INFLUENCE OF DIETARY COMPOSITION ON COCCIDIOSIS VACCINATION EFFICACY IN BROILERS

A Dissertation

by

JASON THOMAS LEE

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

DOCTOR OF PHILOSOPHY

December 2006

Major Subject: Poultry Science

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Approved by

Chair of Committee,	David Caldwell
Committee Members,	Luc Berghman
	Allen Byrd
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ABSTRACT

Influence of Dietary Composition on Coccidiosis Vaccination Efficacy in Broilers.

(December 2006)

Jason Thomas Lee, B.S., Texas A&M University;

M.S. Texas A&M University

Chair of Advisory Committee: Dr. David J. Caldwell

This research program included a series of experiments to investigate the effect of starter diet protein level on the performance of broilers vaccinated with Coccivac®-B and subsequently challenged with a mixed species *Eimeria* challenge compared to non-vaccinated broilers. Pre-challenge performance data indicates that vaccination may decrease body weights and increase feed conversion ratio (FCR) with vaccination. The time period associated with the observed effects is between 13 to 17 d of age. This reduction in performance of vaccinated broilers versus non-vaccinated broilers was eliminated by the conclusion of the experiments (27 d) in the higher protein diets. Vaccination was effective at generating protective immunity against the *Eimeria* challenge evidenced by significantly increased body weight gains, improved feed conversions, reduced post-challenge mortality, and reduced lesion development in vaccinated broilers compared to non-vaccinated.

The final experiment included the comparison of Coccivac®-B to Bio-Cox® (salinomycin) for controlling field strain *Eimeria* in broilers reared on two different

dietary rations varying in protein concentration. Diet A had a lower protein concentration than Diet B. On day 14, *Eimeria* collected from commercial broiler farms in Texas were spray applied to the litter in all pens. Broilers reared on Diet B were heavier at Day 40 while body weights at day 50 were similar for all groups. Broilers fed Diet B had lower FCR during the starter and finisher diets. Broilers fed salinomycin had lower FCR for the starter and grower diets while vaccinated broilers had lower FCR during the withdrawal period. Cumulative FCR for the entire grow out period were similar for all groups.

These data indicate that vaccination can be utilized as an anticoccidial preventive and are suggestive that reduced protein concentration of starter diets can lead to significant losses in broiler performance when utilizing a vaccination program to prevent coccidiosis. Feeding an appropriately formulated diet while vaccinating broilers with Coccivac®-B as an alternative to the use of salinomycin yields at least equivalent if not elevated performance in the presence of field-strain *Eimeria* during grow-out with no effect on the cost of production.

DEDICATION

I dedicate this dissertation to my wife and my entire family for the support that they have given to me over the past four years. Their continued advice, emotional, and monetary support has enabled me to continue my education. Thank you.

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Finally and most importantly, I express my love and gratitude to my wife, parents, family, and friends for their words of encouragement and their many prayers throughout my life and education.

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CHAPTER I

INTRODUCTION

Intestinal parasitism is a major stress factor leading to malnutrition, lowered performance, and reduced production efficiency of livestock and poultry (Yun et al., 2000). Coccidiosis is an intestinal infection caused by an obligate intracellular protozoan parasite belonging to several different species of *Eimeria*. Developmental stages of *Eimeria* alternate between the external environment and endogenously within the host (Lillehoj and Lillehoj, 2000). Coccidiosis is recognized as the parasitic disease that has the greatest economic impact on the commercial poultry industry. In 1995, it was estimated to cause a loss of approximately 40 million pounds on production in the UK of 625 million broilers with an estimated $800 million dollars of lost revenue worldwide (Williams, 1999). The U.S. broiler industry is estimated to lose between $450 million (Allen and Fetterer, 2002a) and $1.5 billion (Yun et al., 2000) annually from coccidial infections in floor reared poultry. From this estimate, 17.5% of these costs were due to the cost of prophylaxis and treatment in broilers and broiler-breeders (Williams, 1998) and 80% were due to losses of feed conversion and weight gain even in the presence of drug-treatment strategies (Vermeulen et al., 2001).

Infections with *Eimeria acervulina, E. maxima,* and *E. tenella* are diagnosed frequently in intensively reared poultry (McDougald et al., 1997), and commercial chicken flocks which are found to be free from coccidiosis are extremely rare (Williams,

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This dissertation follows the style of Poultry Science.

1999). Seven *Eimeria* species have been recognized to infect chickens: *Eimeria acervulina, E. maxima, E. tenella, E. brunette, E. necatrix, E. mitis,* and *E. praecox.* Each species has its own characteristic prevalence, site of infection, pathogenicity, and immunogenicity (Rose and Long, 1980). All species, however, parasitize the epithelial cells of the intestinal mucosa and cause pathological changes varying from local destruction of the mucosal barrier and underlying tissue (often associated with some degree of inflammation resulting in endothelial lesions), to systemic effects such as blood loss, shock syndrome, and even death (Vermeulen et al., 2001). Coccidial infections may be classified in one of three ways: (1) as clinical coccidiosis, characterized by mortality, morbidity, diarrhea, and/or bloody feces; (2) as subclinical coccidiosis, by definition not immediately obvious, but causing reductions in weight gain and feed conversion efficiency of the host, without overt signs of disease; or (3) as coccidiasis, a mild infection causing no adverse effects on the host (Williams, 2002).

To date, the poultry industry has relied heavily on the use of anticoccidial feed additives for prevention of coccidiosis outbreaks. Resistance, in some capacity, to all of the anticoccidial drugs introduced thus far for use in commercial poultry has developed in field strain *Eimeria* (Chapman, 1997). Consumers are becoming increasingly concerned about drug residues in poultry products (McEvoy, 2001), resulting in increased pressure from a percentage of consumers to ban drugs from animal feeds. For these reasons, there is a pressing need to move away from chemotherapeutic control of coccidiosis in favor of non-medicated forms of control such as vaccination (Williams, 2002). Due to the complexity of the life cycle of *Eimeria* which consists of intracellular,

extracellular, asexual, and sexual stages or pathways, it is not surprising that host immunity is complex and involves many components of non-specific and specific immunity (Lillehoj, 1998). Such complexities associated with the integrated host response to the parasite's developmental stages must be taken into account during vaccine development.

Live oocyst vaccination is currently the only viable alternative to the use of anti-coccidial drugs. This control strategy has been used by the poultry industry for over 50 years, primarily in broiler breeder and replacement layer stock (Chapman et al., 2002). The basis for vaccination is the fact that, after an infection, the host is immune to subsequent infections by the same species (Yun et al., 2000). Live oocyst vaccines may be comprised of attenuated or non-attenuated *Eimeria* strains. Attenuation can be obtained by passing parasites through embryonated eggs or by selection for precocity (Williams, 2002). Both non-attenuated and attenuated vaccines provide solid immunity to coccidial infection when applied carefully under good rearing conditions (Shirley and Long, 1990). Live oocyst vaccination has been shown to be an effective tool for the generation of immunity and protection against subsequent *Eimeria* challenge evidenced by increased body weight gain (Danforth, 1998; Crouch et al., 2003; Williams, 2003), reduced feed conversion (Crouch et al., 2003), and reduced intestinal lesion development following *Eimeria* challenge (Danforth, 1998; Crouch et al., 2003; Williams, 2003). Not all reports of live oocyst vaccine usage have been uniformly positive however, leading to a general reluctance in implementing vaccination programs in US broiler production due to reports of reduced performance (Allen and Fetterer, 2002a). Non-attenuated vaccines

in broilers have been shown to decrease weight gain and increase feed conversion ratio as compared to medicated birds during starter period (Danforth, 1998; Williams, 2002). Other researchers have reported negative effects on cumulative broiler performance when using live oocyst vaccines compared to medication evidenced by reduced final body weights (Danforth et al., 1997; Waldenstedt et al., 1999a) and increased feed conversion ratios (Williams et al., 1999; Waldenstedt et al., 1999a). This reported diminished performance was related to mild coccidia infection associated with live oocyst vaccination. Other contradictory reports have indicated that vaccinated broilers performed similar to if not better than medicated broilers (Danforth, 1998; Williams and Gobbi 2002), and that vaccination can lead to significantly lower mortality rates compared to medication (Williams et al., 1999).

Dietary manipulation has been shown to be an effective method of improving broiler performance during coccidial infection (Allen et al., 1998). The manipulations reported to date focused mainly upon sources of fat containing high concentrations of n-3 fatty acids and betaine. Betaine is a naturally occurring, nontoxic amino acid derivative that functions as a dietary source of methyl groups and aids in cell volume regulation under osmotic stress (Saarinen et al., 2001). Increasing dietary protein levels during periods of clinical coccidiosis has been shown to improve broiler performance (Sharma et al., 1973), but such a strategy to date has not been evaluated during vaccination. Therefore, the proposed research program includes a series of five experiments with the following objectives:

1. To evaluate performance parameters affected by varying levels of dietary protein in starter diets of broilers vaccinated with a commercially available anticoccidial vaccine, Coccivac®-B[1].
2. To compare growth parameters of vaccinated and non-vaccinated broilers fed varying starter diet protein levels.
3. To evaluate the effect of vaccination on the generation of immunity and protection against a subsequent mixed species *Eimeria* challenge measured by growth characteristics and intestinal lesion development.
4. To compare effective live oocyst vaccination to the use of a common ionophore coccidiostat, salinomycin by measuring performance parameters in broilers fed two dietary rations varying in protein concentration.

[1] Schering-Plough Animal Health, 556 Morris Avenue, Summit, NJ 07901-1330 USA

CHAPTER II

LITERATURE REVIEW

Life Cycle of *Eimeria* spp.

The life cycles of typical *Eimeria* spp. can be divided into three phases of development: sporogony, merogony, and gametogony (Hammond, 1973). Sporogony usually takes place outside of the host. Oocysts are the exogenous stages that are usually shed in the feces of a definitive host, and sporogony is the process by which a one celled sporont (zygote) within the oocysts wall undergoes a series of divisions to form sporozoites which either lie free in the oocysts wall or may be contained within sporocysts (Current et al., 1990). For species that require sporogony outside of the host, environmental conditions must contain oxygen, moisture, and optimum temperatures. Only sporulated oocysts that contain sporozoites are infective to the definite host. The route of infection is through oral ingestion by the bird. Once ingested, the permeability of the oocyst's environmentally resistant wall is altered by mechanical grinding in the ventriculus allowing sporozoites to be released from the sporocysts by the action of pancreatic enzymes, predominantly trypsin, and bile salts. Trypsin acts to degrade the Stidea body which allows for the release of sporozoites into the intestine of the definitive host, while bile salts promote sporozoite activity and motility (Current et al., 1990).

Once in the intestinal lumen, sporozoites travel to a species-specific site of infection and actively penetrate enterocytes and cells of the lamina propria. Active penetration of sporozoites into host cells involves the organelles of the apical complex (Hammond, 1973). Once inside the host cell, sporozoites round up into a uninucleate

meront and begin the second stage of development, merogony. Merogony is the asexual proliferative phase of *Eimeria* spp. It is initiated when several mitotic nuclear divisions occur and is completed when elongated merozoites are released from the surface of the meront by multiple fission (Current et al., 1990). Host cell damage inflicted by merozoites leaving the enterocyte, characterized by loss of host cell cytoplasm, proves to be a much more damaging act than the initial event associated with invasion of host cells by sporozoites (Hammond, 1973). Once released from the host cell, free merozoites actively penetrate enterocytes near the initial site of infection and begin the next generation of asexual reproduction. The number of asexual generations varies amongst individual *Eimeria* spp. and most have a characteristic number of asexual generations ranging from two to four (Current et al, 1990).

Merozoites of the final generation of merogony enter host cells and initiate the sexual portion of the endogenous cycle (gamogony) by developing into male and female gamonts, the microgamonts and macrogamonts, respectively (Current et al., 1990). Microgamonts undergo nuclear divisions and ultimately results in the production of many microgametes that exit the host cell. Microgametes contain a nucleus, two or three flagella, actively seek out, and penetrate host cells that contain mature macrogametes. Macrogamonts do not undergo nuclear divisions, but increase considerably in size within the host cell allowing for the proliferation of cellular organelles include wall-forming bodies that are involved in the subsequent formation of an oocyst's wall (Current et al., 1990). The macrogamete is fertilized by the penetrating microgamete which results in the formation of a zygote. Following fertilization, the environmentally resistant oocyst's

wall is formed by the wall-forming bodies. Oocysts are then released by the host cell and enter the environment. The life cycle of *Eimeria* spp. will continue with sporogony of oocysts in the environment. *Eimeria* have an enormous capacity to multiply in the intestine due to their complex life cycle. One oocyst of *E. tenella* can give rise to more than 100,000 oocysts in a single generation (Chapman, 1993).

Eimeria infection in poultry is host and site specific within individual species. Intestinal tissue damage caused by the different species of *Eimeria* will vary. *Eimeria acervulina* and *E. mivati* will penetrate and cause lesions in the upper part of the small intestine (duodenum), *E. maxima* and *E. necatrix* cause lesions in the midgut (jejunum), and *E. tenella* and *brunetti* will cause lesions in the lower gut (ceca and large intestine) (Witlock and Ruff, 1977). Sporozoites of *E. acervulina* penetrate the duodenum of chickens from a point just before the curvature of the loop to about 2.5 cm from the pancreatic duct (Doran, 1966). *Eimeria acervulina* has been characterized as having 4 asexual generations (Vetterling and Doran, 1966). These generations are differentiated by merozoite production, size, and time after inoculation. *E. maxima* has been shown to invade the anterior intestine (Joyner, 1982) and its meronts occur in the epithelial cells of the villi of the small intestine while its gamonts are displaced toward the center of the villi and come to lie in their interior (Levine, 1982). *E. tenella*, the most pathogenic species in chicken, has three asexual generations within its life cycle and is localized to the villar epithelial cells and submucosa of the ceca causing hemorrhagic enteritis, and even death (Levine, 1982).

Structural changes due to coccidial infection are similar regardless of species. Common observations are villus flattening or atrophy, crypt hyperplasia, and decreases in villus to crypt ratios (Fernando et al., 1983; Morris et al., 2004). *Eimeria* infections also cause varying degrees of malaise, diarrhea, nutrient malabsorption, reduction of growth rate (Allen et al., 1973; Witlock et al., 1975), and reduced intestinal viscosity (Waldenstedt et al., 2000). Coccidia which infect and develop in crypt stem cells, particularly of the lower small intestine, cecum, and colon are highly pathogenic because they tend to destroy the crypt stem cells that they invade. This destruction prevents renewal of villus epithelium resulting in the denuding of villi with attendant fluid loss, hemorrhage, susceptibility to bacterial invasion, and subsequent formation of necrotic lesions (Ruff and Allen, 1990). Functionally mature epithelial cells of the intestine contain enzymes, such as disaccharidases, which are responsible for the terminal stages of digestion and provide the substrates for absorption (Eichholz and Crane, 1965). Increased mitotic activity leading to increased cell turnover is the most common change reported in coccidia infections (Ruff and Allen, 1990; Morris et al., 2004). Goblet cell hyperplasia and increased numbers of enteroendocrine cells are also detected. As such infections not only cause loss of absorptive area due to villar flattening, but the mucosal population also has a decreased percentage of functionally absorptive cells (Ruff and Allen, 1990). Physical changes within each segment of the intestinal tract are summarized below.

Duodenum

The normal morphology of the duodenal villi range from fingerlike to spatulate-shape while villi parasitized by *E. acervulina* are acutely truncated with no distinct tips as in normal intestines (Witlock and Ruff, 1977; Michael and Hodges, 1975). Infections caused by *E. mivati* are not as severe and widespread as *E. acervulina* although infected birds have similar lesions. The villar area which displayed morphological alteration was the tip of the duodenal villi in *E. mivati* infections and sloughing of the epithelium exposes the lamina propria (Witlock and Ruff, 1977). In some observations made by Witlock and Ruff (1977), the entire villus tip was removed exposing the lamina propria core and oocysts were found within the damaged epithelial cells surrounding the lamina propria core. Diminution of the mucosal layer in infected tissue becomes progressively more evident from 4-6 day post-infection, but by day 14 the intestine had recovered and appeared normal compared to uninfected birds (Allen and Danforth, 1984).

Jejunum

Normal villi in this region are shorter, broader, and apically squared off versus villi of the duodenum. *E. maxima* are reported to undergo development beneath the villar epithelium (Long, 1959). Jejunal damage caused by *E. maxima* can vary from isolated patches of exposed connective tissue of the lamina propria to epithelial sloughing caused by *E. maxima* underlying the epithelial cells (Whitlock and Ruff, 1977). Oocyst formation of *E. maxima* occurs underneath the epithelium which may account for the large numbers of epithelial cells seen in the mucoid exudate characteristic of *E. maxima* infection (Whitlock and Ruff, 1977). *E. necatrix* causes

damage by two distinct means: 1. the tips of the villi are eroded and large numbers of degenerating epithelial cells can be seen and 2. isolated villi are greatly enlarged with portions of lamina propria extruding through the villus tip as a result of pressure exerted by the large developing meront in the lamina propria (Whitlock and Ruff, 1977). Stockdale and Fernando (1975) reported similar observations during an *E. necatrix* infection of ruptured villar epithelium resulting in exposure of the lamina propria, which may allow leakage of blood components into the lumen causing blood streaked intestinal contents.

Ileum, Large Intestine, and Ceca

Uninfected villi of the ileum appear apically pointed, compact, and differ only slightly from those of the large intestine that are blunter, wider, and thicker (Whitlock and Ruff, 1977). *E. brunetti* causes severe damage in both the ceca and large intestine while *E. tenella* causes most damage to the ceca. Almost immediately after invasion of merozoites into crypt epithelium cells by *E. tenella*, the cells begin the sequence of morphological changes including swelling and loss of microvilli, leading to transformation within the lamina propria into giant cells with giant nuclei (Fernado et al., 1983). During an *E. brunetti* infection, the villi of both the ileum and large intestine are completely disrupted and eroded, exposing the underlying connective tissue of the lamina propria causing extensive coagulation necrosis with accompanying sloughing of the mucosa (Whitlock and Ruff, 1977). Villar morphology is completely lost with the disassociation of the epithelial cells and sloughing while *E. brunetti* can be seen embedded in the tissue debris (Whitlock and Ruff, 1977). *E. brunetti* causes extensive

damage to the intestinal mucosa which may account for the mortality associated with this infection.

Damaged to the intestine caused by *Eimeria* spp. is thought to be involved in increasing the susceptibility of chickens to outbreaks of necrotic enteritis (NE) caused by *Clostridium perfringens* Type A and C (Williams et al., 2003), and cases of NE associated with *Clostridium perfringens* have increased in countries that have stopped using antibiotic growth promoters (Van Immerseel et al., 2004; Williams, 2005). The acute form of NE leads to increased mortality in broiler flocks which can account for 1% mortality losses per day for several consecutive days during the last weeks of the rearing period (Van Immerseel et al., 2004). Experimental models developed to initiate NE in broilers usually involve some form of infection with an *Eimeria* spp. to cause intestinal damage and allow for *Clostridium perfringens* establishment and proliferation in the intestine which often coincides with peak coccidial infection (Al-Sheikhly and Al-Saieg, 1980; Williams et al., 2003; McReynolds et al., 2004). In the model developed by Williams et al. (2003), a virulent coccidial challenge with *E. maxima* sufficient to cause clinical coccidiosis exacerbated a virulent clostridial challenge. Williams et al. (2003) also observed reduced NE lesion development following a virulent clostridial challenge in Paracox-5 vaccinated broilers due to preventing severe coccidial lesions that might predispose birds to NE.

Physiological Changes in the Intestinal Epithelium during Coccidiosis

Coccidial infections in poultry have been shown to decrease the growth rate of chickens by 15 to 20% during mild coccidiosis and up to as much as 30 to 40% during

severe coccidiosis (Willis and Baker, 1981a). This period of decreased growth rate is followed by period of compensatory growth during the recovery from the infection (Panda and Combs, 1964). As a result of this increased growth rate, birds recovering from coccidial infections frequently grow rapidly enough that their body weight catches up to that of non-infected birds, although it takes considerable time for this compensatory growth to occur (4-6 weeks post-infection) (Turk, 1974). In the case of chronic infections where doses of the infective parasite are given over a period of several days, both the period of absorption impairment as well as the period of above normal nutrient absorption are prolonged (Turk and Stephens, 1970). The time interval required for infected chickens to undergo compensatory gain so as to equal gain measured in uninfected broilers is probably different at present, as the broiler of 2006 is extremely different in terms of genetic make-up than the broiler of 1974.

Intestinal damage due to a coccidial infection will first become visible on the 4th or 5th d post-infection. The most severe and widespread lesions will occur on the 6th and 7th d post-infection, and complete resolution or healing will be evident by the 14th to 21st d post-infection (Turk, 1974). The following sections will discuss the physiological changes (enzyme activity and nutrient absorption) that take place in each section of the intestine during an infection and discuss the compensatory effects associated with other segments of the small intestine during an infection.

Duodenum

Eimeria acervulina is the primary species that affects the small intestine of chickens. There have been several reports describing the changes in gut physiology

which occur during an *E. acervulina* infection. Infection with *E. acervulina* during the acute phase of the disease (d 4 to d 8 post-infection) causes a significant reduction in the retention of percent protein, percent ether extract and percent gross energy of the diet with a concomitant significant increase in the concentration of ether extract and gross energy of the excreta (Sharma and Fernando, 1975). Coccidial infections of the duodenal loop markedly affect zinc, oleic acid, and calcium absorptions while effects on sulfur amino acid requirements (Willis and Baker, 1981b), protein and amino acid absorption were minimal (Turk, 1974). Other reports contradict Turk (1974) with respect to amino acid and protein absorption. Sharma and Fernando (1975) and Ruff et al. (1976) observed significant reductions in both protein and amino acid absorption rates during *E. acervulina* infection. Ruff et al. (1976) observed significant reductions in methionine absorption in the duodenum of *E. acervulina* challenged chickens compared to levels of non-challenged and *E. tenella* challenged chickens. Ruff and Wilkins (1980) reported similar observations of decreased glucose and methionine absorption during both *E. acervulina* and *E. mivati* infections. Reductions in amino acid digestibility were recently confirmed by Persia et al. (2006), while also reporting a reduction in metabolizable energy levels of diets in *E. acervulina* infected chickens.

Turk and Stephens (1967a) reported that absorption of zinc and oleic acid was typically increased at the beginning of the infection, and markedly dropped as the infection proceeded toward the acute phase, reaching a minimum at the time of the most severe intestinal damage (inflamed duodenum and general lack of muscle tone). Zinc and oleic acid absorption levels were reduced to 25-40% of that of uninfected birds, but

about the time that visible intestinal damage was repaired absorption rates for zinc and oleic acid increased to levels well above those occurring in the uninfected birds (Turk, 1974). Zinc absorption rates of birds that had recovered from coccidial challenge were roughly double when compared to uninfected birds while oleic acid levels increased 25% compared to uninfected birds. These increased levels of absorption were maintained for two weeks which corresponds with the time period of compensatory growth seen in coccidial challenged birds (Turk, 1974). Compensatory gain of broilers infected with *E. acervulina* was also observed by Adams et al. (1996a) between 6 and 12 d post-infection which followed a period of depressed growth from 3 to 6 d post-infection.

E. acervulina infection also slows the rate at which nutrients enter the bloodstream through the lumen of the intestine (Turk and Stephens, 1967a). The interference of nutrient absorption has been attributed to the reduced levels of plasma carotenoids observed in *E. acervulina* infected chickens (Ruff and Fuller, 1975). The maximum amount of oleic acid appeared in the bloodstream at or after 8 h post-ingestion in challenged birds while it appeared between 1 and 2 h post-ingestion in non-challenged birds. Partial responsibility for delayed absorption is due to decreased gut motility (Aylott et al., 1968). Turk (1974) hypothesizes that decreased digestive tract motility may be responsible for the increase in nutrient absorption that is seen for the first three days of infection. This slow digestive tract rate would permit the nutrients to be in contact with the intestinal mucosa for a longer period of time.

Liver glycogen levels are also significantly reduced during the acute phase (5-6 d post-infection) of an *E. acervulina* infection followed by significantly increased levels during the recovery phase (6-8 d post-infection) (Ruff and Allen, 1982). Allen and Danforth (1984) also observed reduced rates of glucose and octanoic acid oxidation in infected birds. Mitochondria isolated from infected birds oxidized both octanoic and α-ketoglutaric acids at lower rates than mitochondria from control chickens (Allen and Danforth, 1984). The reduced rates of these reactions in mitochondria from infected epithelial tissue indicate that *Eimeria acervulina* caused some kind of mitochondrial lesion associated with the matrix or inner membrane (Allen and Danforth, 1984). Allen and Danforth (1984) observed a shift in metabolism due to coccidial infection in the duodenum by *E. acervulina*. *E. acervulina* infection caused a four-fold increase in the ratio of metabolism of glucose through the glycolytic pathway versus the tricarboxylic acid (TCA) pathway at d 4 post-infection. These findings indicate a shift in metabolism away from mitochondrial oxidation towards oxidation of glucose through the pentose phosphate pathway (Bloom and Stetten, 1953).

Periods of malabsorption observed in chickens have been attributed to epithelial destruction through *Eimeria* infection. Allen reported (1987) significant reductions in total mucosal dry weights of upper intestinal segments from *Eimeria acervulina* infected chicks compared to controls at d 3, 5, and 7 post-infection while the lower segment significantly increased in total mucosal dry weight by d 7 post-infection. These findings agree with reports of increased ileal length at 4 d post-infection as well as increased fresh and dry weight at 7 days post *E. acervulina* infection (Allen, 1984). Allen (1984)

also observed increased fresh and dry weights of jejunal segments during *E. acervulina* infection although increased jejunal weights were not observed during severe infections. *E. acervulina* infection also decreased total alkaline phosphatase and sucrase activity in the upper and middle segments compared to controls at 3, 5, and 7 d post-infection (Allen, 1987). Decreased maltase and sucrase activity in the duodenum at 5, 6, and 7 d post-infection with *E. acervulina* have also been reported (Major and Ruff, 1978; Adams et al., 1996a). Maltase (Adams et al. 1996a) and sucrase (Allen, 1987) activity significantly increased in the ileum 7 d post-infection with *E. acervulina*. At 7 d post-infection, more mucosal cells were recovered from ileal segments from infected chicks than from controls (Allen, 1984) Whole cells and mitochondrial preparations of these cells oxidized octanoic acid at higher rates than did controls (Allen, 1984). The increased mass and metabolic activity of the more distal portions of the small intestine provides a means for compensatory absorption of nutrients during *E. acervulina* infection (Allen, 1984).

The periods of time of either impaired or compensatory nutrient absorption correspond directly with the changes in mitochondrial structure of the mucosal cells. During the first 10 days of infection, mitochondria from infected birds swelled and appeared to lose their internal structure with increased numbers of sickle and dumbbell-shaped mitochondria (Turk, 1974). These mitochondria were most numerous on d 6 post-infection or at the time of greatest absorption impairment. During the recovery phase, mitochondria appeared to resume normal shape and function, and many developed rod-like extensions from the body of the mitochondria. After recovery from

E. acervulina infection (d 21 post-infection), mitochondria were larger than normal, coincided with compensatory nutrient absorption, and then regressed to normal size (Turk, 1974). Allen and Danforth (1984) also found that mitochondrial structure changed throughout *Eimeria acervulina* infection. Mitochondria of parasitized cells did not alter in appearance but non-parasitized cells recovered from infected birds differed considerably from mitochondria recovered from uninfected birds. Non-parasitized cells from infected birds contained mitochondria that were swollen on d 5 and 6 post-infection (Allen and Danforth, 1984), possibly indicating increased activity of non-parasitized cells in infected animals.

Jejunum

Two main *Eimeria* species parasitize the jejunum of the bird (*Eimeria maxima* and *Eimeria necatrix*). Infections of either species caused decreased absorption of nutrients and physiological changes in the jejunum. *E. maxima* infection depressed feed intake and reduced body weight gain during the acute phase of infection (5-7 d post-infection) compared to pair-feeding controls (Chapman et al., 1982). *E. maxima* also has been related to decreased glucose and methionine absorption in the jejunum 7 d post-infection (Ruff and Wilkins, 1980). *E. necatrix* infection in broilers caused decreased zinc (Turk and Stephens, 1966) and protein absorption (Turk, 1972) while calcium (Turk, 1973), oleic acid (Turk and Stephens, 1967b), and amino acid absorption was less or unaffected (Turk and Stephens, 1969). Patterns of zinc and protein digestion and absorption patterns were similar to patterns observed in the duodenum throughout the infection. Increased zinc absorption was observed during the first three days post-

infection and returned to normal rates after the recovery phase (Turk, 1974). Protein absorption remained equal to uninfected birds for the first three days but dramatically dropped at d 6 post-infection (58% level of controls) and returned to levels significantly increased (171% level of controls) to uninfected birds upon recovery resulting in compensatory gain (Turk, 1974). The decrease in protein digestion and absorption was only observed during jejunal infections while absorption of amino acids was not affected when fed as individual amino acids. Zinc absorption rates were more significantly reduced during a jejunal infection dropping to a rate 34% observed in the controls (Turk and Stephens, 1966). Oleic acid and calcium absorption were affected to a lesser extent. Jejunal infections reduced oleic acid and calcium absorption to a level 65% to the control which suggests the duodenum has an increased role in fat digestion as compared to the jejunum (Turk, 1974). Jejunal infections dramatically impair the bird's ability to digest proteins, absorb amino acids, and zinc indicating the jejunum's role in the digestion of proteins and absorption of zinc.

Infection with *E. mitis* caused malabsorption of glucose and methionine in the region of the yolk sac diverticulum up to 52% of control levels (Ruff and Edgar, 1982). Reduced absorption became evident 3 d post-infection, continued through 5 d post-infection although no visible lesions were evident except reduced body weight of challenged chickens.

Ileum, Large Intestine, and Ceca

Coccidial infections of the ileum, ceca, and large intestine are primarily caused by *E. tenella* and *E. brunetti*. *E. tenella* or *E. brunetti* infections cause significantly

reduced liver glycogen levels in broilers (Ruff and Allen, 1982). *E. brunetti* caused a decrease in calcium (Turk, 1973), glucose, and methionine absorption rates in the ileum during peak infection (Ruff and Wilkins, 1980). Similar to the other sections of the small intestine, increased absorption rates were observed for nutrients during the first three days of the infection and again following the recovery phase. During an *E. tenella* infection (localized to the ceca), only minute changes were seen in absorption rates of calcium, oleic acid, zinc, and amino acids (Turk, 1972), indicating that *E. tenella* infections have little affect on most nutrient absorption (Turk, 1974). Witlock (1982) observed significantly increased mucosal wet and dry weights of the lower intestine as early as 4 d post-infection over control birds, corresponding with a twofold increase in mucosal thickness and muscular thickness. Coccidial infections lead to increased total mucosal dry weights on d 7 post-infection versus 3 and 5 d post-infection indicating an increase in epithelial cell proliferation rate (Allen, 1987).

Conway et al. (1993) observed *E. tenella* infection significantly reduced plasma carotenoids, lipids, and proteins as well as body weight gain and feed efficiency. Ruff and Fuller (1975) determined that reduced carotenoid levels during *E. tenella* infection were associated with leakage through the damaged wall of the cecum. These data suggested that *E. tenella* has an effect on nutrient absorption which contradicts Turk (1974), although Conway et al., (1993) did not measure nutrient absorption. Plasma carotenoid, lipid, and protein levels may be reduced by another mechanism such as anorexia or bleeding through the gross lesions associated with an *E. tenella* infection. Baba et al. (1993) observed increased mannose residues present on the cecal mucosa

which may allow adhesion of more Type 1 fimbria *Salmonella* to colonize the intestine during an *E. tenella* infection, an action which potentially has implications on human food safety, as described by Kogut et al. (1994).

Host Immune System

The complete immune response of chickens to either a primary or secondary infection with coccidia is very complex and involves cells from both innate and specific systems. All *Eimeria* species induce an acquired immune response that includes both humoral and cell mediated components. It is mainly accepted that cell mediated components comprise the majority of the immune response resulting in immunity generation. Because *Eimeria* are intestinal parasites, the gut-associated lymphoid tissue (GALT) is the first line of defense against infection. The GALT is a multi-layered tissue continuously exposed to food antigens, the normal microbial flora, and ingested pathogens (Yun et al., 2000). The outer layer of the GALT consists of epithelial cells and lymphocytes situated above the basement membrane. The lamina propria lies below the basement membrane and contains lymphocytes and the submucosa (Yun et al., 2000). The GALT contains many cell types (epithelial, lymphoid, antigen presentation cells, and natural killer (NK) cells) in specialized lymphoid organs (Peyer's Patches, cecal tonsils, and the bursa of Fabricius). There are three general functions of the GALT in a host defense against pathogenic infections including coccidiosis: 1. processing and presentation, 2. production of intestinal antibodies, and 3.activation of cell-mediated immunity (Brandtzaeg et al. 1987). Twenty-five percent of the intestinal mucosa is composed of lymphoid tissues, which appear as discrete or aggregated lymphoid

follicles or as scattered lymphocytes in the intestinal epithelium, lamina propria, or lymph (Mowat and Viney, 1997). Cellular communication networks within the GALT are bi-directional with lymphocyte secreting and responding to cytokines that stimulate or inhibit the activities of other lymphocytes and non-lymphoid cells (Yun et al., 2000).

The specialized lymphoid organs present in the GALT allow for a site of antigen interaction with immune cells of the host. Peyer's patches are nodules of lymphoid tissue in the submucosa of the intestine overlaid by a specialized epithelium. This epithelium provides an important site for contact between foreign antigens and lymphoid tissues, and contains B cells, T cells, and antigen presenting cells (Yun et al., 2000). Peyer's patches have been characterized by Burns (1982) as having thickened villi, flattened epithelium lacking goblet cells, accumulation of lymphocytes in encapsulated germinal centers, and diffuse lymphoid tissues. Peyer's patches contain Microfold (M) cells that allow for antigen uptake and the initiation of immune responses. M cells lack a developed microvillus border and glycocalyx coat allowing free access to luminal pathogens (Yun et al., 2000). Lectin-like molecules on the surface of M cell serve as selective binding sites for pathogens. M cells deliver antigens from the lumen of the intestine directly to intraepithelial lymphocytes and subepithelial macrophages by an active transepithelial vesicular transport system (Gebert, 1997).

Cecal tonsils are located at the ileocecal junction and are the major lymphoid tissue in the cecum of the chicken. Cecal tonsils constitute the largest collection of GALT in the chicken with the lymphocyte profile containing 45-55% B cells and 35% T cells indicating a role in both antibody production and cell mediated immune functions

(Yun et al., 2000). The cecal tonsil's immunological maturation and overall size are dependent on the degree of antigenic stimulation with increasing numbers of lymphocytes appearing as a function of age due to migration caused by antigen stimulation (del Cacho et al., 1993). Continuous backflow from the urodaeum of the cloaca stimulates the cecal tonsils with new antigen.

Epithelial Cells

Historically, epithelial cells have been regarded as passive cells with three main functions: 1. absorb essential nutrients from digested foods; 2. compose a major part of the first line of defense against ingested pathogens due to their constant contact with the external environment; and 3. undergo continuous cycle of cell death and regeneration, thereby eliminating cells damaged by the digestive process or harmful environmental agents (Yun et al., 2000). Recently, reports have indicated that epithelial cells are important regulators of natural and acquired immunity by the secretion of chemicals and cytokines (McGee et al., 1993; Panja et al., 1995; Reinecker et al., 1997). Normal intestinal epithelial cells can process soluble polypeptides and activate CD8+ T cells with the expression of antigen with major histocompatibility complex (MHC) class I molecules. It has been reported that intestinal epithelial cells can express MHC class II molecules (Bland, 1988) and present soluble antigens to CD4+ T cells (Kaiserlian et al., 1989). Epithelial cells are poor antigen presenting cells because they are unable to express critical co-stimulatory molecules needed for CD4+ T cell activation (Sanderson et al., 1993). With the lack of co-stimulatory molecules present on the epithelial cells, interactions between intestinal epithelial cells and CD4+ T cells may result in anergy.

Intestinal epithelial cells provide early signals important for initiation and regulation of the inflammatory response following parasitic invasion at the intestinal surface. Following invasion, epithelial cells produce several cytokines including IL-1, IL-6, IL-8, Tumor Necrosis Factor (TNF)-α (Panja et al., 1995), prostaglandins, and complement cascade components (Keelan et al., 1998). These cytokine secretions cause chemotaxis of innate and specific immune cells to the site of infection, and aid in the initiation of the inflammatory response. While intestinal epithelial cells are central to the intestinal immune response, the role of intestinal epithelial cells in *Eimeria* infections is largely unknown.

Cells of the Innate Immune System

Innate immunity is responsible for the elimination of parasites during the early phase of primary infection (Lillehoj and Trout, 1994). Macrophages, one of the professional antigen presenting cells, process soluble antigens into immunogenic peptides, display appropriate peptide-major histocompatibility complexes (MHC) on their surface, and express costimulatory molecules (B7-1 and B7-2) to amplify T cell activation (Yun et al., 2000). Macrophages have the capacity to synthesize and secrete the largest number of communication or signaling molecules of any cell type (Oppenheim and Shevach, 1990). These cytokines play a major role in the up regulating and coordinating a complete immune response. Upon ingestion of *Eimeria acervulina* oocysts, significant numbers of sporozoites are found within macrophages following both primary and secondary infections (Vainio and Lassila, 1989; Trout and Lillehoj, 1995). Macrophage phagocytosis in immune chickens depends on *Eimeria* species.

Increased macrophage uptake of *E. tenella* sporoziotes in immunized chicks has been observed but the same trend does not exist with respect to *E. acervulina.* Increased macrophage uptake of *E. acervulina* sporozoites in *E. acervulina* immunized chicks did not occur (Onaga and Ishii, 1980). Macrophages from immune hosts were activated and digest sporozoites in a complement dependent mechanism (Bekhti and Pery, 1989) and sporozoites stimulated macrophages produced increased amounts of TNF-α (Zhang et al., 1995). Thus, macrophages modulate the host response to *Eimeria* spp. through production of these soluble mediators (Lillehoj and Trout, 1996). However, macrophages may induce pathology by mediating an over amplified inflammatory response against foreign agents. Local intestinal pathology results from degranulation products, reactive oxygen intermediates, and lysosomal enzymes released by activated leukocytes (Klasing, 1991).

Several characteristics of mast cell function illustrate the importance of these cells as integral mediators of mucosal immune responses (Caldwell et al., 2004). Mast cell characteristics include: 1.the location of intestinal mast cells at the interface between the external and internal environments of the animal; 2. the capacity of mast cells to release pro-inflammatory and anaphylactic mediators; 3. and the capacity of the cells to undergo multiple cycles of mediator release during prolonged responses in the intestine (Abraham and Arock, 1998). Mucosal mast cells have been observed to increase in number and activity during *Eimeria* challenge (Caldwell et al., 2004; Morris et al., 2004). Morris et al. (2004) observed significantly increased mast cell counts on d 3 and 4 post-*Eimeria acervulina* challenge. In rats, increased mast cell counts during *Eimeria*

challenge led to increased release of soluble serine protease (Huntley et al., 1985). Petrone et al. (2002) observed increased numbers of mast cells during the acute inflammatory response caused by *E. tenella*. Although this evidence indicates that mast cells may play an integral role in the immune response to *Eimeria*, there is a lack of research to completely identify the precise role. A Factor that compounds the evaluation of mast cell activity is the difficulty of identification due to mast cell degranulation before or during fixation of the tissues not allowing the absorption of the stain (Morris et al., 2004).

NK cells are a sub-population of lymphocytes which lack the antigenic specificity of B- or T-lymphocytes and exhibit spontaneous cytotoxicity against a wide variety of target cells (Reynolds and Ortaldo, 1989). NK cells may constitute an active part of the first line of defense in the intestine because of their close proximately to sites where antigens are introduced. The intestinal epithelium of chickens contains a subpopulation of NK cells that mediate spontaneous cytotoxicity. This indeed suggests that they are an important component to intestinal immunity during coccidiosis (Lillejoh, 1989). Their effects are mediated by several mechanisms including: 1. secretion of immunoregulatory cytokines (including Interferon-γ); 2. lysis of parasitized host cells; and 3. direct inhibition of the growth of microorganisms via interactions with T cells (Bancroft, 1993). Increased NK cell activity in *Eimeria* inoculated chickens has been shown to occur during the later phase of a primary infection (8 d post infection) which correlates with the time of reduced oocyst production (Lillehoj, 1989). NK cell activity significantly increases at 1 and 3 d post-infection upon secondary infection (Lillehoj,

1989; Lillejoh and Lillejoh, 2000). Lillehoj (1989) determined that NK cells may be involved in immuno-surveillance against and in the elimination of *Eimeria* spp. following infection. Increased NK cell activity is accompanied by increased number of intraepithelial lymphocytes expressing CD8+ receptors (Lillehoj and Bacon, 1991).

Cells of the Specific Immune System

The principal cells of specific immunity are B and T cells with each cell type obviously being responsible for various functions during the generation of immunity in an animal. Depending on the properties of the pathogen, the specific system will utilize a humoral response, cell-mediated response, or a combination of the two in order to clear the pathogen from the body. Within the intestinal mucosa, lymphocytes are present in two anatomic compartments: epithelium and lamina propria (Befus et al., 1980). Many researchers have concluded that while both immune responses are present during *Eimeria* infections, cell-mediated immunity is the predominant response responsible during coccidiosis while humoral immunity plays a minor role (Lillehoj and Trout, 1996; Brake et al., 1997b; Lillehoj and Lillehoj, 2000; Yun et al., 2000; Allen and Fetterer, 2002a).

The importance of T cells in the immune response to *Eimeria* has been heavily investigated to identify their role in generating immunity against coccidiosis. As in mammals, chicken T cells can be phenotypically separated into CD4+ (helper/inducer T cells) and CD8+ (cytotoxic/suppressor T cells) subpopulations (Lillehoj and Trout, 1994). Further divisions of these subpopulations are based on two different TCR molecules, αβ or γδ TCR. T helper cells are responsible for assisting with both antibody

synthesis and delayed-type hypersensitivity, whereas T cytotoxic cells mediate cytotoxicity (Lillehoj and Trout, 1994 Turk (1974). T cytotoxic cells recognize foreign antigen in the context of MHC class I molecules and T helper cells recognize antigens in association with MHC class II molecules (Lillehoj and Trout, 1994). In contrast to T cells in the peripheral blood, 75-80% of intraepithelial lymphocytes express the CD8+ phenotype while only 5-15% posses the CD4+ phenotype (Selby et al., 1984) and 50% of the CD8+ intraepithelial lymphocytes may express the γδ TCR (Jarry et al., 1990). The concentration of CD8+ T cells in intestine is presumably higher because most parasites are ingested so CD8+ T cells can come in contact with intracellular parasites at this location. Activation of γδ TCR cells may be inherently unique and separable from that of αβ TCR cells because γδ TCR cells may recognize non-peptide ligands which do not stimulate αβ TCR cells (Tanaka et al., 1995). Therefore, γδ TCR cells can mediate specific cellular immune functions without the requirement for antigen processing allowing recognition of invading pathogens and damaged cells directly (Schild et al., 1994).

Cell mediated immune responses are the most effective immune response against intracellular parasites such as *Eimeria* (Bessay et al., 1996). Cell mediated immune responses include both antigen specific activation of T cells and non-specific activation of NK cells and macrophages. *Eimeria* infection seems to rapidly induce, locally at the site of the parasite development, a dramatic modification of the proportion of T-cell subsets in intraepithelial leukocytes, accompanied by systemic variations that are generally opposing, in the lymphocyte populations (Bessay et al., 1996). Increased

numbers of αβ CD8+ T cells are observed following primary exposure to coccidia with significantly increased αβ TCR and γδ CD8+ T cells upon secondary exposure to *E. tenella* (Vervelde and Jeurissen, 1995; Bessay et al., 1996). Increased levels of CD8+ T cells were related to decreased numbers of oocysts shed (Bessay et al., 1996). *Eimeria* challenge caused increased percentages of CD4+, CD8+, and γδ TCR percentages in the intestine (Bessay et al., 1996). During *E. maxima* challenge, increased T lymphocyte levels were associated with two peaks. The first peak taking place on d 3 to 5 post-infection and the larger second peak taking place on d 11 (Rothwell et al., 1995). Increased T-cell subpopulations (CD4+ and CD8+) and TCR markers (γδ and αβ) were observed in both peaks with CD4+ cells exclusively in the lamina propria and CD8+ in the lamina propria and the epithelium (Rothwell et al., 1995).

Investigations aimed at understanding the cell mediated immune response to coccidiosis utilized two inbred lines of chickens; one line more resistant (SC) to coccidial infection and one line more susceptible (TK) to coccidial infection. CD4+ T cells increased significantly and more rapidly post primary infection in SC chickens compared to TK chickens (Choi et al., 1999). Increased CD8+ T cells were observed in both strains of chickens following primary infection and a continued increase in SC chickens was observed with a marked decrease in TK chickens as the infection persisted (Choi et al., 1999). Intestinal CD4+ and CD8+ T cells isolated from regions of the intestine infected with coccidia produced elevated IFN-γ mRNA and protein during the early phase of infection (Choi et al., 1999). This cytokine plays an important role in the recruitment and up regulation of macrophages to secrete IL-2 causing clonal expansion

of activated T cells. These investigations indicate that CD8+ T cells are important in protection against coccidiosis and that genetic variation within different strains of birds can result in different localized changes in T cell subpopulations during coccidiosis.

The intestine is the largest immunological organ in the body containing 70-80% of all immunoglobulin-producing cells with external intestinal secretions predominantly consisting of mainly IgA and IgM (Yun et al., 2000). The majority of IgA in the intestinal lumen is produced by plasma cells in the lamina propria and transported across epithelial cells. IgA inhibits colonization of microorganisms in the intestinal lumen and neutralizes intracellular viruses as it passes through the epithelial cell. Chickens infected with *Eimeria* produce parasite specific IgM, IgY, and IgA antibodies in the circulation and mucosal secretions. Increased parasite specific antibody production by intestinal B cells shortly after infection with coccidiosis has been documented (Lillejoh and Lillejoh, 2000). Significantly increased sIgA and biliary IgA are detected within a week of oral infection and reached maximal levels around 8-14 days later persisting an additional 2 months (Lillejoh and Ruff, 1987). SIgA levels are significantly higher in intestinal regions that are parasitized versus non-parasitized indicating humoral activation against the parasite (Girard et al., 1997). Antibodies may serve an indirect role in immunity by reducing infectivity as a consequence of parasite agglutination, neutralization, steric hindrance, reduced motility, induction of conformational changes in the parasite's host cell receptor molecules, and inhibition of intracellular parasite development (Lillehoj and Lillehoj, 2000). However, the role of humoral immunity in response to coccidiosis has been readily debated. Agammaglobulinemic chickens produced by hormonal and

chemical bursectomy verify that parasite specific antibodies are not required for resistance to reinfection with coccidia (Lillehoj, 1987).

Cytokine production plays an important role in mounting a complete and full immune response of both the innate and specific systems. IFN-γ stimulates proliferation and differentiation of hematopoietic cells and enhances nonspecific immunity to parasites (Lillejoh and Lillejoh, 2000). IFN-γ regulates specific immunity by enhancing expression of MHC class II antigens and has been used as measure of cell mediated response to coccidiosis (Choi et al., 1999). IFN-γ has potent effect on macrophage activation by inducing macrophage cytostatic activity and induction of macrophage nitric oxide production by oxygen dependent mechanism. In *Eimeria* infected chickens, SC chickens (more resistant strain) produced greater amounts of nitric oxide compared with infected TK chickens (less resistant strain) (Lillehoj and Li, 2004). The production of nitric oxide, a highly active free radical, has been correlated with increased lesion development during coccidiosis outbreaks (Allen, 1997). Peaks in nitric oxide production correspond to the time when mucosal damage is the highest indicating that intestinal lesion development is in part due to the host immune response (Lillejoh and Lillejoh, 2000).

While IFN-γ has a potent role in a host immune response, several other cytokines are secreted as well. Transforming Growth Factor (TGF)-β is another cytokine produced by chickens in response to *Eimeria* infection. Three different isoforms of TGF-β have been identified: β1, β2, and β4. TGF-β4 has been observed to increase 5 to 8 fold during coccidiosis. TGF-β4 may play a role in promoting growth of the intestinal villi with

high levels corresponding to periods of increased cell proliferation. TNF-α production which is secreted by macrophages follows a biphasic pattern, with the first peak corresponding to disease pathogenesis and the second peak associated with the development of protective immunity (Byrnes et al., 1993). IL-1 production during infection with *E. maxima* or *E. tenella* is constant with identical concentrations regardless of oocyst dose (Byrnes et al., 1993). Secretion of IL-15 by macrophages serves to promote γδ-CD8+ T cells and NK proliferation and activation. These cytokine activities play an important role in immunity to coccidiosis (Choi et al., 1999).

Since the complete immune response to a coccidial infection is so complex, there are many aspects of the response that are poorly understood. Overall, the immune response is extremely comprehensive consisting of innate and specific responses with cooperativity between both segments of the immune system. Future research is needed to identify the exact role of the innate and specific systems in the immune response and the exact responsibility each plays.

Cross Species Protection

Host immunity is species specific, and little to no cross-protection is observed against heterologous species (Lillejoh and Lillejoh, 2000) and in some cases different strains of the same species (Shirley, 1989). Lillehoj (1986) showed that cells from chickens which were immune to *E. tenella* responded well to antigens from *E. tenella* but poorly to *E. acervulina* antigens suggesting that the lack of cross-protection may be due to the absence of cross-reactive T cells. Prowse (1991) observed increased interferon production in spleen cells from *E. tenella* immunized birds four to eight times

greater to *E. tenella* antigen compared to *E. acervulina* antigen. However, interferon production in spleen cells from *E. acervulina* immunized birds was similar in response to both *E. acervulina* and *E. tenella* antigens. Prowse (1991) determined that the lack of cross species protection is not due to the lack of cross-reactive T cells and suggested that there must be other factors involved in the expression of resistance. *E. maxima* is the most immunogenic of the avian *Eimeria* species and has been shown to exhibit a much higher degree of immuno variability as compared to other *Eimeria* species. Increased immuno variability of *E. maxima* may provide the basis for observed lack of cross-protective immunity among geographically isolated strains (Martin et al. 1997; Barta et al., 1998). Allen et al. (2005) determined that *Eimeria* fecundity is important in providing good immune stimulation and should be carefully monitored when characterization of the unique immune potentials of *Eimeria* strains is performed.

Williams (1973) observed that chicken flocks that were heavily infected with *E. acervulina* were often lightly infected with other species. During simultaneous inoculation of *E. acervulina* with E. *brunetti*, *E. maxima*, *E. tenella* or *E. necatrix* resulted in lower oocyst production by all of the species except *E. acervulina* compared to oocyst production during individual infections (Williams, 1973). In chickens immunized with *E. acervulina*, challenge doses of *E. acervulina* have a slight enhancement of invasion compared to naïve chickens (Augustine and Danforth, 1986). Augustine (1996) observed enhanced invasion by *E. tenella* in *E. acervulina* immunized chickens compared to naïve chickens although no effect was observed during simultaneous infection with *E. acervulina* and *E. tenella*. While the enhanced invasion

of sporozoites of the immunized species is not of concern because subsequent development by the sporozoites would be limited. The invasion enhancement of other *Eimeria* species is of concern because of the lack of the immunity to this species which may lead to increased disease. Vaccines may protect against challenge with the target species but, at the same time, actually amplify infection by species of *Eimeria* that are not included in the vaccine (Augustin 1996). Therefore, this action must be accounted for in vaccine development.

Live Oocyst Vaccination

The worldwide poultry industry provides a substantial proportion of the nutritional needs of the human population. To keep pace with the increasing demand for the high-quality, low cost protein source that poultry represents, intensive rearing practices have been developed in the past few decades (Lillehoj et al., 2000). A major consequence of these practices has been an increase in the incidence of diseases, including coccidiosis. Coccidiosis has historically been controlled with the inclusion of a number of different dietary coccidiostats. Medication via the feed has proved to be convenient, efficient in terms of labor, and as such cost effective, thus enabling large numbers of chickens to be reared in intensive conditions of the modern poultry industry (Chapman, 1993). Most coccidiostats inhibit asexual stages of the life cycle of *Eimeria*. Ionophorous antibiotics including monensin, salinomycin, narasin, lasalocid and maduramicin are currently the most widely used drugs for the control of coccidiosis. The mechanisms of action is their capability of transporting cations through biological membranes affecting a diverse range of cellular processes dependent upon ion transport

(Chapman, 1993). Diclazuril, the latest coccidiostat to become available, has been shown to be highly effective at improving performance parameters of broilers raised on litter contaminated with *Eimeria* spp. (Conway et al., 2002a). However, its use does not allow for the generation of immunity to *Eimeria tenella* (Conway et al., 2002b). Monensin has been shown to affect the ultrastructure of *Eimeria* spp. evidenced by vacuoles in the cytoplasm, separation of plasma membrane layers, and dense bands in the refractile bodies reducing the ability of cellular invasion of the parasite (Augustine et al., 1992). Use of monensin resulted in improved growth during *E. acervulina* infection (Willis and Baker, 1981a). Even though these drugs remain effective now, resistance has begun to arise to all them (Chapman, 1993).

Infections with coccidia parasites have been calculated to cost the US poultry industry in between $450 million and $1.5 billion (Yun et al., 2000) annually. Approximately 80% of the cost is due to losses in feed conversion and weight gain even in the presence of drug treatment strategies (Vermeulen et al., 2001). Decreased performance characteristics indicate the decreased effectiveness of anticoccidials indicating a need for new strategic concepts to control coccidiosis (Williams et al., 1999; Vermeulen et al., 2001; Allen and Fetterer, 2002a; Chapman et al., 2002; Williams, 2002).

Live oocyst vaccines are currently the only commercially available option for the control of coccidiosis in the poultry industry other than anticoccidial feed additives (Williams, 1998). Live oocyst vaccines are comprised of either virulent *Eimeria* species (Ex. Coccivac® and Immucox®) or attenuated *Eimeria* species (Ex. Paracox® and

Livacox®). Coccidia may be attenuated in any of three ways presently known: by passaging through embryonating chicken eggs, gamma irradiation, or selection for precocity (Williams, 1998). Passaging through eggs is possible for only a few *Eimeria* species. This type of attenuation may be associated with loss of immunogenicity of the line, and may not produce a stable attenuation (Shirley, 1993). The irradiation method is unlikely to lead to a commercial vaccine since the attenuation seems to depend to some extent simply on killing oocysts. This method has not been shown to be stable (Shirley, 1993). The best method of attenuation is by selection for precocity (Jeffers, 1975). The essential characteristics are decreased prepatent time or a reduced reproductive potential resulting in attenuation of virulence, maintenance of immunogenicity, and genetically control stability (William, 1998). All live vaccines are currently recommended for administration when chicks are quite young. Even though vaccines are given at a young age, in some circumstances, heavy wild-type oocyst contamination of a poultry house might produce clinical disease before a vaccine has established protective immunity (Williams, 1998).

There are several factors to take into consideration when evaluating anticoccidial vaccination. These factors include species inclusion, immunogenic variation in strains of *Eimeria* species, and the development of immunity in chickens as a result of the vaccine (Williams, 2002). All species that are likely to be encountered in commercial production of broilers should be included in any vaccine. Currently, *E. acervulina, E. maxima,* and *E. tenella* are included in all commercially available live oocyst vaccines. If a species is present in a particular rearing environment and not included in a vaccine,

that species may be able to cause disease in certain circumstances (Williams, 2002). Immunogenic variation is another aspect that is of concern with the implementation of anticoccidial vaccination. Incomplete protection due to immuno-variability between coccidial species present in the vaccine and those found in poultry rearing facilities has emerged as a potential complicating factor associated with vaccination (Martin et al., 1997; Caldwell et al., 2004).

Williams (1998) states that an advantage to the use of attenuated vaccines is that local virulent strains challenge the birds and fresh oocysts accumulate in the litter. Genetic recombination between vaccinal and wild coccidia would be expected to increase, and that this series of events with the reintroduction of attenuated oocysts with each new vaccination doubtlessly produces oocysts heterozygous for precocity (Williams, 1998). This recombination possibly reduces the pathogenicity of the resident coccidial population. The use of Coccivac® in poultry houses where drug-resistant parasites are problematic, increases the sensitivity of the resident coccidial population after several flocks have been vaccinated (Chapman, 1994). Due to genetic recombination, residual oocysts carried over to each successive flock are likely to become more drug-sensitive and perhaps less virulent during each vaccination cycle (Williams, 1998; Chapman et al., 2002). While several vaccines, both attenuated and non-attenuated, have been used to treat broiler breeders, there has been a general reluctance to use them in broilers and heavy roaster birds because of reports of reduced weight gain and increased feed conversion ratios compared to prophylactically medicated birds (Allen and Fetterer, 2002a). Several published reports have evaluated

the use of live oocyst vaccination for control for coccidiosis in broilers and are summarized below.

Williams (1994) investigated the safety of the use of an attenuated vaccine (Paracox®) on broiler performance. Vaccination was compared to a commonly used anticoccidial feed additive, nicarbazin. Broilers were housed in an environment free of extraneous virulent coccidial species. Oocysts were not present in medicated broilers at anytime within the experiment indicating that no extraneous *Eimeria* were present and that cross contamination by the vaccine strain did not occur. At the conclusion of the growout period (d 54), vaccinated broilers performed better than medicated broilers with a 10.4% increase in body weight, 7.2% reduction in feed conversion, and 44.4% reduction in mortality. Broilers were sampled throughout the experiment for the detection of lesions associated with vaccination. Lesions were only present in a small percentage of vaccinated broilers (4%). Recycling of the attenuated coccidia occurred in vaccinated broilers with oocysts present in the litter between 5 and 33 d after vaccination with a single peak between 5 and 12 d after vaccination. Williams (1994) concluded that these results reflect the safety of the Paracox® vaccine.

Danforth et al. (1997a) evaluated the generation of protective immunity in three different strains of male and female chickens immunized at 1 day of age with a drug-resistant field isolate of *E. maxima* in both battery and floor pen trials. The generation of immunity due to immunization with 2500 oocysts/bird was evidenced by reduced lesion development and increased weight gain in all three strains of chickens. In the floor pen trial, there was no interference of immunity generation with inclusion of three different

anticoccidial shuttle programs including narasin, nicarbazin, or monensin. Broilers were determined to be immunized upon *E. maxima* challenge based on lesion development and weight gains. There were no differences in performance parameters between any of the three different shuttle programs utilized by Danforth et al. (1997a).

If live oocyst vaccination was to be adopted by the commercial broiler industry, a route of administration that allows for a uniform distribution of the vaccine to all chicks needed to be identified. Danforth et al. (1997b) investigated several delivery techniques of the Immucox® vaccine which included gavage, spray cabinet, conventional Vetech delivery system, or gel-delivery system. A series of experiments conducted to evaluate these delivery systems indicated that the gel-delivery system with Immucox® vaccine improved uniformity of the primary vaccination infection. The gel-delivery system elicited an overall enhanced protective immune response in roaster birds compared to the other delivery systems (Danforth et al., 1997b). Vaccinated broilers (Immucox plus 500 oocysts of a local field strain of *E.maxima*) via gel-delivery and reared on litter seeded with a local strain of *E. maxima* had similar growth characteristics at d 70 to medicated broilers (Maxiban/Monteban shuttle program) raised on similar litter. However, the average gain to d 35 was significantly lower in the vaccinated group compared to the medicated group. The results of this series of experiments indicated that the other immunization techniques showed protective immune responses during a challenge infection, but the gel-delivery system was the only vaccinated group that yielded body weight gains similar to non-immunized controls.

The generation of immunity with live oocyst vaccination as a result of protection against an *Eimeria* challenge was further investigated and reviewed by Danforth (1998). Immunization of male and female chickens with 2500 oocyst/bird *E. maxima* at 1 d of age allowed for protection upon subsequent challenge with 25000 oocysts at d 10. Vaccinated broilers had significantly increased weight gains and lower lesion scores 7 d post-challenge compared to non-immunized chickens although vaccination led to a check in body weight gain pre-challenge.

Live oocyst vaccines must be comprised of *Eimeria* species strains which are in a particular geographic region due to lack of cross-protection observed between different strains of the same *Eimeria* species (Danforth, 1998). This observation was observed by Danforth et al. (1997b) when using Immucox. The *E. maxima* strain present in the vaccine would not elicit protection against the field strain *E. maxima* present in Maryland. Thus a modification had to be made to the vaccine with the addition of 500 oocyst of the Maryland field strain to the vaccine in order to generate efficient immunity upon field strain challenge. This represents an extremely important aspect that must be considered when adopting a live oocyst vaccination program.

Waldenstedt et al. (1999a) evaluated the used of a live attenuated anticoccidial vaccine (Paracox®) compared with an anticoccidial ionophore (Narasin) during a 36 d grow out of broilers isolated from extraneous *Eimeria* parasites. At the conclusion of the study, vaccinated broilers were lighter in terms of body weight (4.6%) and had a higher feed conversion ratio (2.5%) compared to unvaccinated medicated broilers. This reduction in performance could not be avoided with the inclusion of virginiamycin into

the diet of vaccinated broilers. One other observation made by Waldenstedt et al. (1999a) was that throughout the experiment cecal clostridial counts were considerably higher in vaccinated unmedicated broilers than in unvaccinated birds given narasin.

Williams et al. (1999) investigated the use of an attenuated anticoccidial vaccine compared to anticoccidial drug shuttle programs in a large field trial. Virginiamycin was included in all diets. The parameters evaluated included feeding cost, feed conversion, mortality, coefficient of variation of body weight, culled birds, total water consumption, and birds rejected during processing. No significant differences were observed in any of the parameters evaluated except feed conversion and mortality. Vaccination of broilers yielded a significantly higher feed conversion ratio although no difference was observed in feeding cost. Significantly lower mortality was observed in the vaccinated broilers compared to the medicated shuttle programs. This difference was probably observed due to two necrotic enteritis outbreaks that were observed in medicated broilers while necrotic enteritis was not observed in vaccinated broilers. One of these outbreaks was associated with a coccidiosis infection. The investigators concluded that there must be some ionophore and virginiamycin resistant *Eimeria* and *Clostridium prefringens* present in the affected houses. The final mean weights of the birds, often used as a major criterion in commercial poultry trials, were of no relevance in this experiment because the feed consumption of the birds was controlled in an attempt to achieve even, predetermined weights throughout the trials (Williams et al., 1999).

Williams and Gobbi (2002) conducted another field trial evaluating the use of the vaccine Paracox® when directly compared to a medication shuttle program. The

medication shuttle program consisted of nicarbazin in the starter diets and monensin in the grower and finisher diets. Females were reared to 36 d of age and males to 56 d of age. At the conclusion of three flocks, vaccinated broilers were significantly heavier than medicated broilers. This difference was observed at d 27 and persisted through the remainder of grow out for males and females. Vaccinated broilers also had lower feed conversion ratios by 2 and 3 points for females and males, respectively. As well, significantly fewer vaccinated males were rejected at processing compared to medicated broilers. Williams and Gobbi (2002) concluded that anticoccidial vaccination of broilers with Paracox® may afford performance equal to that obtained with a traditional anticoccidial drug shuttle program.

Williams (2003) evaluated the ability of Paracox®, an attenuated vaccine, to generate immunity in broilers judged by lesion development and body weight gain. Upon challenge with several species individually, vaccinated broilers had decreased lesion development and increased weight gain compared to naïve control broilers. Vaccinated broilers had lower coefficients of variation with respect to weight gain compared to naïve control broilers. Upon microscopic evaluation of the lesions in challenge broilers, all intestinal smears from naïve chickens contained merozoites, gametocytes, or oocysts. Intestinal smears from vaccinated broilers containing intestinal lesions indicated that the majority of the lesion (68%) exhibited no parasites with the remaining intestinal smears (32%) containing very few parasites. Williams (2003) concluded that fully immune birds may exhibit gross lesions with few or no endogenous parasites after virulent challenges suggesting that the paucity of parasites in lesions

occurring in immune birds is due to an immune response of the host rather than to the genetically controlled reduced fecundity of an attenuated challenge organism.

Further investigation by Crouch et al. (2003) of the attenuated vaccine, Paracox®-5, validated the findings of Williams (2003). Broilers were vaccinated on 1 d of age and challenged with an individual species on 28 d of age with one of the following species: *E. acervulina, E. maxima, E. mitis,* or *E. tenella*. Performance was improved with vaccination compared to non-vaccination 7 d post-challenge evidenced by increased body weight gains and reduced feed conversion. Lesion scores were also significantly lower in vaccinated broilers compared to non-vaccinated broilers. Lesions were not present in the majority of vaccinated broilers while all non-vaccinated broilers exhibited lesions with the majority having lesion scores of 3 or 4. In a second study, seeder birds were added to pens of either vaccinated or non-vaccinated broilers at 14 d of age. Vaccinated broilers had significantly increased body weight gains and lower feed conversion ratios. Vaccinated broilers were similar to the negative control broilers in which seeder birds were not added. These data demonstrate the protective efficacy of a live coccidiosis vaccine (Paracox-5) based on precocious lines of *E. acervulina, E. maxima, E. mitis,* and *E. tenella*, administered to 1 d old chicks against all constitutive vaccinal species (Crouch et al. 2003).

An attenuated vaccine developed by Li et al. (2004) containing *E. acervulina, E. maxima,* and *E. tenella* which were resistant to monensin was evaluated to determine the performance characteristics of chickens vaccinated with ionophore tolerant oocysts (Li et al., 2005). Chickens were vaccinated at 3 d of age by gavage or drinking water with a

virulent *Eimeria* challenge 3 weeks post-vaccination. Vaccination with gavage required a lower dose as compared to the drinking water in order to generate appropriate levels of immunity. Chickens vaccinated with the ionophore tolerant vaccine with either method and fed a medicated diet (monensin) had significantly higher final body weights 4 weeks post-infection as compared to medicated and vaccinated chickens. A vaccine containing ionophore resistant *Eimeria* proved to be efficacious and provided satisfactory protection and in combination with the use of ionophores and could be a useful adjunct to planned immunization in the control of coccidiosis (Li et al., 2005).

Recently, the effects of vaccination with an attenuated vaccine on the intestinal microbial ecology of broilers were investigated. Oviedo-Rondon et al. (2006) compared the microbial ecology of both vaccinated and non-vaccinated broilers fed diets supplemented with essential oils. Their results indicate that vaccination by itself causes small changes on intestinal microbial communities and that coccidia infection results in drastic shifts in microbial communities. Inclusion of the essential oils modulated microbial communities during coccidial challenges and avoided drastic changes in these communities after coccidia infection independent of vaccination.

Possible vaccines for the control of coccidiosis have been evaluated on several criteria including lesion development and oocyst shedding. These criteria while acceptable for the evaluation of drug efficacy in chickens are not completely appropriate for the determination of vaccine efficacy (Williams and Catchpole, 2000). Many times chickens that are immune to *Eimeria* species may produce mild lesions and have small amount of oocysts shedding but will have no check in body weight gain or feed

conversion ratio. Williams and Catchpole (2000) developed a protocol for the evaluation of possible anticoccidial vaccines. The judgment of immunity development was based on feed conversion and body weight gain of vaccinated birds following a subsequent challenge of an *Eimeria* species present in the vaccine. The benefits of the protocol over previous ad hoc experimental designs are: 1. immunization is carried out with multivalent vaccines of *Eimeria* species up to the maximum of seven that may infect chickens; 2. assessments of immunity are carried out for each species separately so results can not be confounded; 3. the criteria of efficacy are those that are crucial to demonstrate commercial usefulness; 4. the possibility of drawing erroneous conclusions based upon inappropriate criteria such as oocyst production or lesion scores is avoided; 5. because the same criteria are used for each species, direct comparisons may be made amongst immunities to all of the species in the vaccine being tested (Williams and Catchpole, 2000). While this protocol is useful in determining the efficacy of a vaccine to produce immunity, the application in the field for this type of protocol is impossible because poultry houses more likely than not contain multiple species of *Eimeria*.

Allen et al. (2004) also development a protocol to measure the effectiveness of anticoccidial vaccination in the generation of immunity in broilers and subsequent protection against a field strain challenge to one of three different strains of *E. maxima*. The calculation of the protective index included four independent measurements. The measurements included weight gain and lesion score which are two important parameters used to measure the effectiveness of a treatment such as vaccination to control coccidiosis. The two other factors used to calculate the protective index were

plasma carotenoids and plasma $NO_2^- + NO_3^-$. Decreases in plasma carotenoids are recognized as sensitive indicators of coccidiosis-associated pathology in the small intestine (Allen, 1992) and plasma $NO_2^- + NO_3^-$ concentrations have been found to increase during primary infection in a dose related manner (Allen, 1997). These values when taken at 6 d post-infection tend to be significantly correlated. The protective index (PX) is calculated for each chicken using the following algorithm: $PX = (N_{gain} + N_{carotenoids}) - (N_{square\ root\ lesion\ score} + N_{[NO2- + NO3-]})$ (Allen et al., 2004). Mean PX values of unchallenged groups cluster around 0 with mean PX values of protected chickens statistically similar to those from unchallenged groups and unprotected chickens have a mean PX value highly negative compared to the other groups (Allen et al., 2004). This protocol allows for the evaluation of different strains of *Eimeria* for the possible inclusion in anticoccidial vaccines allowing for the measurement of cross protection across strains. This protocol can also be used to rank the effectiveness of different anticoccidial treatments.

The use of anticoccidial vaccines has been investigated and while their effectiveness at generating immunity is not questioned, there is debate within the literature of the expected performance of vaccinated broilers compared to anticoccidials. Some researchers have observed reduced growth characteristics evidenced by decreased weight gain (Danforth et al., 1997b; Danforth, 1998; Waldenstedt et al., 1999a; Williams, 2002) and increased feed conversion ratios (Williams et al., 1999; Waldenstedt et al., 1999a) while other reports indicate that vaccinated broilers perform equally or better than medicated broilers (Danforth, 1998; Williams and Gobbi, 2002).

Dietary Modulation for Improved Performance during Coccidiosis

The effects of coccidiosis on nutrient absorption, body weight gain, and feed conversion have been well document. Research has focused on dietary modulation during periods of coccidiosis for the improved performance of broilers during infection (Allen et al., 1998). Fat absorption has been shown to decrease during *E. acervulina* infection (Sharma and Fernando, 1975). The addition of 0.4 g/kg of cholic acid can significantly improve fat digestion during an *E. acervulina* infection although no effect on performance parameters was observed (Adams et al., 1996b). Substitution of animal fat in the diet with coconut oil, which has a high concentration of medium-chain triacylglycerols, improved fat digestion during *E. acervulina* infection and consequently led to increased body weight gain and a reduced feed conversion (Adams et al., 1996b). The reasons for these improvements in fat digestion are related to the characterization of the lipid. Medium-chain triacylglycerols have greater water solubility, enter enterocytes without hydrolysis as a result of their smaller molecular size, and present a greater surface for lipase enzyme action, and short chain length results in more efficient absorption by the diseased mucosal surface (Babayan, 1987).

The search for new methods of control of coccidiosis in chickens has led to investigations of possible compounds with anticoccidial properties. Inclusion of *Artemisia annua* (annual wormwood) leaves at a dietary level of 5% protected chickens from *E. tenella* infection with reduced lesion development, and 1% inclusion indicated a possible protection against post-vaccine reductions in body weight and feed efficiency (Allen et al., 1997). *Artemisia annua* has been noted for its anti-malaria properties and

its use to treat human malaria. While this inclusion level is probably not realistic, isolation of the active compounds and addition of these compounds into the diet at significantly lower concentrations may in fact be realistic. The inclusion of artemisinin (17 ppm), camphor (119 ppm), and 1,8-cineole (119 ppm) significantly increased weight gain and reduced lesion scores of chickens during a mixed species challenge of *E. acervulina* and *E. tenella* (Allen et al., 1997).

In the past decade, considerable research has focused on the effects of betaine (trimethyl glycine) on chicken performance with and without anticoccidial feed additives during coccidial challenge. It has been used to replace a portion of the sulfur amino acid requirement (Pesti et al., 1979) as well as being an osmoprotectant to allow cells to tolerate osmotic stress (Bagnasco et al., 1986). Matthews et al. (1997) investigated the addition of betaine in broilers diets in combination with monensin and *Eimeria* challenge. The addition of 0.1% betaine significantly increased weight gain and feed intake of *E. acervulina* infected chickens and the addition of 0.5% betaine led to improved feed efficiency. The addition of 0.1% of betaine in combination with monensin (55 ppm) significantly improved feed efficiency, and the addition of betaine tended to increase feed efficiency of uninfected chickens. When the level of monensin was increased to 110 ppm, feed efficiency of uninfected chickens increased while decreasing feed efficiency in uninfected chickens when used without monensin. Betaine may have an effect on *E. acervulina*-infected chickens but no conclusive evidence indicates an improvement of monensin efficacy when fed in combination with betaine (Matthews et al., 1997).

Betaine has been shown to have beneficial effects when fed in combination with salinomycin (Augustine et al., 1997). Addition of betaine at 0.15% in combination with salinomycin at 66 ppm resulted in improved performance characteristics than either fed alone. Reduced invasion by *E. acervulina* and *E. tenella* as compared with untreated controls was also observed. This enhanced effect observed with betaine in combination with the ionophore, salinomycin, was not observed when betaine was used in combination with another commonly used ionphore, narasin (Waldenstedt et al., 1999b). Inclusion of betaine alone led to increased live weight of broilers but no positive effect in combination with narasin was observed.

Matthews and Southern (2000) observed inconsistent results with respect to dietary betaine inclusion (0.075%). In two experiments consisting of an acute and chronic infection with *E. acervulina*, betaine inclusion had no effect on performance parameters in the initial experiment while broilers fed betaine exhibited increased weight gain, feed efficiency, and lesion development in the replicate experiment. Betaine fed alone did not consistently affect growth performance in chronic or acute coccidiosis-infected chicks (Matthews and Southern, 2000).

Betaine supplementation has been shown to positively influence plasma betaine concentrations which were significantly decreased during an *E. maxima* infection (Fetterer et al., 2003). The increased levels of betaine resulted in consistently higher chick weights than control chicks and were significantly increased during *E. maxima* challenge. Klasing et al. (2002) investigated the effects of dietary betaine on performance parameters, intestinal osmolarity, number of leukocytes, villus height, and

immune cell function during an *E. acervulina* infection. Inclusion of betaine decreased osmolarity of the duodenum which was increased with coccidial infection and increased the numbers of intraepithelial leukocytes. Coccidial challenge resulted in decreased villus height but this was ameliorated with the inclusion of 1.0g/kg of dietary betaine. Betaine inclusion increased nitric oxide release by peripheral blood heterophils and peritoneal macrophages and increased the release of chemotactic factors from heterophils. Klasing et al. (2002) demonstrated that at least part of the protective effect of betaine can be attributed to enhancement of monocyte chemotaxis and nitric oxide production by heterophils and macrophages.

N-3 fatty acids have been observed to reduce lesion development (Allen et al., 1996) and increase weight gain in broilers (Allen et al., 1996; Korver et al., 1997) fed increased dietary concentration of n-3 fatty acids following an *E. tenella* infection. Further studies orchestrated by Danforth et al. (1997c) indicated that *E. tenella* development in chickens fed high n-3 fatty acid diets showed ultrastructural degeneration of asexual and sexual parasite stages and the early release and shedding of asexual and sexual parasite developmental stages into the cecal lumen. Ultrastructural degeneration was characterized by cytoplasmic vacuolization, chromatin condensation within the nucleus, a lack of parasitophorous vacuole delineation, and in some cases complete loss of parasite ultrastructural organization (Danforth et al., 1997c). Allen et al. (1996) speculate that when fed to birds beginning at 1 d of age, diets high in n-3 fatty acids would cause oxidative destruction of the parasite. Persia et al. (2006) also observed beneficial effects of the addition of 10% fish meal, which is high in n-3 fatty

acids, in chicken diets resulting in improved performance of chicks during *E. acervulina* infection. These authors also investigated the use of a prebiotic-type product, which contains dairy and yeast fractions and dried fermentation extracts, on performance. Improved performance was observed in the absence of coccidial challenge but no beneficial effects were observed in chickens challenged with *E. acervulina*.

Vitamin E has shown to function as an antioxidant and immunomodulator in chickens (Boa-Amponsem et al., 2000), but Allen and Fetterer (2002b) determined that vitamin E supplementation did not alleviate the negative effects on broiler performance and development of mucosal lesions associated with *E. maxima* challenge. The authors base this ineffectiveness of feeding a fat-soluble form of vitamin E is that it is malabsorbed during *E. maxima* infection and becomes less biologically available to infected tissues during an acute infection.

Vitamin A deficiency in broiler diets results in decreased numbers of intraepithelial lymphocytes expressing CD3, CD4, and CD8 surface markers and resulted in more surface Ig-A expressing cells than non-deficient controls (Dalloul et al., 2002). Upon introduction of challenge with *E. acervulina*, vitamin A deficient broilers showed lower CD4 expressing cells and increased oocyst shedding compared to non-deficient broilers. An increase in CD8 cells was not observed in vitamin A deficient broilers but was observed in non-deficient broilers (Dalloul et al., 2002). These authors concluded that vitamin A deficiency compromises local immune defenses of challenge broilers, as reflected in lymphocyte profiles and oocyst shedding.

In conclusion, considerable research has focused on dietary modulation in an effort to improve broiler performance during periods of clinical coccidiosis, mainly focusing on micronutrients and neglecting the effects of dietary protein levels. Dietary supplementation with micronutrients is confounded by the negative effect on intestinal absorption caused by all of the coccidia species (Allen and Fetterer, 2002b). In addition, the anorexia associated with coccidial infection can result in decreased feed intake and may also affect the intestinal levels of betaine and other micronutrients (Allen and Fetterer, 2002a). Dietary modulation aimed at improved broiler performance during periods of coccidiosis focused on protein level specifically is unclear.

CHAPTER III

THE EFFECT OF PROTEIN LEVEL ON GROWTH PERFORMANCE OF LIVE OOCYST VACCINATED BROILERS FOLLOWING A SUBSEQUENT MIXED SPECIES *EIMERIA* CHALLENGE

Introduction

Coccidiosis is an intestinal disease of intensively reared livestock cause by coccidial parasites of the genus *Eimeria*. The disease causes intestinal epithelium lesions, reduction of body weight, reduced feed efficiency, and often overt morbidity and mortality (Guzman et al., 2003). Economically, the most important members of *Eimeria* which infect chickens worldwide are *E. acervulina, E. brunette, E. maxima, E. mitis, E. praecox, E. necatrix,* and *E. tenella* (Shirley et al., 2005). Infections with *E. acervulina, E. maxima,* and *E. tenella* are diagnosed frequently in intensively reared poultry (McDougald et al., 1997). It has been estimated that coccidiosis costs the world's commercial chicken producers at least $800 million each year (Williams, 1998) with approximately 80% of this cost due to poor performance (Williams, 1999). As the world's poultry production continues to grow so do the concerns about the control of coccidiosis, since commercial chicken flocks found to be free from coccidia are extremely rare (Williams, 2002). Historically, the poultry industry has relied on anticoccidial drugs for the control of coccidiosis but resistance of coccidia has developed to all of the anticoccidial drugs introduced so far (Chapman, 1997). Consumers are becoming increasingly concerned about drug residues in poultry products (McEvoy, 2001), resulting in increased pressure from a percentage of consumers to remove drugs

from animal feeds. For these reasons, there is a pressing need to move away from chemotherapeutic control of coccidiosis in favor of non-medicated forms of control such as vaccination (Williams, 2002).

Vaccination against coccidiosis is not a new concept and has been used in the poultry industry for around 50 years (Shirley et al., 2005). Live oocyst vaccination using attenuated and non-attenuated *Eimeria* is currently the only commercially available vaccination strategy for control of coccidiosis in poultry. These vaccines provide solid immunity to coccidial infection when applied carefully under good rearing conditions (Shirley and Long, 1990). Despite their proven success in eliciting effective protection against coccidiosis in replacement and breeding flocks, these vaccines have not been universally accepted by the U.S. poultry industry for meat producing broiler and heavy roaster bird flocks (Danforth, 1998). The reluctance of broiler producers to adopt anticoccidial vaccination strategies is related to several reports on measured performance parameters associated with vaccination, weight gain and feed efficiency. Performance of vaccinated broilers has not always equaled that of medicated broilers (Danforth, 1998; Williams, 2002). The reduced performance is related to mild coccidia infection associated with live oocyst vaccination. Increasing protein level during periods of clinical coccidiosis has been shown to improve broiler performance (Sharma et al., 1973) but such a strategy to date has not been evaluated during live oocyst vaccination. The objective of the current research was the evaluation of dietary protein level on broiler performance and lesion development during live oocyst vaccination with Coccivac®-B and subsequent mixed species *Eimeria* challenge.

Materials and Methods

A series of four experiments were designed to evaluate the effect of selected levels of dietary protein on broiler performance and immunity generation while utilizing a vaccination program for the prevention of coccidiosis. Growth parameters of vaccinated broilers were compared to non-medicated non-vaccinated broilers prior to and following a mixed species *Eimeria* challenge. For each of the following experiments, broiler chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum*. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Texas A&M University laboratory animal care committee. Prior to chick placement, grow out facilities were thoroughly cleaned and disinfected. Fresh pine shavings were used for bedding material. Pens were equipped with one 30 lb tube feeder and nipple drinkers. In experiments that included a mixed species *Eimeria* challenge, dose titration of the challenge inoculum was performed prior to experiment to identify a dose sufficient to cause identifiable gross lesions in vaccinated broilers.

Experiment 1

Experiment 1 was a randomized block design consisting of 5 dietary protein levels (20, 21, 22, 23, and 24%) with eight replicates of each protein level for a total of 40 pens. Each replicate contained 25 chicks for a total of 1000 chicks placed. During diet formulation, diets were formulated on an isocaloric basis and careful consideration was given to maintain constant amino acid to protein ratios throughout all 5 dietary treatments (Table 3-1). The 23% dietary treatment met or exceeded NRC (1994)

Table 3-1. Calculated nutrient concentrations of experimental diets used in Experiments 1 through 4.

Nutrient					
Protein (%)	20.0	21.0	22.0	23.0	24.0
Methionine	0.44	0.47	0.50	0.52	0.55
TSAA	0.78	0.82	0.86	0.90	0.94
Lysine	1.06	1.12	1.19	1.26	1.33
Threonine	0.75	0.79	0.83	0.87	0.91
Arginine	1.32	1.40	1.48	1.55	1.63
Tryptophan	0.24	0.25	0.27	0.28	0.30
Calcium	0.90	0.90	0.90	0.90	0.90
Available Phos.	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20
ME (kcal/kg)	3200	3200	3200	3200	3200

specifications for a broiler starter diet. On day of hatch, all chicks were individually weighed (top and bottom 5% discarded), wing banded and vaccinated with Coccivac®-B by oral gavage. Once vaccinated, chicks were randomly assigned to treatment groups using chick weight. Broiler chicks were fed dietary treatment for 21 d at which time pen weights were taken and feed consumption determined for the calculation of feed conversion ratios.

Experiment 2

The experimental design utilized for Experiment 2 was a 3 x 2 x 2 factorial designed to determine the effect of one of three dietary protein levels (20, 22, and 24%), vaccination (vaccination compared to non-medicated non-vaccination), and mixed species *Eimeria* challenge (21 d of age) on broiler chick performance. This experimental design generated a total of 12 treatment groups and each group was replicated in triplicate. Each replicate contained 25 chicks for a total of 900 chicks placed. Dietary treatments were identical to those utilized in Experiment 1 for the three protein levels selected (Table 3-1). On day of hatch, chicks were individually weighed (top and bottom 5% discarded), wing banded, and assigned to treatment groups. Chicks assigned to vaccinated treatment groups were vaccinated using Coccivac®-B in a commercial spray cabinet that vaccinates 100 bird chick trays. A red food coloring dye was added to the vaccine and chicks were allowed to preen for 1 hr before placement to allow for vaccine uptake. On d 21, pen weights were taken and feed consumption determined for the calculation of feed conversion ratios. Half of the treatment groups were challenged with a mixed species challenge containing *Eimeria acervulina* (6×10^5),

Eimeria maxima (4 x 10^5), and *Eimeria tenella* (2 x 10^5) sporulated oocysts. On d 27 (6 days post-challenge), pen weights were taken, feed consumption determined, and 10 broilers from each replicate necropsied for the quantitative assessment (scoring) of intestinal lesion development (Johnson and Reid, 1970).

Experiment 3

The experimental design for Experiment 3 was a replicate of Experiment 2 with some slight alterations. All treatment groups remained the same although replicate number was decreased from three to two, increased bird number per replicate from 25 to 30, and increased the number of birds necropsied per replicate from 10 to 15. On day of hatch, chicks were weighed, wing banded, and assigned to treatment group based on chick weight. Chicks were vaccinated in a commercial spray cabinet for 100 bird chick trays. A red food coloring dye was added to the vaccine and chicks were allowed to preen for 1 hr before placement to allow for vaccine uptake. On d 20, pen weights were taken, feed consumption determined, and chicks were challenged with a mixed species challenge consisting of *Eimeria acervulina* (6 x 10^5), *Eimeria maxima* (4 x 10^5), and *Eimeria tenella* (2 x 10^5) oocysts. On d 26 (6 days post-challenge), pen weights were taken, feed consumption determined, and 15 broilers from each replicate necropsied for the determination of intestinal lesion development (Johnson and Reid, 1970).

Experiment 4

With the results of the previous three experiments, Experiment 4 investigated the effect of starter diet duration on broiler chick performance during an anti-coccidial vaccination program with subsequent *Eimeria* challenge. The experimental design was a

3 x 2 x 2 factorial with the variables of starter period duration (13, 17, and 21 d), vaccination (vaccinated compared to non-medicated non-vaccinated), and a mixed species *Eimeria* challenge (challenge and non-challenged). Each treatment was replicated in triplicate for a total of 36 pens. Each replicate contained 25 chicks placed for a total of 900 chicks placed. Again, chicks were individually weighed, wing banded, and assigned to treatment groups by weight. A red food coloring dye was added to the vaccine and chicks were allowed to preen for 1 hr before placement to allow for vaccine uptake. Pen weights and feed consumption were determined on days 13, 17, 21, and 27. The 22% starter diet used in the previous three experiments was fed to broilers for either 13, 17, or 21 days depending on treatment. At the conclusion of the starter period, rations were switched to a grower diet formulated to meet the specifications of the high nutrient density diet specified in Leeson and Summers (2005) with the exception of the energy value being maintained at 3200 kcal/kg. On d 21, broilers were challenged with a mixed species challenge consisting of *Eimeria acervulina* (1.25×10^6), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (5×10^4) sporulated oocysts. On d 27 (6 days post-challenge), pen weights were taken, feed consumption determined, and 10 broilers from each replicate pen were necropsied for the determination of intestinal lesion development (Johnson and Reid, 1970).

Statistical Analysis

Experiment 1

Data were analyzed using SPSS Version 11.0 for Windows (SYSTAT, 2001) for all experiments. Statistical significance was determined by one-way analysis of variance

and means were separated by Duncan's multiple range test. The threshold for statistical significance was $P \leq 0.05$.

Experiment 2

Body weights and feed conversion ratios for d 21 data were analyzed using a one-way analysis of variance due to the presence of a significant interaction between protein level and vaccination. Significant differences were determined at $P \leq 0.05$ and means were separated using a Duncan's Multiple Range Test. Similarly, due to an interaction between challenge and vaccine, data collected on d 27 including post-challenge body weight gain, feed conversion ratios, mortality, and lesion scores were analyzed using a one-way analysis of variance with differences deemed significant at $P \leq 0.05$. Means were separated using a Duncan's Multiple Range Test. Post-challenge mortality was subjected to a square root arc sin transformation before analysis.

Experiment 3

Body weight and feed conversion ratios for d 20 were analyzed using a 3 x 2 factorial analysis of variance. No significant interaction was observed as in Experiment 2. Post-challenge body weight gains, feed conversion ratios, and lesion scores were analyzed using a one-way analysis of variance because of a significant interaction between vaccine and challenge. Post-challenge mortality was analyzed in a similar fashion following a square root arc sin transformation. Significance was determined at $P \leq 0.05$, and means were separated using Duncan's Multiple Range Test.

Experiment 4

Pre-challenge data were analyzed using a 3 x 2 factorial analysis of variance. Differences in main effects were deemed significant a $P \leq 0.05$ and means were separated using a Duncan's Multiple Range Test. Post-challenge data were analyzed using a one-way analysis of variance due to a significant interaction present between vaccine and challenge. Means were separated using a Duncan's Multiple Range Test at $P \leq 0.05$. Post-challenge mortality was analyzed in similar fashion following a square root arc sin transformation.

Results

Experiment 1

Average broiler body weights at d 21 increased as protein level in the diet increased (Table 3-2). The 20% protein starter diet yielded lower ($P<0.05$) average body weights compared to all other treatments. The 24% protein diet yielded higher ($P<0.05$) average body weights compared to the 21 and 22% protein starter diets while the 21, 22, and 23% protein starter diets were similar. Feed conversion results yielded an inverse relationship as body weight with increasing protein level reducing mortality corrected feed conversion of vaccinated broilers at 21 d of age. The 20% protein diet resulted in a higher ($P<0.05$) feed conversion ratio compared to all other treatments. Increasing dietary protein concentration to 21% lowered ($P<0.05$) feed conversion ratio compared to the 20% protein level. A further increase in protein level to 22 and 23% further reduced ($P<0.05$) feed conversion ratios while the 24% protein starter diet resulted in a

Table 3-2. Average body weights (g) and mortality corrected feed conversion ratios ± SEM of live oocyst vaccinated (Coccivac®-B) broilers at day 21 fed selected concentrations of protein (Experiment 1).

Protein (%)	Body Weight (g)	Feed:Gain
20	585 ± 12^{c}	1.57 ± 0.03^{a}
21	665 ± 5^{b}	1.40 ± 0.02^{b}
22	679 ± 11^{b}	1.35 ± 0.01^{c}
23	689 ± 14^{ab}	1.34 ± 0.02^{c}
24	720 ± 9^{a}	1.27 ± 0.01^{d}

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

lower ($P<0.05$) mortality corrected feed conversion ratio compared to all other levels. Average body weights and mortality corrected feed conversion ratios of vaccinated broilers fed 20, 22, and 24% dietary protein levels were significantly different from each other at 21 d of age. Therefore, these three protein levels were selected for use in Experiments 2 and 3.

Experiment 2

Average body weights of broilers at 21 d followed a similar trend as reported in Experiment 1, increasing dietary protein level increased average body weights of broilers (Table 3-3). In non-vaccinated broilers, increases ($P<0.05$) in average body weights were observed with each increase in dietary protein level. The 20% protein level resulted in the lowest body weight and the 24% protein level resulted in the heaviest body weights. Body weights for vaccinated broilers followed a similar trend. The 20% protein level resulted in lower ($P<0.05$) body weights than the 22 and 24% starter diets. An increase in body weight due to increased protein concentration from 22% to 24% was not observed in vaccinated broilers. Vaccinated broilers fed the lowest protein concentration in the starter diet were the lightest broilers compared to all other treatments. Vaccination of broilers reduced ($P<0.05$) body weights at the 20 and 24% protein levels compared to the non-vaccinated broilers fed the same protein level. However, the vaccinated broilers fed 22% protein were similar to non-vaccinated broilers fed the same protein concentration.

Mortality corrected feed conversion ratios at 21 d also followed similar trends as in Experiment 1; increasing protein level reduced feed conversion ratios of both

Table 3-3. Average body weights (g) and mortality corrected feed conversion ratios ± SEM of non-vaccinated and vaccinated (Coccivac®-B) broilers at day 21 fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Treatment	Body Weight (g)	Feed:Gain
20	Non-vaccinated	664 ± 18^{c}	1.40 ± 0.03^{b}
20	Vaccinated	579 ± 8^{d}	1.60 ± 0.02^{a}
22	Non-vaccinated	733 ± 33^{b}	1.30 ± 0.01^{cd}
22	Vaccinated	709 ± 14^{bc}	1.34 ± 0.02^{bc}
24	Non-vaccinated	808 ± 10^{a}	1.26 ± 0.01^{d}
24	Vaccinated	737 ± 17^{b}	1.31 ± 0.02^{cd}

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

vaccinated and non-vaccinated broilers (Table 3-3). Broilers fed the 20% protein diet yielded higher ($P<0.05$) feed conversion ratios compared to the 22% and 24% levels regardless of vaccination. Vaccination caused an increase ($P<0.05$) in feed conversion at lowest protein level investigated. However, at the two highest protein levels fed, there were no differences between vaccinated and non-vaccinated broilers with respect to feed conversion at the same dietary protein level.

Body weight gain and mortality corrected feed conversion ratios during the challenge period (21 d to 27 d) were similar for non-vaccinated and vaccinated non-challenge broilers at all protein levels (Table 3-4). In challenged broilers, vaccination increased ($P<0.05$) body weight gain compared to non-vaccinated broilers at all protein levels investigated. Non-vaccinated challenge broilers had similar weight gain at all protein levels during the challenge period which were lower ($P<0.05$) than all other treatments. Within the vaccinated challenge broilers, increased ($P<0.05$) weight gains were observed in broilers fed the 24% protein diet compared to the 20% protein level while the 22% level was intermediate. Mortality corrected feed conversion ratios during the challenge period for vaccinated challenge broilers were similar at all protein levels. In lieu of challenge, vaccinated broilers yielded similar feed conversion ratios as all non-challenged treatment groups. Challenge of non-vaccinated broilers resulted in higher ($P<0.05$) feed conversion ratios compared to all other treatment groups. In non-vaccinated challenged broilers, post-challenge feed conversion ratios were decreased ($P<0.05$) with each increase in dietary protein level. Increased protein concentration improved broiler performance during *Eimeria* challenge with increased body weight

Table 3-4. Average body weight gains (g), mortality corrected feed conversion ratios (FCR), and mortality ± SEM of non-vaccinated (NV) and vaccinated (V) (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 21 fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Vaccine	Chall[1]	BW Gain (g)	FCR Day 21-27	FCR Day 1 -27	Post-challenge Mortality[2]
20	NV	No	392 ± 7^{a}	1.59 ± 0.02^{d}	1.49 ± 0.03^{d}	0.07 ± 0.07^{bc}
20	V	No	377 ± 32^{a}	1.61 ± 0.05^{d}	1.60 ± 0.02^{c}	0.00 ± 0.00^{c}
22	NV	No	402 ± 8^{a}	1.39 ± 0.16^{d}	1.38 ± 0.01^{e}	0.00 ± 0.00^{c}
22	V	No	406 ± 16^{a}	1.52 ± 0.04^{d}	1.41 ± 0.02^{e}	0.00 ± 0.00^{c}
24	NV	No	415 ± 15^{a}	1.49 ± 0.02^{d}	1.35 ± 0.01^{e}	0.07 ± 0.07^{bc}
24	V	No	396 ± 12^{a}	1.49 ± 0.02^{d}	1.35 ± 0.01^{e}	0.00 ± 0.00^{c}
20	NV	Yes	63 ± 17^{d}	5.80 ± 0.46^{a}	1.78 ± 0.03^{a}	0.35 ± 0.09^{a}
20	V	Yes	236 ± 21^{c}	2.05 ± 0.09^{d}	1.74 ± 0.05^{a}	0.16 ± 0.08^{abc}
22	NV	Yes	96 ± 6^{d}	4.61 ± 0.22^{b}	1.67 ± 0.01^{b}	0.33 ± 0.03^{a}
22	V	Yes	278 ± 19^{bc}	1.89 ± 0.03^{d}	1.50 ± 0.01^{d}	0.07 ± 0.07^{bc}
24	NV	Yes	84 ± 28^{d}	3.91 ± 0.60^{c}	1.59 ± 0.05^{c}	0.23 ± 0.12^{ab}
24	V	Yes	316 ± 24^{b}	1.90 ± 0.03^{d}	1.50 ± 0.01^{d}	0.19 ± 0.10^{abc}

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

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

[2] Reported values are a result of a square root arc sin transformation of the observed mortality rates.

gain in vaccinated broilers and decreased feed conversion ratios in non-vaccinated broilers.

Cumulative mortality corrected feed conversion ratios for 1 to 27 d were similar in non-vaccinated and vaccinated non-challenged broilers at both 22 and 24% protein levels. Non-challenged broilers fed the 20% protein diet had a higher ($P<0.05$) feed conversion ratio compared to the 22 and 24% protein levels. Vaccination caused an increase ($P<0.05$) in cumulative feed conversion at the 20% level in non-challenged broilers but had no adverse effect in non-challenged broilers fed the 22 and 24%. In challenged broilers, vaccination led to a decrease ($P<0.05$) in cumulative feed conversion ratio in the 22 and 24% protein fed broilers compared to non-vaccinated challenge broilers while the 20% protein level was similar for both vaccinated and non-vaccinated challenged broilers. Increased dietary protein led to decreased ($P<0.05$) cumulative feed conversion ratios in non-vaccinated challenged broilers. Vaccination resulted in decreased ($P<0.05$) mortality in the 22% protein fed challenged broilers compared to the 20 and 22% non-vaccinated challenge broilers while all other challenge groups had similar mortality rates.

Overall, lesion development was decreased in vaccinated challenged broilers compared non-vaccinated challenged broilers (Table 3-5). Overall lesions in the upper small intestine indicative of *E. acervulina* were minimal. All vaccinated challenged broilers had ($P<0.05$) lower lesion development in the upper small intestine compared to the 22 and 24% protein fed non-vaccinated challenged broilers. Mid intestinal lesion development associated with *E. maxima* was decreased ($P<0.05$) in the 24% protein fed

Table 3-5. Lesion scores of non-vaccinated and vaccinated (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 21of half of the treatment groups fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Vaccination	Challenge[1]	Upper	Mid	Lower
20	Non-vaccinated	No	0.26 ± 0.08^{bc}	0.40 ± 0.02^{e}	0.00 ± 0.00^{d}
20	Vaccinated	No	0.00 ± 0.00^{d}	0.87 ± 0.05^{de}	0.23 ± 0.12^{d}
22	Non-vaccinated	No	0.00 ± 0.00^{d}	0.64 ± 0.16^{e}	0.04 ± 0.04^{d}
22	Vaccinated	No	0.00 ± 0.00^{d}	0.87 ± 0.04^{de}	0.03 ± 0.03^{d}
24	Non-vaccinated	No	0.10 ± 0.05^{cd}	0.47 ± 0.02^{e}	0.00 ± 0.00^{d}
24	Vaccinated	No	0.00 ± 0.00^{d}	0.60 ± 0.02^{e}	0.07 ± 0.04^{d}
20	Non-vaccinated	Yes	0.13 ± 0.08^{cd}	2.13 ± 0.46^{a}	2.17 ± 0.11^{a}
20	Vaccinated	Yes	0.00 ± 0.00^{d}	1.90 ± 0.09^{ab}	0.60 ± 0.15^{c}
22	Non-vaccinated	Yes	0.33 ± 0.09^{b}	1.83 ± 0.22^{ab}	2.03 ± 0.11^{a}
22	Vaccinated	Yes	0.03 ± 0.03^{d}	1.57 ± 0.03^{bc}	0.77 ± 0.17^{c}
24	Non-vaccinated	Yes	0.53 ± 0.15^{a}	1.93 ± 0.60^{ab}	2.03 ± 0.11^{a}
24	Vaccinated	Yes	0.00 ± 0.00^{d}	1.20 ± 0.03^{cd}	1.40 ± 0.20^{b}

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

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

vaccinated challenged broilers compared to the 24% non-vaccinated challenged broilers. Numeric decreases in mid intestinal lesion scores were observed in vaccinated challenge broilers at the 20 and 22% levels compared to non-vaccinated challenged broilers. Lower intestinal lesion development associated with *E. tenella* challenge was decreased ($P<0.05$) in vaccinated challenge broilers compare to non-vaccinated challenge broilers at all protein levels. Lower intestinal lesion scores were similar for all non-vaccinated challenge broilers while vaccinated challenged broilers fed 24% protein had significantly increased lower intestinal lesion scores compare to the 20 and 22% vaccinated challenge broilers. An inverse relationship existed between mid and lower intestinal lesion development in vaccinated challenged broilers fed different dietary protein levels. Mid intestinal lesions decreased with increasing protein level while lower intestinal lesion development increased with increasing protein level. Lesions were observed in a small percentage of non-challenged broilers. The lesions present in the non-vaccinated non-challenge broilers may be attributed to the close proximity in which broilers were reared while the lesions present in the vaccinated non-challenged broilers may be due to continued cycling of vaccine oocysts or the close proximity of rearing. Housing both challenged and non-challenged broilers in close proximity to each other was essential in order to assure no environmental or pen-related effects on performance throughout the duration of the experiment.

Experiment 3

Experiment 3 was conducted to further investigate the inverse relationship between mid and lower intestinal lesion development and protein level in challenge

broilers as well as to validate the results observed in Experiment 2. The interaction observed in Experiment 2 between protein level and vaccination was not observed in Experiment 3 pre-challenge. Therefore, main effects will be discussed as opposed to individual treatments. Average body weights on d 21 of broilers fed 22 and 24% protein starter diets were higher ($P<0.05$) compared to the 20% protein level (Table 3-6). Vaccinated broilers had lower ($P<0.05$) body weights at 21d as compared to non-vaccinated broilers. Mortality corrected feed conversion ratios followed similar patterns observed in the previous two experiments. Broilers fed a starter diet containing 20% protein had increased ($P<0.05$) feed conversion ratios compared to 22 and 24% protein fed broilers, and vaccination increased ($P<0.05$) feed conversion ratios at d 21.

Body weight gains were similar for non-vaccinated and vaccinated non-challenged broilers regardless of protein level with one exception. The 20% vaccinated broilers gained less ($P<0.05$) weight during the six day post challenge period (20 d to 26 d) compared to the 22% non-vaccinated broilers (Table 3-7). Vaccinated challenge broilers gained more ($P<0.05$) body weight during the challenge period compared to all non-vaccinated challenged broilers. Body weight gain for all non-vaccinated challenged broilers was similar at all protein levels. The 22% vaccinated challenged broilers gained more ($P<0.05$) body weight compared to the 20 and 24% vaccinated challenged broilers. Body weight gain of the 22% vaccinated challenged broilers were increased to a level similar to several non-challenged treatment groups.

Mortality corrected feed conversion ratios during the challenge period were similar for all non-challenge treatments, thus vaccination did not have an adverse effect

Table 3-6. Average body weights (g) and mortality corrected feed conversion ratios ± SEM of non-vaccinated and vaccinated (Coccivac®-B) broilers at day 20 fed diets containing three different protein concentrations (Experiment 3).

Body Weight (g)

Protein	Treatment		
	Non-Vaccinated	Vaccinated	Mean
20	720 ± 11	679 ± 14	699 ± 11[b]
22	780 ± 5	750 ± 9	765 ± 7[a]
24	793 ± 9	739 ± 5	762 ± 12[a]
Mean	762 ± 11[a]	723 ± 11[b]	

Feed:Gain

Protein	Treatment		
	Non-Vaccinated	Vaccinated	Mean
20	1.49 ± 0.01	1.55 ± 0.02	1.52 ± 0.01[a]
22	1.34 ± 0.01	1.42 ± 0.04	1.38 ± 0.03[b]
24	1.30 ± 0.01	1.38 ± 0.02	1.34 ± 0.02[b]
Mean	1.38 ±0.03[b]	1.45 ±0.03[a]	

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

Table 3-7. Average body weight gains (g), mortality corrected feed conversion ratios (FCR), and mortality ± SEM of non-vaccinated (NV) and vaccinated (V) (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 20 fed diets containing three different protein concentrations (Experiment 3).

Protein (%)	Vaccine	Chall[1]	BW Gain (g)	FCR Day 20-26	FCR Day 1 -26	Post-challenge Mortality[2]
20	NV	No	391 ± 5^{abc}	1.66 ± 0.01^{c}	1.55 ± 0.01^{cd}	0.00 ± 0.00^{b}
20	V	No	382 ± 5^{bc}	1.70 ± 0.05^{bc}	1.61 ± 0.01^{bc}	0.14 ± 0.13^{b}
22	NV	No	441 ± 30^{a}	1.52 ± 0.02^{c}	1.40 ± 0.01^{e}	0.00 ± 0.00^{b}
22	V	No	438 ± 15^{ab}	1.53 ± 0.01^{c}	1.43 ± 0.01^{e}	0.00 ± 0.00^{b}
24	NV	No	414 ± 24^{abc}	1.52 ± 0.02^{c}	1.38 ± 0.02^{e}	0.00 ± 0.00^{b}
24	V	No	429 ± 8^{abc}	1.52 ± 0.05^{c}	1.43 ± 0.04^{e}	0.00 ± 0.00^{b}
20	NV	Yes	197 ± 26^{e}	2.65 ± 0.23^{a}	1.72 ± 0.05^{a}	0.41 ± 0.04^{a}
20	V	Yes	305 ± 3^{d}	1.99 ± 0.06^{b}	1.68 ± 0.01^{ab}	0.18 ± 0.00^{ab}
22	NV	Yes	191 ± 3^{e}	2.57 ± 0.04^{a}	1.58 ± 0.01^{cd}	0.13 ± 0.13^{b}
22	V	Yes	380 ± 2^{c}	1.60 ± 0.06^{c}	1.51 ± 0.03^{d}	0.13 ± 0.13^{b}
24	NV	Yes	193 ± 5^{e}	2.47 ± 0.04^{a}	1.55 ± 0.01^{cd}	0.10 ± 0.09^{b}
24	V	Yes	309 ± 17^{d}	1.80 ± 0.03^{bc}	1.51 ± 0.01^{d}	0.10 ± 0.09^{b}

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

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

[2] Reported values are a result of a square root arc sin transformation of the observed mortality rates.

on feed conversion during this period of grow-out. Introduction of a mixed species challenge resulted in an increase ($P<0.05$) in feed conversion ratios of non-vaccinated broilers compared to all other treatments groups. An effect of protein level on feed conversion ratio of non-vaccinated challenged broilers was not observed. Feed conversion of 20% vaccinated challenged broilers was higher ($P<0.05$) compared to the 22% vaccinated challenged broilers. Vaccinated challenged broilers fed 22 and 24% protein diets resulted in feed conversion ratios similar to all non-challenged treatments. Cumulative mortality corrected feed conversion ratios for vaccinated non-challenged broilers were similar to non-vaccinated non-challenged broilers within each protein treatment. Non-challenged broilers fed 20% protein yielded higher ($P<0.05$) feed conversion ratios compared to the 22 and 24% protein levels. Non-challenged broilers fed 22 and 24% protein levels had lower ($P<0.05$) feed conversion ratios than all challenged treatments. Although numeric reductions in cumulative feed conversions were observed in vaccinated challenged broilers compared to non-vaccinated challenged broilers, these did not reach the level of significance. Post-challenge mortality was increased ($P<0.05$) in 20% non-vaccinated challenged broilers compared to all other challenged groups and non-challenged groups with the exception of the 20% vaccinated challenged broilers.

As in Experiment 2, in most instances, lesion development was reduced in vaccinated challenged broilers compared to non-vaccinated broilers (Table 3-8). Lesion development in the upper small intestine associated with *E. acervulina* was reduced ($P<0.05$) in vaccinated challenged broilers compared to non-vaccinated challenged

Table 3-8. Lesion scores of non-vaccinated and vaccinated (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 20 fed diets containing three different protein concentrations (Experiment 3).

Protein (%)	Vaccination	Challenge[1]	Upper	Mid	Lower
20	Non-vaccinated	No	0.00 ± 0.00^{c}	0.33 ± 0.10^{d}	0.03 ± 0.03^{d}
20	Vaccinated	No	0.10 ± 0.06^{c}	0.50 ± 0.10^{cd}	0.10 ± 0.06^{cd}
22	Non-vaccinated	No	0.10 ± 0.06^{c}	0.73 ± 0.15^{cd}	0.03 ± 0.03^{d}
22	Vaccinated	No	0.23 ± 0.10^{c}	1.03 ± 0.20^{bc}	0.07 ± 0.05^{cd}
24	Non-vaccinated	No	0.03 ± 0.03^{c}	0.77 ± 0.11^{cd}	0.07 ± 0.05^{cd}
24	Vaccinated	No	0.00 ± 0.00^{c}	0.87 ± 0.11^{c}	0.00 ± 0.00^{d}
20	Non-vaccinated	Yes	1.29 ± 0.20^{a}	1.55 ± 0.18^{a}	1.00 ± 0.16^{a}
20	Vaccinated	Yes	0.23 ± 0.08^{c}	1.87 ± 0.21^{a}	0.37 ± 0.09^{bc}
22	Non-vaccinated	Yes	1.03 ± 0.22^{a}	1.90 ± 0.21^{a}	1.00 ± 0.15^{a}
22	Vaccinated	Yes	0.07 ± 0.05^{c}	0.90 ± 0.15^{c}	0.20 ± 0.09^{cd}
24	Non-vaccinated	Yes	0.57 ± 0.11^{b}	1.50 ± 0.20^{ab}	0.80 ± 0.15^{a}
24	Vaccinated	Yes	0.10 ± 0.06^{c}	0.83 ± 0.21^{cd}	0.50 ± 0.12^{b}

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

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

broilers at all protein levels. The highest dietary protein level, 24%, resulted in reduced upper intestinal lesion development in non-vaccinated challenge broilers compared to the two lower protein levels. Reductions ($P<0.05$) were observed in 22% and 24% protein fed vaccinated challenged broilers compared to all other challenged groups. No protection with respect to *E. maxima* challenge was observed in 20% protein fed vaccinated broilers, as lesion development in this group was similar to non-vaccinated challenge groups. Vaccinated challenged broilers fed either 22 or 24% protein had lower ($P<0.05$) lesion development associated with *E. maxima* compared to non-vaccinated challenged groups. Lower intestinal lesions associated with *E. tenella* were reduced ($P<0.05$) due to vaccination. Vaccinated challenged broilers had lower ($P<0.05$) lesion scores compared to non-vaccinated challenged broilers regardless of protein level. Due to the interaction present, it is difficult to use these data to completely identify the effect of protein level on lesion development due to challenge.

Experiment 4

Results from the previous three experiments indicate that performance parameters of vaccinated broilers were not enhanced by increasing the protein level above 22%. Experiment 4 investigated the effects of the duration of the starter phase on broiler performance during vaccination and subsequent challenge. Average body weights for vaccinated and non-vaccinated broilers were similar ($P>0.05$) at 13 d of age (Table 3-9). Vaccinated broilers had lower ($P<0.05$) average body weights compared to non-vaccinated broilers at 17 d of age. At 17 d of age, no differences existed between broilers fed the starter diet for 13 d as opposed to 17 d. On 21 d of age, vaccinated

Table 3-9. Average body weights ± SEM of non-vaccinated and vaccinated (Coccivac®-B) broilers fed a starter diet for three different durations (Experiment 4).

Starter Duration (d)	Treatment	Body Weight (g)
Day 13		
	Non-vaccinated	373 ± 4
	Vaccinated	375 ± 3

Day 17

Duration (d)	Treatment		
	Non-vaccinated	Vaccinated	Means
13	562 ± 7	537 ± 10	549 ± 7
17	566 ± 5	551 ± 8	559 ± 5
Means	564 ± 4[a]	546 ± 7[b]	

Day 21

Duration (d)	Treatment		
	Non-vaccinated	Vaccinated	Means
13	757 ± 16	715 ± 19	736 ± 14
17	767 ± 10	757 ± 14	762 ± 8
21	777 ± 6	739 ± 15	760 ± 18
Means	767 ± 6[a]	737 ± 13[b]	

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

broilers had lower (P<0.05) average body weights compared to non-vaccinated broilers while duration of starter period had no effect on average body weight. Mortality corrected feed conversion ratios at 13 d were similar (P>0.05) for vaccinated and non-vaccinated broilers (Table 3-10), and similar (P>0.05) results were observed on 17 d and 21 d. Vaccination had no adverse effects on feed conversion ratios during the first 21 d of age. Increased starter period duration resulted in decreased feed conversions on d 21. Broilers switched to the grower diet on d 13 had an increased (P<0.05) feed conversion compared to broilers switched to the grower diet on d 17 and d 21. This was the only adverse effect observed associated with shorter starter phase duration.

During the challenge period (21d to 27d of age), body weight gains were similar for all non-vaccinated and vaccinated non-challenged treatments. In challenged broilers, all vaccinated treatment groups gained more (P<0.05) body weight than non-vaccinated broilers (Table 3-11). No effect of starter phase duration was observed on weight gain during the challenge period. Mortality corrected feed conversion ratios were similar for vaccinated and non-vaccinated non-challenged broilers during the challenge period. In challenged broilers, vaccination resulted in decreased (P<0.05) feed conversions compared to non-vaccinated broilers post-challenge. Cumulative feed conversion ratios (1d to 27d) were similar for all non-challenged treatments. All cumulative feed conversion ratios were higher (P<0.05) in challenged broilers compared to non-challenged broilers. Cumulative feed conversion ratios in vaccinated challenged broilers fed the starter diet for 21 d duration period were reduced (P<0.05) compared to the non-vaccinated challenged broilers fed the starter diet for 13 and 17 d. Post-challenge

Table 3-10. Mortality corrected feed conversion ratios ± SEM of non-vaccinated and vaccination (Coccivac®-B) broilers fed a starter diet for three different durations (Experiment 4).

Starter Duration (d)	Treatment	Feed:Gain
Day 1 to 13		
	Non-vaccinated	1.10 ± 0.01
	Vaccinated	1.10 ± 0.01

Day 1 to 17

Duration (d)	Treatment		
	Non-vaccinated	Vaccinated	Means
13	1.30 ± 0.01	1.32 ± 0.02	1.31 ± 0.02
17	1.28 ± 0.02	1.30 ± 0.02	1.29 ± 0.02
Means	1.29 ± 0.01	1.31 ± 0.02	

Day 1 to 21

Duration (d)	Treatment		
	Non-vaccinated	Vaccinated	Means
13	1.40 ± 0.02	1.47 ± 0.04	1.44 ± 0.03[a]
17	1.36 ± 0.02	1.37 ± 0.01	1.37 ± 0.01[b]
21	1.34 ± 0.01	1.37 ± 0.02	1.35 ± 0.01[b]
Means	1.37 ± 0.01	1.41 ± 0.02	

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

Table 3-11. Average body weight gains (g), mortality corrected feed conversion ratios (FCR), and mortality ± SEM of non-vaccinated (NV) and vaccinated (V) (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 21 fed a starter diet for three different durations (Experiment 4).

Duration (d)	Vaccine	Chall[1]	BW Gain (g)	FCR Day 21-27	FCR Day 1 -27	Post-challenge Mortality[2]
13	NV	No	418 ± 63[a]	1.54 ± 0.19[b]	1.46 ± 0.07[c]	0.07 ± 0.07[b]
13	V	No	435 ± 25[a]	1.34 ± 0.07[b]	1.41 ± 0.05[c]	0.00 ± 0.00[b]
17	NV	No	448 ± 32[a]	1.44 ± 0.05[b]	1.41 ± 0.01[c]	0.00 ± 0.00[b]
17	V	No	403 ± 24[a]	1.55 ± 0.02[b]	1.44 ± 0.01[c]	0.00 ± 0.00[b]
21	NV	No	409 ± 48[a]	1.72 ± 0.24[b]	1.46 ± 0.06[c]	0.00 ± 0.00[b]
21	V	No	441 ± 15[a]	1.47 ± 0.04[b]	1.43 ± 0.02[c]	0.00 ± 0.00[b]
13	NV	Yes	81 ± 11[c]	5.77 ± 0.50[a]	1.73 ± 0.02[a]	0.49 ± 0.04[a]
13	V	Yes	242 ± 14[b]	2.01 ± 0.22[b]	1.62 ± 0.03[ab]	0.14 ± 0.07[b]
17	NV	Yes	102 ± 2[c]	5.43 ± 0.32[a]	1.73 ± 0.01[a]	0.49 ± 0.02[a]
17	V	Yes	210 ± 11[b]	2.70 ± 0.14[b]	1.65 ± 0.01[ab]	0.14 ± 0.07[b]
21	NV	Yes	113 ± 36[c]	4.84 ± 0.58[a]	1.67 ± 0.03[ab]	0.36 ± 0.04[a]
21	V	Yes	271 ± 22[b]	2.27 ± 0.12[b]	1.59 ± 0.05[b]	0.08 ± 0.08[b]

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

[1] Mixed species challenge contained *Eimeria acervulina* (1.25×10^6), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (5×10^4) sporulated oocysts.

[2] Reported values are a result of a square root arc sin transformation of the observed mortality rates.

mortality was higher ($P<0.05$) in non-vaccinated challenged groups compared to all other treatments while mortality for vaccinated challenged broilers was similar to all non-challenged broilers. An effect of starter phase duration on post-challenge mortality was not observed. Lesion development associated with the mixed species challenge was unaffected by starter phase duration time. However, lesion development was reduced ($P<0.05$) in the upper and lower intestinal segments in vaccinated challenged broilers compared to non-vaccinated challenged broilers (Table 3-12). While lesion development associated with the *E. maxima* challenge was numerically reduced in vaccinated challenged broilers compared to non-vaccinated challenged broilers, only broilers switched to grower on d 13 of age reached the level of significance.

Discussion

Increasing dietary protein level improved broiler performance at 21 d of age, determined by body weights and feed conversion, regardless of vaccination. This observation was expected as many reports have correlated crude protein level of diets to broiler performance (Sterling et al. 2003; Vieira et al., 2004). Crude protein level of diets is of extreme importance due to the cost associated with increasing the protein level in diets, which leads to impacts on costs as well as revenues during broiler meat production (Eits et al., 2005). In Experiment 1, a linear relationship was observed with respect to body weight and feed conversion associated with increased crude protein level to 21 d of age in vaccinated broilers. Broilers fed 20, 22, or 24% protein in the starter diet to 21 d of age differed significantly in performance characteristics. Therefore, these

Table 3-12. Lesion scores of non-vaccinated and vaccinated (Coccivac®-B) broilers six days post mixed species *Eimeria* challenge at day 21 fed a starter diet for three different durations (Experiment 4).

Duration (day)	Vaccination	Challenge[1]	Upper	Mid	Lower
13	Non-vaccinated	No	0.03 ± 0.03^{c}	0.13 ± 0.06^{d}	0.00 ± 0.00^{d}
13	Vaccinated	No	0.00 ± 0.00^{c}	0.60 ± 0.11^{c}	0.10 ± 0.06^{d}
17	Non-vaccinated	No	0.00 ± 0.00^{c}	0.07 ± 0.05^{d}	0.00 ± 0.00^{d}
17	Vaccinated	No	0.03 ± 0.03^{c}	0.63 ± 0.10^{c}	0.03 ± 0.03^{d}
21	Non-vaccinated	No	0.00 ± 0.00^{c}	0.13 ± 0.06^{d}	0.00 ± 0.00^{d}
21	Vaccinated	No	0.00 ± 0.00^{c}	0.47 ± 0.09^{cd}	0.03 ± 0.03^{d}
13	Non-vaccinated	Yes	1.40 ± 0.18^{ab}	2.30 ± 0.15^{a}	2.10 ± 0.15^{a}
13	Vaccinated	Yes	0.23 ± 0.09^{c}	1.70 ± 0.20^{b}	0.63 ± 0.15^{c}
17	Non-vaccinated	Yes	1.47 ± 0.21^{a}	2.37 ± 0.17^{a}	2.23 ± 0.16^{a}
17	Vaccinated	Yes	0.13 ± 0.08^{c}	2.07 ± 0.20^{ab}	1.13 ± 0.21^{b}
21	Non-vaccinated	Yes	1.13 ± 0.13^{b}	2.13 ± 0.15^{a}	2.30 ± 0.14^{a}
21	Vaccinated	Yes	0.10 ± 0.06^{c}	2.07 ± 0.18^{ab}	1.13 ± 0.17^{b}

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

[1] Mixed species challenge contained *Eimeria acervulina* (1.25×10^{6}), *Eimeria maxima* (4×10^{5}), and *Eimeria tenella* (5×10^{4}) sporulated oocysts.

protein levels were selected for use in subsequent experiments to compare growth characteristics to non-vaccinated broilers during field strain *Eimeria* challenge. Non-vaccinated broilers indicated similar patterns in performance characteristics as vaccinated broilers with improvement in performance characteristics related to increased protein level of the diet. Vaccination tended to reduce body weight and increase feed conversion compared to non-vaccinated broilers prior to challenge. However, in Experiment 2 where an interaction was observed and data were analyzed by one-way analysis, vaccinated broilers fed 22% protein had similar growth characteristics compared to the non-vaccinated broilers fed the 22% starter diet. Reduced body weights and increased feed conversion ratios during the early stages of growth due to vaccination have been reported by other investigators (Danforth, 1998; Williams, 2002). However, other published reports have indicated that compensatory gain in vaccinated broilers during subsequent dietary periods results in similar if not improved performance characteristics at the completion of grow-out (Danforth, 1998; Williams et al., 1999; Williams and Gobbi, 2002; Williams, 2002).

During the six day challenge period which began on d 21 (Experiment 2 and 4) or d 20 (Experiment 3), vaccination did not adversely effect body weight gain and feed conversion ratios. Similarly, increasing dietary protein had no effect on performance parameters during this six day period. Over the duration of the experiment, feed conversion ratios of non-challenged broilers were unaffected due to vaccination with respect to the 22 and 24% dietary level. Non-challenged broilers regardless of immunization status fed the 20% dietary protein level had increased feed conversion

ratios compared to broilers fed the 22 and 24% dietary protein levels. However, vaccination significantly increased feed conversion ratio of vaccinated broilers fed 20% protein compared to non-vaccinated broilers fed 20% protein in one experiment. Diets containing the lowest protein level in these experiments tended to have a larger impact on performance characteristics due to vaccination. The highest dietary protein level (24%) did not necessarily translate to the highest level of performance in vaccinated and challenged chickens, as the 22% protein level was often indistinguishable from the 24% level. In Experiment 2, performance values of chickens fed a 22% dietary protein level did not differ between vaccinated and non-vaccinated groups prior to or during challenge.

The generation of immunity through vaccination improved performance of broilers during *Eimeria* challenge has been widely reported (Brake et al., 1997b; Weber and Evans, 2003; Williams, 2003; Shirley et al., 2005) and was evident by significantly higher body weight gain, reduced feed conversion ratios, reduced lesion development, and in some cases reduced mortality post-challenge compared to non-vaccinated broilers. Improved growth characteristics during the challenge period in vaccinated broilers led to significantly improved cumulative feed conversion ratios (d 1-27) in broilers fed the two higher protein levels in Experiment 2.

In Experiment 2, increasing dietary protein tended to reduce lesion development associated with *E. maxima* while tending to increase lesion development associated with *E. tenella* in vaccinated chickens. This trend was also observed in Experiment 3, decreased lesion development associated with *E. maxima* was observed with increased

protein level although lesion development in the lower intestine was not different. This may be attributed to the overall lower lesion development observed in Experiment 3 in the lower intestine. These data tend to indicate an inverse relationship in lesion develop associated with *E. maxima* and *E. tenella* during a mixed species challenge in vaccinated broilers with increasing protein level. Further investigation into the relationship of dietary protein level and lesion development is needed before additional conclusions can be drawn.

The results obtained from the first three experiments indicate that a dietary protein inclusion rate of 22% in the starter diet allows for similar growth characteristics of vaccinated and non-vaccinated broilers. Diets in these experiments were not changed according to broiler age and the starter diet was fed to d 27 which is not applicable in modern poultry production. With this in mind, Experiment 4 focused on the relationship between vaccination and the duration of the starter period. Vaccination had no effect on body weights and feed conversion ratios on d 13 of age. Growth depression associated with vaccination was observed on 17 d of age, which corresponds with the second cycling of vaccine oocysts in the rearing environment, and continued to d 21 (Williams, 2002). Growth depression in vaccinated broilers fed 22% protein was also observed in Experiment 3 but not in Experiment 2. Interestingly, feed conversion ratios were unaffected due to vaccination through d 21 with vaccinated and non-vaccinated broilers having similar feed conversion ratios. Body weights were unaffected with respect to broiler age when changed to a grower diet although broilers changed to a grower diet on d 13 had a significantly higher feed conversion compared to broilers changed on d 17 or

21. Day of dietary change from starter to grower had no effect on body weight gain, feed conversion ratio, or mortality during the challenge period although vaccination did significantly improve all these characteristics in challenged broilers. Non-vaccinated challenged broilers had a significantly higher mortality rate compared to vaccinated challenged broilers, a finding supported by the observations of Williams et al. (1999).

Within these experiments, broilers immunized with Coccivac®-B had significant protection against lesion development associated with *E. acervulina* and *E. tenella.* Reductions in lesion scores, both numeric and significant, in the mid intestinal segments were observed but not to as great an extent as lesions present in the other two sites of infection. This is most likely attributed to the immunogenic variability observed with *E. maxima*. In order to gain significant immunization with the use of a commercially available live oocyst vaccine, Danforth et al. (1997b) altered the vaccine used by the addition of *E. maxima* strains isolated locally.

Data from this series of experiments indicate that vaccination with a live oocyst vaccine is an effective tool for the generation of immunity to field strain *Eimeria* challenge. Vaccination resulted in improved growth parameters in immunized compared to non-immunized broilers following challenge. Further observations indicate that while duration of the starter period does not affect growth characteristics, dietary protein level is an important factor to consider when utilizing a vaccination program for the prevention of coccidiosis in order to maximize growth characteristics. Increasing dietary protein levels may be one management consideration which might reduce or

eliminate the adverse effects on broiler performance due to vaccination during the starter period.

CHAPTER IV

EVALUATION OF COCCIVAC®-B OR SALINOMYCIN FOR CONTROL OF FIELD STRAIN *EIMERIA* CHALLENGE IN BROILERS ON TWO DIFFERENT (SEASONAL) FEEDING PROGRAMS

Introduction

Coccidiosis is recognized as the parasitic disease that has the greatest economic impact on poultry production (Allen and Fetterer, 2002a). Infection with coccidia parasites have been calculated to cost the US poultry industry between $450 million (Allen and Fetterer, 2002a) and $1.5 billion (Yun et al., 2000) annually. Approximately 80% of this cost is due to a decrease in performance in the presence of drug treatment strategies (Vermeulen et al., 2001). There are several species of *Eimeria* that cause coccidiosis in chickens with the most prevalent being *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella*. All *Eimeria* species parasitize the epithelial cells of the lining the intestine causing pathological changes varying from local destruction of the mucosal barrier to systemic effects such as blood loss, shock, and death (Vermeulen et al., 2001). The infection leads to economic losses resulting from malabsorption of nutrients evidenced by decreased weight gain, increased feed conversion, and in severe infections, possibly increased mortality. Historically, the poultry industry has prevented and controlled coccidiosis through prophylactic chemotherapy by including anticoccidial feed additives in rations fed to production broilers. However, even with the implementation of shuttle programs for these anticoccidial feed additives, drug resistant

strains continue to emerge across the U.S. and the world forcing considerable interest in the development of alternative methods of control (Williams, 2002).

Live oocyst vaccination is currently the only viable alternative to the use of anti-coccidial drugs and has been used by the poultry industry for over 50 years, primarily for broiler breeder and replacement layer stock (Chapman et al., 2002). The basis for their use is the fact that, after an infection, the host is immune to subsequent infections by the same species (Yun et al., 2000). Live oocyst vaccination has been shown to be an effective tool for the generation of immunity and protection against subsequent *Eimeria* challenge evidenced by increased body weight gain (Danforth, 1998; Crouch et al., 2003; Williams, 2003), reduced feed conversion (Crouch et al., 2003), and reduced lesion development following challenge (Danforth, 1998; Crouch et al., 2003; Williams, 2003) in vaccinated chickens as compared to non-vaccinated chickens. However, there has been a general reluctance in implementing vaccination programs in US broiler production due to reports of reduced performance (Allen and Fetterer, 2002a). Non-attenuated vaccines in broilers have been shown to decrease weight gain and increase feed conversion ratios when compared to medicated birds during the starter period of grow-out (Danforth, 1998; Williams, 2002). Negative effects on cumulative broiler performance have been reported when using live oocyst vaccines compared to medication evidenced by reduced final body weights (Danforth et al., 1997; Waldenstedt et al., 1999a) and increased feed conversion ratios (Williams et al., 1999; Waldenstedt et al., 1999a). Although, Danforth (1998) and Williams and Gobbi (2002) observed that vaccinated broilers performed similar to if not better than medicated broilers, and that

vaccination can lead to significantly lower mortality rates compared to medication (Williams et al., 1999). Previous research has indicated that varying dietary protein levels can influence performance during clinical coccidial infection (Sharma et al., 1973).

The objective of this study was to determine dietary influence on broiler performance parameters reared on litter containing locally isolated field strains of *Eimeria* while utilizing two methods of coccidiosis control. The experimental approach to this trial included a comparison of the efficacy of salinomycin or Coccivac®-B when administered with one of two different diets that vary in protein concentration.

Materials and Methods

A 2 x 2 factorial base design comparing two dietary regimens (Table 4-1) with two anticoccidial control measures (ionophoric chemotherapy or vaccination) was used to investigate broiler performance as measured by body weight and feed conversion. The dietary regimen consisted of four dietary phases: starter (1-14 d of age), grower (15-29 d of age), finisher (30-40 d of age), and withdrawal (41-50 d of age). Salinomycin inclusion rate was 60 g/ton for the starter and grower diets and was reduced to 50 g/ton in the finisher diet. The withdrawal diet did not include salinomycin. Chicks were placed in a total of 40 pens (10 replicated/treatment) with dimensions of 6 ft x 6 ft. With subtracting 1 square foot for feeder space in each pen, 35 square feet of rearing space per pen and a placement density of 0.8 sq. ft / bird required that 43 birds be placed per individual pen. The total number of birds placed was 1,720. Every attempt was made to allow pens to be representative of industry operations. Pens included one 30 lb tube

Table 4-1. Calculated nutrient concentrations of experimental diets fed to broilers given one of two coccidiosis control measure (salinomycin or Coccivac®-B).

	Starter[1]		Grower[2]		Finisher[3]		Withdrawal	
Diet	A	B[4]	A	B	A	B	A	B
Protein (%)	21.5	22.0	20.0	19.6	16.5	17.8	15.8	17.5
Methionine	0.58	0.59	0.55	0.56	0.44	0.50	0.42	0.49
TSAA	0.95	0.97	0.90	0.90	0.74	0.82	0.72	0.81
Lysine	1.22	1.27	1.14	1.12	0.90	1.00	0.85	0.95
Threonine	0.84	0.86	0.78	0.76	0.64	0.69	0.62	0.68
Arginine	1.44	1.47	1.32	1.29	1.05	1.15	1.01	1.13
Tryptophan	0.25	0.26	0.23	0.22	0.17	0.19	0.17	0.19
Calcium	1.00	1.00	0.91	0.91	0.81	0.81	0.72	0.80
Available Phos.	0.51	0.51	0.46	0.46	0.40	0.43	0.35	0.40
Sodium	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23

Table 4-1 Continued

	Starter[1]		Grower[2]		Finisher[3]		Withdrawal	
ME (kcal/kg)	3080	3080	3135	3135	3190	3190	3245	3245

[1] Starter diets contained 50g/ton of BMD. Diets that utilized salinomycin for anticoccidial prevention contain 60g/ton of salinomycin.
[2] Grower diets contained 25g/ton of BMD and 45g/ton of roxarzone. Diets that utilized salinomycin for anticoccidial prevention contain 60g/ton of salinomycin.
[3] Finisher diets contained 25g/ton of BMD and 34g/ton of roxarzone. Diets that utilized salinomycin for anticoccidial prevention contain 50g/ton of salinomycin.
[4] Contained 0.25 lb/ton of Ethoxyquin and Vit E-125.

feeder and nipple drinker system. Fresh pine shavings were used as the litter material for each pen. Broiler chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum*. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Texas A&M University laboratory animal care committee, and grow out facilities were thoroughly cleaned and disinfected before initiation of trial.

On day of placement, chicks in vaccinated groups were sprayed with the commercially available live oocyst coccidiosis vaccine, Coccivac®-B, using a Spraycox 2 machine at the hatchery. Chicks were allowed to preen for at least one hour prior to placement on litter in floor pens. On d 14, the litter in all pens was contaminated with field strain *Eimeria* using a garden sprayer. The inoculum with a target dose of 40,000 oocysts/chick consisting of *E. acervulina, E. maxima,* and *E. tenella* was prepared from oocysts previously isolated from commercial broiler houses in Texas. On d 21, 7 d post-spray application of oocysts, fresh fecal samples were obtained from a representative sample of pens to ensure oocyst uptake.

Body weights and feed conversion ratios were determined on days of dietary changes during grow-out. Therefore, all birds and feed were weighed on day of placement, and then on d 14, 29, 40, and 50 of grow-out to calculate performance parameters related to the experimental objective of the trial.

Statistical Analysis

Statistical analysis was determined using the general linear model of SPSS V. 11.0 (SYSTAT, 2001). Data were analyzed using 2 x 2 factorial analysis of variance

with differences of main effects deemed significant at $P \leq 0.05$. A significant interaction was present between diet and anticoccidial control method on d 14 body weights, thus this data was analyzed using a one-way analysis of variance. Means were separated using a Duncan's Multiple Range Test with a significance value of $P \leq 0.05$.

Results and Discussion

Body Weight

With respect to vaccination, body weights of broilers fed diet B were heavier ($P<0.05$) than broilers fed diet A on d 14 of age. Direct comparison was done on d 14 because of a significant interaction present between anticoccidial prevention method and dietary regimen. Broilers medicated with salinomycin fed both diet A and B were similar ($P>0.05$) to both vaccinated treatments on d 14 (Table 4-2). Through the remainder of the study, body weights were similar between both coccidiosis control methods, although body weights of vaccinated broilers were numerically higher compared to medicated broilers at the conclusion of the study. With respect to dietary regimen, broilers fed diet B were heavier ($P<0.05$) on d 40, but these differences did not persist until d 50 with both dietary regimens yielding similar body weights at the completion of grow-out. At the conclusion of the experiment on d 50, no significant effect ($P>0.05$) was observed with respect to anticoccidial control measure or dietary regimen.

Mortality Corrected Feed Conversion Ratio

Differences were observed in mortality corrected feed conversion ratio during the starter period with respect to both factors, dietary regimen and anticoccidial control

Table 4-2. Body weights (kg) of broilers fed two diets varying in protein level with two control methods for coccidiosis.

Body Weight (kg)			
Day 14			
Treatment		Diet	
	A	B	Mean
Salinomycin	0.50 ± 0.00[ab]	0.50 ± 0.00[ab]	0.50 ± 0.00
Coccivac®-B	0.49 ± 0.01[b]	0.51 ± 0.00[a]	0.50 ± 0.00
Mean	0.50 ± 0.00	0.50 ± 0.00	
Day 29			
Treatment		Diet	
	A	B	Mean
Salinomycin	1.68 ± 0.01	1.68 ± 0.01	1.68 ± 0.01
Coccivac®-B	1.65 ± 0.01	1.68 ± 0.01	1.67 ± 0.01
Mean	1.67 ± 0.01	1.68 ± 0.01	
Day 40			
Treatment		Diet	
	A	B	Mean
Salinomycin	2.58 ± 0.02	2.63 ± 0.02	2.61 ± 0.01
Coccivac®-B	2.60 ± 0.02	2.65 ± 0.02	2.62 ± 0.02
Mean	2.59 ± 0.01[b]	2.64 ± 0.01[a]	

Table 4-2 Continued

Body Weight (kg)

Day 50			
Treatment		Diet	
	A	B	Mean
Salinomycin	3.27 ± 0.01	3.30 ± 0.03	3.29 ± 0.02
Coccivac®-B	3.31 ± 0.03	3.36 ± 0.03	3.33 ± 0.02
Mean	3.29 ± 0.02	3.33 ± 0.02	

[a,b] Means with different superscripts differ at P<0.05.

measure (Tables 4-3 and 4-4). Medicated broilers had a lower ($P<0.05$) feed conversion during the starter period compared to vaccinated broilers, and broilers fed diet B had a lower ($P<0.05$) feed conversion compared to broilers fed diet A. During the grower period (d 14 to d 29), medicated broilers yielded a lower ($P<0.05$) feed conversion ratio compared to vaccinated broilers although no differences were observed with respect to diet. During the finisher period (d 29 to d 40), broilers fed diet B yielded a lower ($P<0.05$) feed conversion ratio compared to broilers fed diet A, while no differences were observed during the finisher period with respect to coccidial control measure. During the withdrawal period (d 40 to d 50), vaccinated broilers had a lower ($P<0.05$) feed conversion ratio compared to broilers that were fed the medicated diet that contained salinomycin. This increase in feed conversion is probably attributed to removal of salinomycin during the withdrawal phase. No dietary effects were observed for the withdrawal period.

When evaluating cumulative feed conversion during grow-out, vaccinated broilers yielded a higher ($P<0.05$) feed conversion ratio compared to medicated broilers at d 29, while no dietary effects were observed. On d 40, significant differences were observed with respect to both factors. Diet B fed broilers had a lower ($P<0.05$) feed conversion ratio compared to diet A, and medicated broilers had a lower ($P<0.05$) feed conversion compared to vaccinated broilers. At the conclusion of grow-out (d 50), there were no differences between coccidiosis control methods although diet B yielded a lower ($P<0.05$) feed conversion compared to diet A. The use of Coccivac®-B as a coccidiosis

Table 4-3. Cumulative mortality corrected feed conversion ratios of broilers fed two diets varying in protein level with two control methods for coccidiosis.

Mortality Corrected Feed Conversion

Day 14

Treatment	Diet		
	A	B	Mean
Salinomycin	1.21 ± 0.01	1.20 ± 0.01	1.21 ± 0.00[b]
Coccivac®-B	1.23 ± 0.00	1.21 ± 0.00	1.22 ± 0.00[a]
Mean	1.22 ± 0.01[a]	1.20 ± 0.00[b]	

Day 29

Treatment	Diet		
	A	B	Mean
Salinomycin	1.44 ± 0.01	1.46 ± 0.01	1.45 ± 0.00[b]
Coccivac®-B	1.48 ± 0.01	1.47 ± 0.01	1.47 ± 0.00[a]
Mean	1.46 ± 0.01	1.46 ± 0.00	

Day 40

Treatment	Diet		
	A	B	Mean
Salinomycin	1.66 ± 0.01	1.63 ± 0.01	1.65 ± 0.01[b]
Coccivac®-B	1.69 ± 0.01	1.66 ± 0.01	1.67 ± 0.00[a]
Mean	1.68 ± 0.01[a]	1.65 ± 0.00[b]	

Table 4-3 Continued

Mortality Corrected Feed Conversion

Day 50

Treatment	Diet		
	A	B	Mean
Salinomycin	1.90 ± 0.01	1.88 ± 0.01	1.89 ± 0.01
Coccivac®-B	1.91 ± 0.01	1.87 ± 0.01	1.89 ± 0.01
Mean	1.90 ± 0.01[a]	1.88 ± 0.01[b]	

[a,b] Means with different superscripts differ at $P<0.05$.

Table 4-4. Dietary period mortality corrected feed conversion ratios of broilers fed two diets varying in protein level with two control methods for coccidiosis.

Mortality Corrected Feed Conversion			
Starter			
Treatment	Diet		
	A	B	Mean
Salinomycin	1.21 ± 0.01	1.20 ± 0.01	1.21 ± 0.00[b]
Coccivac®-B	1.23 ± 0.00	1.21 ± 0.00	1.22 ± 0.00[a]
Mean	1.22 ± 0.01[a]	1.20 ± 0.00[b]	
Grower			
Treatment	Diet		
	A	B	Mean
Salinomycin	1.54 ± 0.01	1.56 ± 0.01	1.55 ± 0.01[b]
Coccivac®-B	1.58 ± 0.01	1.58 ± 0.01	1.58 ± 0.01[a]
Mean	1.56 ± 0.01	1.57 ± 0.01	
Finisher			
Treatment	Diet		
	A	B	Mean
Salinomycin	2.06 ± 0.02	1.94 ± 0.01	2.00 ± 0.02
Coccivac®-B	2.06 ± 0.01	1.97 ± 0.01	2.01 ± 0.01
Mean	2.06 ± 0.01[a]	1.96 ± 0.01[b]	

Table 4-4 Continued

Mortality Corrected Feed Conversion

Withdrawal			
Treatment	Diet		
	A	B	Mean
Salinomycin	2.86 ± 0.06	2.90 ± 0.06	2.88 ± 0.04[a]
Coccivac®-B	2.73 ± 0.04	2.70 ± 0.04	2.72 ± 0.03[b]
Mean	2.80 ± 0.04	2.80 ± 0.04	

[a,b] Means with different superscripts differ at P< 0.05.

control measure yielded broilers with equivalent performance parameters compared to salinomycin fed broilers regardless of dietary regimen.

The effectiveness of live oocyst vaccination in the generation of immunity and protection against subsequent *Eimeria* infection has been well documented (Danforth, 1998; Crouch et al., 2003; Williams, 2003). The reluctance of implementation of vaccination programs with the US broiler industry is due to reports of reduced performance associated with vaccination (Allen and Fetterer, 2002a). The results of the current experiment indicate that vaccination with a live non-attenuated oocyst vaccine for the control of coccidiosis does not necessarily result in broilers with reduced growth parameters compared to medicated broilers. These results are in agreement with Williams and Gobbi (2002) although these authors observed that vaccination significantly increased broiler body weights compared to a medication shuttle program. Body weights of vaccinated broilers were numerically higher in the present study although not significantly. Danforth (1998) also observed similar cumulative growth parameters in vaccinated roasters compared to roasters fed a medicated diet with a shuttle program consisting of nicarbazin and narasin, although vaccination reduced body weights until d 35 of age. Waldenstedt et al. (1999a) also observed significant reductions in body weight and increased feed conversion ratios of vaccinated broilers compared to medicated broilers at 36 d of age.

Other reports have indicated reduced body weight and increased feed conversion ratios of vaccinated chicks compared to medicated chicks during the starter period (Danforth, 1998; Williams, 2002). A post-vaccination check in body weight gain during

the starter period was not observed in this study with vaccinated broilers fed either dietary regimen having similar body weights at d 14 compared to medicated broilers, although an increase in feed conversion ratio was observed during the starter period in vaccinated chicks compared to salinomycin medicated chicks. This increase in feed conversion ratio due to vaccination was also observed during the grower period; however, the improved performance observed during the withdrawal period due to vaccination eliminated these differences by the conclusion of grow-out. Removal of salinomycin from the withdrawal diet of medicated broilers resulted in increased feed conversion ratios presumably from *Eimeria* exposure in combination with the lack of immunity in medicated broilers. Body weights of vaccinated broilers on d 14 indicate that reductions in early body weight can be eliminated with dietary modulation. In vaccinated broilers, increased protein level in diet B resulted in higher body weights compared to broilers fed diet A on d 14, although this difference did not persist through the remainder of the trial.

The lack of wide spread anticoccidial vaccination programs used within the US broiler industry stems mostly from fears of reduced performance which cause increased costs of production. Upon analysis of these two dietary regimens including the cost of the anticoccidial control measure, no differences were observed in the present experiment with respect to the cost of production per kilogram of live broiler weight when using either salinomycin or Coccivac®-B (Table 4-5). The increased protein level in diet B increased the cost of production compared to the lower dietary protein level included in the diet A regimen. The results of this experiment suggest that substitution

Table 4-5. Production cost including dietary and anticoccidial control cost per kilogram of live weight of broilers fed two diets varying in protein level with two control methods for coccidiosis.

Cost (Cents/kg of live broiler weight)			
Treatment	Diet		
	A	B	Mean
Salinomycin	29.31 ± 0.09	30.12 ± 0.14	29.72 ± 0.12
Coccivac®-B	29.35 ± 0.11	30.00 ± 0.11	29.67 ± 0.11
Mean	29.33 ± 0.07[b]	30.06 ± 0.09[b]	

[a,b] Means with different superscripts differ at $P < 0.05$.

of ionophoric chemotherapy for a vaccination program with a live oocyst coccidiosis vaccine does not reduce performance or increase production cost in broilers reared under simulated industry conditions in the presence of field strain *Eimeria*.

CHAPTER V

CONCLUSIONS

Live oocyst vaccination is currently the only available alternative to the use of anticoccidial drugs for the control of coccidiosis in the poultry industry. Its effectiveness when judged by final growth characteristics in comparison to medication has been heavily debated within the literature. The objective of this research was the evaluation of vaccination and the generation of immunity against subsequent field strain *Eimeria* challenge with a focus on dietary protein level affects on growth parameters of broilers. Vaccination significantly protected broilers against a mixed species challenge of *E. acervulina, E. maxima,* and *E. tenella*. Protection was evidenced by reduced lesion development six d post challenge, increased body weight gain, and reduced feed conversion ratios in vaccinated broilers during the challenge period when compared to non-vaccinated broilers reared under similar grow-out conditions. Decreased body weight gain in vaccinated broilers when compared to non-vaccinated broilers in low protein diets was observed, but increasing the protein level in the diet of vaccinated broilers eliminated this growth depression. The growth depression in vaccinated broilers appeared between d 13 and d 17 after placement. This time period coincides with the second cycling of vaccine strain oocysts within the rearing environment. The threshold established for protein inclusion in the starter diet is 22% before severe affects on growth characteristics are observed. A dietary protein level less than 22% in the starter diet is associated with decreased growth characteristics in vaccinated broilers as compared to non-vaccinated broilers.

During evaluation of a vaccination program in comparison to chemoprophylactic therapy using a common ionophore, salinomycin, during a complete grow-out of broilers, live oocyst vaccination yielded growth parameters equivalent to those of medicated broilers. Increasing dietary protein level to 22% allowed for significantly higher body weights at the conclusion of the starter period in vaccinated broilers compared to vaccinated broilers fed 21.5% dietary protein. However, this difference did not persist throughout the remainder of grow-out. The comprehensive results of these experiments indicate that live oocyst vaccination is a viable alternative to the use of medication for the control of coccidiosis during simulated commercial grow-out conditions. Since we did not observe alterations in growth characteristics or an increase the cost of production associated with vaccination, we feel coccidiosis vaccination should receive greater consideration for use in the US broiler industry. Importantly, when preparing to implement a vaccination program for the control of coccidiosis in commercial broiler production systems, dietary protein level during the starter phase must be closely monitored to ensure good performance.

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