in mid and lower lesion development for broilers receiving vaccine alone, probiotic alone, and vaccine + probiotic (feed). Probiotic-induced reductions in lesion score are likely associated with non-specific improvements in intestinal health through mucosal immune stimulation (Table 5-4). Vaccine-associated lesion score reductions are attributable to the traditionally described generation of protective immunity. Previous findings suggest that even though there were lesions present, they were not associated with clinical disease (Bushell et al., 1990; Bushell et al., 1992). Microscopic lesion scores followed similar trends, with broilers in the vaccine alone, probiotic alone, and vaccine + probiotic groups achieving reductions in microscopic scores in the mid and lower intestinal regions compared to broilers receiving only the ionophore (Table 5-5). These data suggest that co-administration of probiotic during coccidiosis vaccination results in performance parameters that are improved when compared to vaccination alone and indistinguishable from protection conferred by feeding an ionophore in the presence of field strain *Eimeria.*

CHAPTER VI

CONCLUSION

The modern commercial poultry industry, despite many consistent advances that have been achieved in general poultry health, continues to struggle with the enteric disease avian coccidiosis. Advances in control of this enteric pathogen throughout the years have been made through research which has focused upon two primary control methods, anticoccidial chemotherapy and vaccination. Broiler production is clearly the most susceptible sector of the industry to this type of disease due to high density rearing environments that exist throughout the industry. Anticoccidial drug inclusion in rearing diets remains the primary method of control in integrated broiler production, but consumer preferences for more "drug-free" approaches coupled with the continuous development of drug resistance in field strain *Eimeria* are promoting the search for alternative control measures. Vaccination has been used for decades by the industry but to date, the practice has received only limited acceptance in vertically integrated broiler production. The research described in this thesis focused upon improving live oocyst coccidiosis vaccination efficacy through the use of probiotic bacteria. The trials discussed in Chapters III and IV attempted to simulate industry-type conditions to test the effects of both vaccine and probiotics on broiler performance. Vaccination has been reported to cause early-phase of production performance losses due to the low level infection induced by the vaccine to stimulate protective immunity. Such losses in early-phase production are usually followed by a period of compensatory growth during the

last half of grow-out (Danforth et al., 1997a; Mathis, 1999). Results from the trials discussed in Chapters III and IV of this thesis report similar observations regarding the effects of vaccination on broiler performance. In general, body weights and FCR were negatively impacted by vaccination through the grower phase of rearing in both trials. These losses were counteracted by compensatory gains in both body weight and FCR in the later phases of grow-out of these trials. These observations were supported by oocyst output and shedding data in both experimental trials. Vaccinated broilers, including vaccinated broilers receiving probiotic, were associated with peak output prior to d 26 of rearing in both trials. Non-vaccinated broilers were associated with output or shedding that was greater later in grow-out, following d 26. Greater magnitude of output in vaccinated broilers prior to this point in rearing corresponds to diminished performance through the grower phase of rearing. A general absence of shedding or output after the grower phase can be linked to improved performance in vaccinated animals following this time point in both trials.

The positive impact of probiotic administration on vaccination was observed in several different observations of both trials from Chapters III and IV. In the data reported within Chapter III, probiotic administration was observed to have a positive interaction with vaccination for ending broiler body weight during this trial, as the probiotic + vaccine group had the highest body weight at termination (P=.053). This suggests an improvement in vaccine establishment and efficacy when combined with probiotic administration. A positive effect of probiotic administration was also seen on cumulative mortality of broilers within the trial described in Chapter III. In this trial,

broilers within the probiotic alone experimental group had reduced ($P<0.05$) cumulative mortality compared to all other experimental groups. Further, broilers in the vaccine + probiotic group had lower ($P<0.05$) mortality as compared to the vaccine alone experimental group. During the trial reported in Chapter IV, a positive impact of probiotic administration on feed conversion was observed. Broilers receiving probiotic alone were associated with a reduction ($P<0.05$) in FCR during the withdrawal period. This data is similar to work previously published describing positive correlation with respect to performance parameters in broilers receiving probiotic administration (Gao et al., 2008; Awad et al., 2009).

The trial described in Chapter V had a similar experimental design as the previous two trials, but included an ionophore (monensin) in the diet of select experimental groups and also a different route of application for the evaluated probiotic. A feed-based probiotic, comprised of the same culture evaluated in trials described in Chapters III and IV but modified for dietary inclusion prior to crumbling/pelleting was included in two experimental groups. This comparison of feed-applied or water-applied probiotic application resulted in generally improved performance associated with the water application of probiotic in this trial. An interesting observation of this trial was observed with cumulative body weight and FCR when comparing broilers in vaccinated with probiotic groups against broilers receiving dietary ionophore administration. At termination of this trial, values for body weight and FCR in broilers from the vaccine + probiotic (water) group were improved compared to all other experimental groups, including vaccine alone, and were indistinguishable from broilers in ionophore groups.

Vaccination has often been compared with anticoccidial use and the results often range from negative to variable. Other reports have shown that using a vaccine to be as good if not better for performance when compared to anticoccidial drugs (Danforth, 1998; Mathis, 1999; Suo et al., 2006), while others suggest that vaccines do not outperform anticoccidials (Williams et al., 1999). Oocyst output in this trial (Chapter V) supported higher challenge levels were achieved through feed delivery when compared to the trial described in Chapter IV where litter application of oocysts was performed. Patterns of output were similar to the previous two trials (Chapters III and IV) where vaccinated broilers were associated with two early peaks during rearing. Non-vaccinated broilers, again similar to previous observations, were associated with two output peaks later in the trial.

Another area of interest discussed in this thesis relates to the intestinal health parameters measured in broilers receiving a clinical challenge of field strain *Eimeria* to test immunity development later in grow-out. During the trials described in Chapters IV and V, a subset of broilers was pulled from the performance trial approximately one week prior to termination. These animals were challenged and reared for seven days to evaluate the effects of clinical challenge in broilers from different experimental groups in these trials. General trends supported vaccination as being very effective in reducing gross lesion scores, microscopic lesion scores, and weight gain during challenge. These observations were expected and are supported by previous research reporting lesion development in vaccinated broilers (Williams, 2003). Data from the trial described in Chapter V generally showed an increase in lesion development associated with the

ionophore alone group compared to other experimental groups in this trial, confirming that immunity development in these broilers was delayed and ineffective at protecting these animals against clinical *Eimeria* challenge. Direct effects of probiotic administration in minimizing clinical parameters related to infection during *Eimeria* challenge were observed. In the trial described in Chapter IV, probiotic administration alone reduced gross and microscopic lesion development during challenge.

Taken together, the data presented in Chapters III, IV, and V of this thesis suggests that probiotic administration improves coccidiosis vaccine efficacy and reduces the negative effects of challenge by field strain *Eimeria*. These findings suggest that simultaneous probiotic and vaccine administration can improve performance and intestinal health parameters during periods of clinical coccidiosis invasion. More research needs to be performed to validate and extend these findings to assist with improving vaccine efficacy in the future. The future of the poultry industry will rely heavily on research and development of alternative methods of coccidiosis control to ensure that growers are well equipped to minimize and control coccidiosis within the environment of commercial poultry production.

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