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Coccidiosis Vaccination and Nutrient Utilization in Broiler Chickens

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Coccidiosis Vaccination and Nutrient Utilization in Broiler Chickens

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by

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ABSTRACT

Four experiments were conducted to evaluate the interrelationships among coccidiosis vaccination and nutrient utilization in floor-reared broiler chickens. Experiment 1 longitudinally compared the effects of coccidiosis vaccination and a chemical coccidiostat on broiler performance, nutrient digestibility, and intestinal morphology. Coccidiosis vaccination had no significant impact on morphology, overall body weight gain and feed intake of vaccinated birds, although vaccination impaired overall FCR. Vaccination elicited a transient reduction in digestibility of energy and nutrients, particularly for lipids, but vaccinated birds were able to recover from these reductions by 20 d. Experiment 2 assessed the impact of vaccination in digestibility of different feed ingredients and consisted of a basal diet and 3 test diets in which 30% of the basal diet was replaced with either corn, soybean meal, or distillers dried grains with solubles to allow for calculation of nutrient digestibility of individual ingredients by difference. Vaccination negatively impacted ether extract digestibility, particularly for corn. Nutrient digestibility was minimally impacted by vaccination in birds fed soybean meal, whereas ether exact digestibility was minimally impacted and nitrogen and amino acid digestibility were improved by vaccination in birds fed distillers dried grains with solubles. In experiment 3, a fat-free diet was fed to determine the impact of a coccidiosis vaccination model on ileal endogenous fatty acid flow and the values obtained were used to standardize the ether extract and fatty acid digestibility values of birds fed soybean oil or poultry fat diets. The vaccine challenge model negatively impacted digestibility of ether extract and most FA, regardless of the dietary lipid source. However, these results suggest endogenous fatty acid losses account for much of the reduction in lipid digestibility. In experiment 4, the influence of 3 starter diet energy concentrations achieved with varying soybean oil supplementation on nutrient digestibility, growth performance and processing characteristics were evaluated in coccidiosis vaccinated

broilers. Vaccination reduced nutrient digestibility in all diets but did not compromise overall body weight gain, feed intake, or most processing weights. However, vaccinated birds fed higher energy density diets through greater soybean oil supplementation during the starter period had impaired feed efficiency throughout the experiment.

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CHAPTER 1: INTRODUCTION

Coccidiosis, a parasitic disease caused by an intestinal infection from the protozoan parasites of the genus *Eimeria*, continues to be a widespread challenge to commercial poultry production. Previously, coccidiosis was primarily managed with in-feed administration of anticoccidial ionophores but recent consumer demand has shifted much of broiler production in the United States to antibiotic-free production systems in which in-feed ionophores are typically not permitted. Although many antibiotic-free or raised without antibiotics guidelines do permit the use of the "chemical" class of anticoccidials, there is a limited number of these compounds and their overuse can quickly lead to drug resistant *Eimeria* strains (Chapman, 2001). Therefore, live oocyst coccidiosis vaccination is a viable strategy for the control of coccidiosis.

The use of a live *Eimeria* vaccine can promote immunity and reduce the potential for clinical coccidiosis outbreaks to occur during the later growth periods (Chapman et al., 2002). However, mild infections that occur during the process of vaccinal oocyst cycling can lead to nutrient malabsorption and ultimately impair broiler performance. Although this is often referenced, relatively little information on nutrient utilization is available under conditions that mimic those experienced by commercially-vaccinated, floor-reared broiler flocks. Instead, most research to characterize the impact of coccidial challenges on nutrient utilization in broilers has involved a high number of oocysts delivered at a single inoculation time point, which is much more acute and severe compared with the mild infections that occurs within vaccinated commercial broiler flock (Persia et al., 2006; Parker et al., 2007; Adedokun et al., 2016). Additionally, most of these experiments have been conducted in battery cages, which prevents multiple infections during oocyst cycling that occurs under field conditions when birds are reared on litter.

Dietary adjustments, especially during times of intestinal disruption, may promote bird health and performance. As such, understanding the interactions among coccidiosis vaccination, growth performance, and nutrient utilization are of great interest and thus, may reveal strategies that optimize the diet and thus improve overall gastrointestinal health in broilers and broiler performance during periods of subclinical coccidiosis. Therefore, this dissertation will address the interrelationships of coccidiosis vaccination and nutrition in broiler chickens, with a specific focus on identifying the digestibility of individual feed ingredients and different lipid sources which may be altered during coccidiosis vaccination, as well as strategies to improve nutrient utilization and bird performance of floor-reared broilers when administered a live oocyst coccidiosis vaccine at day of hatch.

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CHAPTER 2: LITERATURE REVIEW

COCCIDIOSIS IN POULTRY

Coccidiosis is a parasitic disease caused by an intestinal infection from the protozoan parasites of the genus *Eimeria*. Seven pathogenic species of *Eimeria* commonly infect poultry, 6 of which were described by Tyzzer (1929) and include *E. acervulina, E. tenella, E. mitis, E. praecox, E. maxima, E. necatrix* and the discovery of *E. brunetti* by Levine (1942) added a seventh species. Out of these 7 species, *E. mitis and E. praecox* are not considered highly pathogenic for poultry. Eimeria species vary in infection location, as each species invades the intestine at a specific site. Differences among the species can be identified based on oocyst morphology, host and immune specificity, as well as the appearance and location of lesions within the gastrointestinal tract of the bird (Johnson and Reid, 1970).

Eimeria Life Cycle

The life cycles of *Eimeria* parasites are complex as they occur within and outside of the host and involve both asexual and sexual reproduction (Yun et al., 2000). Coccidiosis infection occurs when a bird ingests a sporulated oocyst. Oocyst sporulation occurs in the environment and requires heat, humidity, and oxygen, and variations in these conditions can influence the reproduction of *Eimeria* (Kheysin, 1972). These optimal oocyst sporulation conditions are common in poultry house facilities, as 95% of *E. acervulina* oocysts were sporulated within 5 days of being excreted into the litter and sporulated oocysts remained viable in the poultry house for 3 weeks (Williams, 1995). Therefore, a commercially reared broiler flock will be susceptible to a coccidiosis infection throughout the majority of their grow out period.

Each sporulated oocyst contains 4 sporocysts that each contain 2 infectious sporozoites (Fayer, 1980). Once the sporulated oocyst is ingested, the permeability of the oocyst's

environmentally resistant, double layered wall is ruptured by mechanical grinding in the gizzard, and 2 sporozoites are released from each sporocyst (Current et al., 1990). The release of sporozoites into the intestine is also facilitated by trypsin, which degrades the body of the oocyst, and bile salts which promote the activity and motility of sporozoites (Current et al., 1990). Once the sporozoites are released, they attach to and invade intestinal epithelial cells where they develop into trophozoites and undergo asexual reproduction (schizogony) to produce merozoites (Hammond, 1973; Fayer, 1980). Merozoites rupture the epithelial cell and proceed to invade and damage new epithelial cells, and the damage caused by merozoites leaving the enterocytes is believed to be greater than that of the initial invasion (Hammond, 1973; McDougald and Reid, 1991). After several generations of asexual division, merozoites undergo sexual reproduction (gameteogony) and develop into male and female gamonts, microgametes and macrogametes, respectively (Current et al., 1990). Microgametes fertilize the macrogametes to produce zygotes that form an environmentally resistant thick wall and develop into unsporulated oocysts. The unsporulated oocyst is then excreted by the bird into the environment where it will sporulate within 48 hours (McDougald and Reid, 1991). Most *Eimeria* species are shed approximately 4 to 5 days post infection, with *E. maxima* shedding occurs from 6 to 9 days post infection (Allen and Fetterer, 2002).

Oocysts can be categorized based on size; smaller oocysts include *E. acervulina*, *E. mivati,* and *E. mitis* and range from 15 to 18 µm in length × 13 to 14 µm in width, medium sized oocysts consist of *E. necatrix*, *E. tenella*, and *E. praecox* and range from 20 to 22 µm in length × 17 to 19 µm in width, and large oocysts represent *E. maxima* and are typically 30 µm in length × 20 µm in width (Reid and Long, 1979). However, with the exception of *E. maxima*, which is noticeably larger than the others, the precise determination of other Eimeria species cannot be

determined based on size alone. As stated previously, each individual *Eimeria* species infects a specific site within the avian intestinal tract, where *E. acervulina*, *E. praecox*, and *E. mivati* infect the duodenum and upper jejunum, *E. maxima* and *E. necatrix* infect the jejunum and ileum, *E. brunetti* infects the lower ileum and large intestine, and *E. tenella* infects the ceca (Witlock and Ruff, 1977). However, the severity of infection can vary on several factors such as the species of *Eimeria*, number of ingested oocysts, as well as the immune status of the bird (McDougald and Reid, 1991).

Morphological Changes in the Intestinal Epithelium During Coccidiosis

Extensive damage to the intestinal epithelial is common during a coccidiosis infection, as coccidia parasites infect, proliferate, and destroy the cells they invade. Birds subjected to a coccidiosis infection will have peak intestinal damage approximately 6 days after infection and improvements in villi morphology are rapidly noticeable following infection (Turk, 1974; Fernando and McCraw, 1973). Previous research has reported the deleterious effects of coccidiosis on intestinal morphology which include shortened and thickened villi and increased crypt depth within the infected region of the small intestine. This consequently decreases the absorptive surface area within the intestinal tract and thus negatively impacts the digestion and absorption of nutrients (McDougald and Reid, 1991; Forder et al., 2007). These morphological changes are often accompanied by rapid intestinal cell turnover to facilitate repair and maintain intestinal integrity but rapid turnover can also aid in pathogen removal from the host (Fernando and McCraw, 1973). Furthermore, increased cell turnover could further increase the energy requirement for intestinal tract maintenance, as enterocyte turnover is energetically demanding (Fernando and McCraw, 1973).

In addition to morphological damage caused by a coccidial infection, additional physiological changes within the intestine can also occur such as reduced digestive enzyme activity (Williams, 2005). Reductions in both pancreatic enzyme activity and enzymatic activity along the mucosal brush border have been reported during periods of rapid enterocyte turnover, as rapid turnover reduces the maturity of digestive enzymes (Eichholz and Crane, 1965; Sharma and Fernando, 1975; Adams et al., 1996a). Increased intestinal acidity can also impair digestive enzyme functions, as *Eimeria*-induced pH reductions can cause intestinal pH to fall below the optima efficiency for digestive enzyme activity (Williams, 2005). Increased intestinal acidity is presumably linked to the increased passage rate time, thereby a possible explanation is increased passage rate does not allow sufficient time for the digesta to be diluted by endogenous secretions to neutralize the acidity of the digesta. Consequently, the reduction in pH will likely impact nutrient digestion and solubility within the lumen.

In addition to being the main site of nutrient digestion and absorption, the intestinal tract also functions as a physical barrier to protect the host against foreign pathogens. The intestinal epithelial barrier is comprised of absorptive epithelial cells that are bound together by tight junction proteins and serve as the first line of defense against the luminal environment. Tight junction proteins regulate the permeability of intestinal barrier by preventing the diffusion of luminal antigens, microorganisms, and their toxins across the epithelium to sub-epithelial tissues (Groschwitz and Hogan, 2009). Coccidia parasites can damage the intestinal epithelial barrier function and consequently increase intestinal permeability (gut leakage). Poor barrier function during coccidiosis leads to an inflammatory response, increased plasma protein leakage into the intestinal lumen, and nutrient malabsorption (Joyner et al., 1975). Increased mucin production has also been reported during a coccidiosis infection, presumably to serve as a protective

mechanism to prevent pathogen adhesion on epithelial surfaces and protect against further infection (Collier et al., 2008; Horn et al., 2009). Consequently, the leakage of plasma proteins and increased mucogenesis can promote the proliferation of intestinal *Clostridium perfringens*, the causative bacterial agent of necrotic enteritis (Williams, 2005). Clostridia utilize plasma proteins, which are a nutrient rich substrate and may adhere to the carbohydrate chains attached to mucin, which can result in bacterial colonization through the intestinal tract (McDougald, 2003; Montagne et al., 2004). Coccidiosis can decrease nutrient digestibility, as well as increase plasma protein leakage and mucin production, all which serve as beneficial nutrients for bacteria and thus promote bacteria proliferation. As such, coccidiosis can predispose birds to other enteric diseases, such as necrotic enteritis (Chapman et al., 2002; Cooper and Songer, 2009).

COCCIDIOSIS CONTROL

Coccidiosis in commercial poultry has typically been controlled with the use of in-feed anticoccidial medication. Commercially-available anticoccidial drugs, each with varied modes of action, can typically be divided into ionophores, which are produced by fermentation and disrupt *Eimeria* membrane function via osmotic balance interference, and chemicals, which are produced by chemical synthesis and impact mitochondrial function and co-factor synthesis (Chapman, 1999). Ionophores have continued to be effective due to "leakage", which allows some parasites to bypass the drug and thus initiate a low-grade cycling of coccidia that allows the bird to develop natural immunity (Chapman, 1997; Hafez, 2008). While ionophores have helped alleviate coccidia resistance, the use of ionophores are often not permitted for poultry raised in antibiotic-free production systems since they exert an antibiotic effect on coccidia parasites. The use of chemical anticoccidials are permitted in antibiotic-free production systems but they prevent oocyst cycling which minimizes the development of the bird's immunity. The beneficial

effects of chemical anticoccidials (sulfaquinonxaline) to control coccidiosis was first reported by Grumbles et al. (1948) but less than seven years later, evidence of resistance was reported by Cuckler et al. (1955). As such, coccidia parasites readily develop resistance to chemical anticoccidials which severely limits their effectiveness (Chapman, 1993). Therefore, this has prompted the use of anti-coccidial vaccines for the control of coccidiosis.

Live oocyst vaccines are a commercially available option for the control of coccidiosis in the poultry industry. A major difference among commercially available live oocyst vaccines is whether the *Eimeria* oocysts used in the vaccine are virulent (nonattenuated) or attenuated. Non-attenuated vaccines are comprised of *Eimeria* oocysts derived from laboratory or field strains and have not been modified to change their natural virulence, whereas attenuated vaccines utilize oocysts that have an artificially reduced virulence (Dalloul and Lillehoj, 2005). Coccidia is typically attenuated either by the passage of parasites through embryonated eggs or by selection for precocity. However, only a few *Eimeria* species are able to pass through the eggs which can cause a loss of immunogenicity of the line and therefore may not be a stable method for vaccine attentuation (Shirley, 1993). Precocity is the preferred method, as these strains have a shortened prepatent time and the number of oocysts produced during infection is reduced (William, 1998). Drawbacks associated with the use of a non-attenuated vaccine include the possibility of the vaccine introducing new *Eimeria* species into the flock and only small oocyst numbers can be administered to the young bird since the natural virulence has not been modified (Dalloul and Lillehoj, 2005). The use of an attenuated vaccine may reduce potential difficulties associated with pathogenic field strains, but due to a lower oocyst yield, attenuated vaccines may not provide successful immunity and can also have a higher cost of production (Williams, 2002; Dalloul and Lillehoj, 2005).

Commercially available coccidiosis vaccines contain live mixtures of *Eimeria* species, typically *E. acervulina*, *E. maxima*, *E. mivati*, and *E. tenella,* and are designed to be sprayed on and ingested by the bird at day of hatch (Tellez et al., 2014). Since immunity to *Eimeria* is species specific and there is little to no cross-protection against multiple species, it's important to vaccinate birds against all *Eimeria* species that a commercial flock may encounter. Successful coccidiosis vaccination requires oocyst cycling, which permits the bird to be exposed to multiple reinfections from the shedding and ingestion of oocysts in the litter and allows the bird to develop immunity to prevent more severe infections that can occur at a later time throughout the grow out period (Williams, 2002). The traditional method of vaccine application is by a spray-cabinet, where the vaccine is sprayed on the bird as an aqueous solution (Chapman et al., 2002). However, poor vaccine distribution is a concern with the use of a spray-cabinet and other alternative means such as gel vaccine application have been implemented, as gel beads are more viscous and may increase vaccine ingestion (Albanese et al., 2018). Vaccine application typically occurs either in the hatchery or at the farm prior to placement. As birds preen they ingest the oocysts which produce a sub-clinical infection within the intestinal tract. Therefore, uniform distribution of vaccine administration is critical for flock immunity, as a non-uniform distribution of the vaccine would result in birds being infected and re-infected at different time points, which is not desirable.

Coccidiosis Vaccination and Broiler Performance

The effectiveness of coccidiosis vaccination at generating immunity has been widely reported (Brake et al., 1997; Williams, 2003; Shirley et al., 2005; Lee et al., 2011) as the generation of immunity though coccidiosis vaccination improved BW gain, reduced FCR, and reduced intestinal lesion scores of broilers subjected to an *Eimeria* challenge compared with non-

vaccinated challenged broilers. The utilization of vaccines in commercial broiler flocks has caused concerns in the industry due to frequent reports of reductions in broiler growth and feed efficiency that are associated with the sub-clinical, vaccine-induced infection (Danforth, 1998; Mathis, 1999; Lehman et al., 2009). Parker et al. (2007) concluded broilers vaccinated at day of hatch had reduced feed intake and body weight gain at d 17 when compared with those broilers given an in-feed ionophore. During a 36 d grow out period, vaccinated broilers had a 4.6% reduction in body weight and an increased feed conversion ratio when compared with anti-coccidial medicated broilers (Waldenstedt et al., 1999). This was further supported by Lehman et al. (2009) who reported coccidiosis vaccinated broilers had reduced body weight gain and increased feed conversion when compared with birds given an in-feed coccidiostat through 8 weeks of age. This also resulted in vaccinated broilers having reduced carcass weights at time of processing (Lehman et al., 2009). On the other hand, Mathis (1999) observed a reduction in body weight gain and poor feed conversion in vaccinated broilers during the first 3 weeks, followed by a compensatory weight gain that diminished any losses by 35-42 d of age, after protective immunity has been established.

Immune Response of Broilers During Coccidiosis Exposure

The avian immune system is comprised of innate and adaptive immune responses (Dalloul and Lillehoj, 2005). Following a coccidia exposure, innate immunity is associated with the early phase of the initial infection, whereas adaptive immunity follows a secondary infection, as it takes longer to initiate due to a specific response to the invading pathogen (Lillehoj et al., 2007). Since *Eimeria* parasites invade the intestine, immune responses are primarily coordinated by the gut-associated lymphoid tissue (**GALT**), which is comprised of the mucosal layer, bursa of Fabricius, and aggregated lymphoid tissue in the Meckel's diverticulum, Peyer's patches, and

cecal tonsils (Yun et al., 2000). The GALT serves as the host's defense against a pathogenic infection by processing and presenting antigens, producing antibodies via the humoral immune system, and cells within the GALT, such as lymphoid and antigen presenting cells found through the intestinal mucosal layer, can activate cell mediated immunity (Brandtzaeg et al., 1987). After infection, a pathogen that has breached the mucosal barrier initiates innate immune cells to induce an inflammatory cytokine response to signal and activate additional immune cells to the infection site. These innate immune cells, mainly macrophages and dendritic cells, function as antigen presenting cells to the adaptive immune system. Therefore, recognition of pathogens by the innate immune system is critical for activation of the adaptive immune system.

The adaptive immune system is comprised of humoral and cell-mediated immunity, which are mediated by B and T cells, respectively. In chickens, B cells develop in the bursa of Fabricius and express immunoglobulins on their surface, whereas T cells primarily develop in the thymus and express the T cell receptor complex on their surface. The binding of an antigen to B cells stimulates the production of immunoglobulins (**Ig**), also known as antibodies (Yun et al., 2000). IgA is the primary antibody in mucosal secretions and parasite specific IgA antibodies have been detected in circulation following coccidiosis infection (Lillehoj and Trout, 1996; Yun et al., 2000). However, circulating antibodies have minimal ability to limit a coccidia infection and therefore cell-mediated immune responses are the most effective during a coccidia infection (Lillehoj and Trout, 1996; Allen and Fetterer, 2002).

Cell mediated immunity is characterized by T lymphocytes, and according to their specific function and cluster of differentiation (**CD**) surface markers, T cells can be further classified as CD4+ or CD8+ cells. CD4+ cells, also known as helper T cells, recognize specific antigens and therefore induce immune responses, while CD8+ cells have a cytotoxic role within

the immune system (Lillehoj and Trout, 1994; Viertlboeck and Göbel, 2008). The activation of these cells is based on the major histocompatibility complex (**MHC**), which is comprised of two types of molecules, class I or II. The MHC molecules are located on cell surfaces and allow specific T cell receptors to recognize antigenic peptides. The CD8+ T cells recognize foreign antigens presented by MHC class I molecules and enable CD8+ T cells to kill cells that produce pathogen derived proteins, whereas CD4+ helper T cells recognize antigens presented with MHC class II molecules (McDonald, 1999). Helper T cells (**T_H**) are differentiated based off the cytokines they secrete and control parasitic replication during the primary infection (Lillehoj and Trout, 1996). Once activated, T_H cells divide into Type 1 (**T_H1**) and Type 2 (**T_H2**) helper T cells. During an inflammatory response, macrophages and T_H1 cells produce pro-inflammatory cytokines, such as interleukin-1 and interleukin-6 (Murray and Wynn, 2011). Additionally, Interferon-γ (**IFN-γ**) is a pro-inflammatory cytokine, typically produced by T_H1, that signals macrophages to synthesize nitric oxide during an immune response (Liew and Cox, 1991). Nitric oxide production from arginine is catalyzed via an enzyme known as inducible nitric oxide synthase and is a highly active free radical molecule that reacts with superoxide anions to produce byproducts that are toxic to pathogens (Moncada et al., 1991; Ovington and Smith, 1992). On the other hand, T_H2 cells produce anti-inflammatory cytokines such as interleukin-10 to suppress the pro-inflammatory response (Murray and Wynn, 2011). The balance between T_H1 and T_H2 is important for a successful immunity and therefore, when one type of T_H response is activated, the other is inhibited.

Alterations in digestion, absorption, and intestinal barrier function are common attributes of intestinal epithelial damage. Highly prolific coccidia parasites have been known to disturb cellular integrity and cause an inflammatory immune response within the intestinal tract (Lillehoj

and Trout, 1996). As previously discussed, an inflammatory response will signal additional immune cells to the site of infection and the inflammatory cells will infiltrate the intestinal mucosa, causing intestinal wall thickening and thus disrupting the ability of the gastrointestinal tract to properly absorb nutrients (Lourenssen et al., 2005; Cobaxin-Cárdenas, 2018). This has been supported by an observed thickness in the lamina propria during a coccidiosis infection (Klassing et al., 2002). Furthermore, production of pro-inflammatory cytokines, such as interleukin-1 can decrease appetite (Klasing and Barnes, 1988; Cartmell et al., 1999). While oocyst and pathogen ingestion would be suppressed as a result of a reduction in feed intake, it would inevitably impair broiler performance as the reduction in feed intake would result in birds consuming less nutrients that are needed for intestinal repair, function of the immune system, and growth. Therefore, an immune response can elicit a multitude of metabolic changes that occur within the bird, resulting in a disruption in nutrient utilization and impair broiler performance.

COCCIDIOSIS AND NUTRIENT UTILIZATION

Reduced nutrient digestion and absorption caused by reduced absorptive capacity of the intestinal tract and intestinal inflammation as previously described are commonly associated with coccidiosis and can negatively impact broiler performance (Preston-Mafham and Sykes, 1970; Major and Ruff, 1978; Ruff and Wilkins, 1980; Adams et al., 1996a; Metzler-Zebeli et al., 2009). Malabsorption of nutrients due to coccidiosis has been reported for glucose, proteins, amino acids, lipids, metabolizable energy, minerals, and carotenoids (Preston-Mafham and Sykes, 1970; Turk, 1973; Sharma and Fernando, 1975; Tyczkowski et al., 1991; Persia et al., 2006). Amerah and Ravindran (2015) challenged birds with *E. acervulina*, *E. maxima*, and *E. tenella at 14 d post-hatch* and observed an overall 16% reduction in mean amino acid apparent ileal digestibility at d 21. Rochell et al. (2016) observed a linear reduction in the apparent ileal

digestibility of all measured amino acids, excluding tryptophan and glycine, when birds were administered increasing amounts of *E. acervulina.* Coccidiosis-induced reductions for glycine, threonine, and cysteine have been commonly reported (Parker et al., 2007; Adedokun et al., 2012) Threonine, as well as cysteine, serine, and proline, are major structural components of intestinal mucin and are involved in intestinal integrity (Adedokun et al., 2012). Increased mucogenesis and enterocyte turnover (Fernando and McCraw, 1973), as well as the activation and heighted immune response during infection, will likely influence the amino acid needs of broilers during coccidiosis and amino acids involved in these processes may exhibit the greatest losses and become limited (Parker et al., 2007).

The increased mucogenesis, rapid turnover of and sloughing of intestinal cells, as well as plasma protein leakage into the lumen caused by coccidiosis will likely increase endogenous amino acid losses (Fernando and McCraw, 1973; Collier et al., 2008; Amerah and Ravindran, 2015). Adedokun et al. (2016) challenged birds with a 12× dose of a coccidiosis vaccine at 14 or 35 d of age, evaluated ileal endogenous amino acid losses one week later and reported endogenous losses were higher in the older birds rather than the younger birds. The vaccine challenge was likely more destructive to the mucosal layer in younger birds which may have impaired endogenous secretions and thus reduced the quantity of ileal endogenous amino acid flow in the intestinal tract. Older birds have a more developed intestinal tract and thus increased mucin production and endogenous secretions in response to an *Eimeria* infection. As such, broiler performance and apparent and standardized ileal digestibility were more impacted in the younger birds. This is likely attributed to younger birds being more susceptible to infection and intestinal inflammation may have reduced mucus secretion and therefore less mucus lining the intestinal wall. Therefore, the impact of coccidiosis infection on amino acid digestibility and

endogenous losses can vary depending on the age of the bird as well as the severity and time of infection (Persia et al., 2006).

Caloric costs of a coccidiosis infection have been reported whereby older broilers (d 35 and d 42) subjected to a severe *Eimeria* infection had a greater energy expenditure than younger broilers. Carbohydrates and lipids are the main dietary energy sources and digestibility of these nutrients has been shown to be negatively impacted during coccidia-exposure. Reductions in ileal digestible energy (**IDE**) were recently demonstrated by Amerah and Ravindran (2015), who reported broilers subjected to a coccidiosis challenge on d 14 had a 1,069 kcal/kg reduction in IDE compared with non-challenged broilers d 21. Reductions in energy are likely attributed to reductions in starch and lipid digestibility, where Amerah and Ravindran (2015) reported cocci-infected broilers had a 18.8% reduction in starch and 96% reduction in lipid digestibility at d 21, when compared with ileal digestibility of non-challenged birds. Adams et al. (1996b) also published lipid digestibility in the excreta was drastically reduced from 86 to 22% when birds were infected with *E. acervulina*. However, apparent digestibility values are not corrected for endogenous lipids present in excreta or ileal digesta and they may underestimate the true digestibility values for birds infected with coccidiosis. Therefore, since significant losses of nutrients, including lipids, will occur throughout the normal digestive process it is important to measure and correct for these losses. To our knowledge, quantification of endogenous fat losses in poultry during coccidia-exposure has not yet been achieved and remains to be determined.

Lipid digestion and absorption are relatively complex processes and reductions in lipid digestibility during coccidia exposure are not well understood. Lipid digestion relies heavily on bile salts because they attach to lipid droplets and increase their surface area for access by enzymatic lipase and therefore are essential for lipid emulsification. Adams et al. (1996b)

infected birds with a coccidiosis challenge at d 18 and suggested bile salt secretion may be reduced during coccidiosis based on their findings that birds receiving supplemental cholic acid had a 43% lipid digestibility at d 21, compared with a 22% lipid digestibility for challenged birds that did not receive supplemental cholic acid. However, cholic acid supplementation did not improve lipid digestion in challenged birds at d 16. Furthermore, Adams et al. (1996b) determined lipid source can influence digestion during a coccidiosis infection, where the replacement of animal fat with coconut oil led to an overall improvement in lipid digestion, as well as improved broiler growth and feed efficiency. However, the replacement of animal fat with soybean oil did not significantly improve lipid digestion and only numerical improvements were observed (Adams et al., 1996b). Coconut oil is a medium chain saturated fatty acid that contains high concentrations of lauric (12:0) and myristic (14:0) acid, soybean oil is an unsaturated fatty acid that contains a high proportion of both linoleic (18:2) and oleic (18:1) acids, and animal fat has a higher proportion of saturated fatty acid relative to soybean meal, as it is comprised mainly of oleic and palmitic (16:0) acids. Due to their smaller molecular size, medium-chain triacylglycerols can enter enterocytes without undergoing hydrolysis and their shorter chain length may allow for a more efficient absorption when the mucosal surface is damaged (Babayan, 1987). Additionally, Sharma and Fernando (1975) infected birds with *E. acervulina* at d 16 and using both a light and electron microscope observed an accumulation of fat globules in duodenal villus epithelial cells 4 and 5 d post-infection. Therefore, they concluded that intestinal villus cells parasitized by the gammonts of *E. acervulina* presumably prevent lipids that were absorbed by enterocytes from being readily incorporated into portomicrons to be secreted into the portal circulation and utilized by the bird.

Marked reductions in lipid digestibility can also influence the absorption of plasma carotenoids, as they are fat-soluble pigments that are absorbed in conjunction with fatty acids as components of mixed micelles. Carotenoids are solely derived from the diet and can be additionally supplemented, as carotenoids add pigmentation to poultry skin and eggs yolks, which is very important in consumer markets in Mexico and China (Liu et al., 2008). During an acute coccidia exposure, reductions in plasma carotenoid concentrations are likely attributed to nutrient malabsorption, as well as impaired micelle transport. Therefore, carotenoid concentrations may serve as an indirect marker of infection severity and sensitive indicator of intestinal damage, where magnitude of reduction will increase relative to the severity of infection (Ruff et al., 1974; Allen et al., 2004). Reductions in lipid digestibility can also impair the absorption of fat soluble vitamins, including vitamin D, which will in turn reduce the absorption of calcium and phosphorus. Therefore, impaired lipid digestibility is not only damaging to itself but can also reduce the absorption of fat soluble compounds.

The experiments described above have evaluated the impact of *Eimeria* infection on amino acid digestibility and energy utilization of complete diets. To formulate broiler diets on a digestible basis, the digestible coefficients are needed for each individual feed ingredient used in the diet. However, it is likely that the impact of mild coccidiosis on nutrient digestibility differs among common feed ingredients and the influence of individual ingredients on digestibility needs to be evaluated. Therefore, it is important to understand how amino acid availability and energy utilization of various ingredients may be impacted by coccidiosis vaccination.

Nutrient Digestibility Methodology

Two commonly used bioassays to evaluate nutrient digestibility of individual feed ingredients include semi-purified diets and practical diet replacement assay. Due to the drawbacks

associated with the bioassays available, it is not easy to quantify the digestibility of individual feed ingredients. A semi-purified diet is more commonly used in amino acid utilization studies, as it allows the test ingredient to serve as the sole source of amino acids (Ravindran et al., 2017). However, potential drawbacks are often associated with the use of semi-purified diets. Reductions in feed intake and enzyme secretions, such as amylase, lipase, and pancreatic secretions, have been reported in broilers fed semi-purified diets (Partridge et al., 1982; Shastak et al., 2014). Semi-purified diets can also increase digesta passage rate, since dextrose is rapidly digested and absorbed (Colnago et al., 1984). Furthermore, feeding a semi-purified diet has been shown to ameliorate an *E. tenella* infection which may be attributed to the rapid passage rate, thus allowing less time for oocysts excystation and consequently resulting in a lighter infection (Colnago et al., 1984). As such, these factors could influence the desired development of coccidiasis induced by the live oocysts vaccine and should be considered when determining the digestibility of nutrients for floor-reared broilers vaccinated for the control of coccidiosis. Alternatively, the practical diet replacement assay allows the digestibility of the test ingredient at interest to be determined by the difference method. The difference method is based on a practical diet replacement with the test ingredient of interest and digestibility values for individual test ingredients are calculated according their proportional contribution to the test diet (Kong and Adeola, 2013; Zhang and Adeola, 2017). This approach is often used for determination of ME_n of individual feed ingredients (Stefanello et al., 2016), but has also been used for the determination of amino acid digestibility (Fan and Sauer, 1995).

DIETARY MODIFICATIONS FOR IMPROVED PERFORMANCE DURING COCCIDIOSIS EXPOSURE

Reduced feed intake and metabolic changes that occur during an intestinal disease can influence the birds' nutritional requirements (Choct, 2009). Dietary adjustments, especially

during times of intestinal disruption, may support and improve the performance of broilers. During periods of reduced amino acid digestibility, broiler diets that contain an increase in the total amino acid supply can lead to an overall improvement in broilers subjected to a coccidiosis infection. Research conducted by Lee et al. (2011) reported coccidiosis vaccinated broilers fed diets that contained protein levels of 23 and 24% had improved BW gain compared with vaccinated broilers fed diets that contained 20 and 21% crude protein levels, during a 21 d period. Increasing the dietary supplemental amino acid concentrations may support the performance of broilers during an infection period, as supplemental amino acids are highly digestible which may be beneficial during times of intestinal damage. Lehman et al. (2009) reported coccidiosis vaccinated broilers had an improvement in bodyweight gain and feed efficiency when supplemented with a gelatin comprised of glycine, serine, and proline during the first 3 weeks of age. This was further evaluated by Adedokun et al. (2016), where reductions in amino acid digestibility in broilers subjected to a 12× coccidiosis vaccine challenge were quantified and then diets were formulated using crystalline amino acids to potentially account for the observed reductions in nutrient digestibility. Adedokun et al. (2016) reported an improvement in feed efficiency in vaccinated broilers fed supplemental lysine, DL-methionine, L-threonine, isoleucine, L-tryptophan, and valine. The high digestibility of supplemental amino acids, in addition to the increased inclusion levels, may benefit broilers when the absorptive capacity of the intestine is impaired and nutrient digestibility is compromised due to a disruption in intestinal integrity.

While dietary modulations have improved protein and amino acid utilization, improvements in lipid utilization during coccidiosis are scarce. Coccidiosis is energetically costly to the bird, as coccidiosis can initiate an immune response, increase the maintenance

energy costs and decrease retained feed energy (Teeter et al., 2008). Therefore, coccidia-exposed birds may benefit from diets that are formulated with an increased energy density but in order to make informed dietary changes reductions in digestibility first need to be characterized. While the research summarized above has provided information on the impact of coccidiosis challenges on nutrient utilization, very few experiments have been validated in conditions that reflect broiler flocks commercially-vaccinated for the control of coccidiosis. Understanding nutrient requirements during periods of intestinal damage and formulating diets to account for the malabsorption of nutrients are imperative to provide nutritional strategies that support broiler performance during the critical stages of vaccination.

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CHAPTER 3: LONGITUDINAL CHARACTERIZATION OF COCCIDIOSIS CONTROL METHODS ON LIVE PERFORMANCE AND NUTRIENT UTILIZATION IN BROILERS

ABSTRACT

An experiment was conducted to quantify the timing and magnitude of potential losses in growth performance, apparent ileal digestibility (**AID**) of nutrients and energy (**IDE**), and intestinal morphology for broilers vaccinated for the control of coccidiosis compared with those provided a chemical coccidiostat. Treatment groups consisted of 3 coccidiosis control methods: unvaccinated, unmedicated (**NC**), in-feed chemical coccidiostat (**PC**), and live oocyst vaccination (**VAC**) at day of hatch and were administered to male Cobb broilers reared in floor pens. Body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined at 12, 16, 20, 28, and 36 d. Blood and ileal digesta were collected from birds in 10 replicate pens of each treatment at 12, 16, 20, and 36 d to evaluate plasma carotenoid and nitric oxide concentrations and determine nutrient AID and IDE. Jejunal samples were taken at 12, 20, and 36 d for morphological measurements. Oocyst shedding in VAC birds was confirmed by increased oocyst counts and decreased carotenoid concentrations ($P < 0.05$) when compared with PC birds, with no differences ($P > 0.05$) in nitric oxide concentrations. At 20 d, BWG and FI were lowest ($P < 0.05$) in VAC birds, intermediate in NC birds, and highest in PC birds, with no differences in FCR ($P > 0.05$). By 28 and 36 d, FCR was higher ($P < 0.05$) for VAC and NC birds but BWG and FI of VAC birds were similar ($P > 0.05$) to PC birds. At d 12, IDE and AID of nitrogen and ether extract were lower ($P < 0.05$) in VAC birds than PC birds. At d 16, AID of nitrogen was similar ($P > 0.05$) between PC and VAC birds, whereas AID of ether extract remained lower in VAC birds than PC birds. No differences in AID of nutrients or IDE were observed ($P > 0.05$) between VAC and PC birds at 20 or 36 d. No differences ($P > 0.05$) in

jejunal morphology were observed at any time point. Overall, VAC elicited a transient reduction in AID and IDE, particularly for lipids, that diminished by d 20.

INTRODUCTION

Coccidiosis, an intestinal parasitic disease caused by protozoa of the genus *Eimeria*, remains one of the most prevalent diseases in commercial poultry production. Traditionally, coccidiosis has primarily been managed with in-feed administration of anticoccidial ionophores, but in the United States, the use of ionophores is typically not allowed under most antibiotic-free poultry production systems. Although these guidelines do currently permit the use of chemical anticoccidial drugs, there is a limited number of these compounds and their overuse can quickly lead to emergence of drug resistant *Eimeria* strains (Chapman, 2001; Kitandu and Juranova, 2006). Therefore, this resistance has increased reliance on live oocyst coccidiosis vaccination, which can induce immunity and reintroduce drug-sensitive strains into the rearing facility, as an important control strategy for coccidiosis (Chapman et al., 2002).

Coccidiosis vaccination involves exposure of young chicks, typically at hatch, to small numbers of live *Eimeria* oocysts to promote immunity and reduce the potential for clinical coccidiosis outbreaks during the later growth periods (Chapman et al., 2002). However, mild infections that occur during the process of vaccinal oocyst cycling, which involves the initial infection, oocyst shedding and sporulation, and reinfection, may compromise broiler growth through reduced feed intake or feed efficiency, and the relatively short lifespan of broilers may be insufficient for compensatory gain (Williams, 1998; Lehman et. al, 2009). Impaired feed efficiency during vaccine cycling is presumably due in part to nutrient malabsorption associated with intestinal damage and inflammation associated with the sub-clinical, vaccine-induced infection. (Lehman et al., 2009; Lee et al., 2011; Adedokun et al., 2016).

Reductions in nutrient and energy digestibility have been reported in cocci-exposed birds, and responses are dependent on diet composition and the type and number of *Eimeria* species administered in the challenge model (Persia et al., 2006; Parker et al., 2007; Amerah and Ravindran, 2015; Rochell et al., 2016; Adedokun and Adeola, 2017). Increasing the dietary concentration of digestible nutrients for which digestibility is impaired is a potential strategy to support the performance of broilers during coccidial vaccine cycling. Indeed, Adedokun et al. (2016) fed increased concentrations of supplemental amino acids to broilers to account for a predicted reduction in amino acid digestibility based on a previous coccidial challenge trial and observed improved feed efficiency of broilers compared those fed a control diet. However, commercial adoption of this approach for floor-reared, vaccinated broilers requires longitudinal characterization of nutrient digestibility to identify appropriate dietary adjustments that may benefit broilers during the critical stages of vaccination.

Most research to characterize the impact of coccidial challenges on nutrient utilization in broilers has been conducted in battery cages equipped with wire flooring, which prevents multiple infections during oocyst cycling (Persia et al., 2006; Parker et al., 2007; Adedokun et al., 2016; Rochell et al., 2016). Additionally, most of these experiments have involved acute challenges that are much more severe and occur later compared with the mild infections that occur within the first 3 weeks of a vaccinated commercial broiler flock. Therefore, the objective of this study was to characterize the timing and magnitude of reductions in growth performance and nutrient utilization in floor-reared broilers throughout different stages of oocyst cycling following live *Eimeria* vaccination of broilers at day of hatch.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

General Bird Husbandry and Diets

One thousand, five-hundred male broiler chicks from a Cobb 500 female line were obtained from a commercial hatchery on day of hatch. All chicks were group-weighed and distributed to 120 floor pens on clean litter with fresh pine shavings. Each floor pen was equipped with a hanging feeder and a nipple drinker line. To ensure sufficient digesta content, 14 birds were placed per pen (0.07 m^2 per bird) in those 30 pens pre-selected for the first collection time point, at 12 d post-hatch, whereas the other 90 pens were placed with 12 birds (0.09 m^2 per bird). Birds were provided access to feed and water *ad libitum* throughout the experiment. The lighting schedule and temperature targets were adjusted according to management guidelines published by the primary breeder (Cobb-Vantress, 2015). Birds were reared up to 36 d post-hatch and fed starter (0 to 14 d), grower (15 to 28 d), and finisher (29 to 36 d) diets based on corn and soybean meal and formulated to meet or exceed published nutrient recommendations (Cobb-Vantress, 2015).

Experimental Treatments

Upon arrival, one-third (500) of the chicks were orally-gavaged with the manufacturer's recommended dose of a live oocyst vaccine (Coccivac®-B52; Merck Animal Health, Intervet Inc. Millsboro, DE, USA). An oral gavage (0.25 mL/bird) was used to provide uniform administration. Throughout the trial, litter was sprayed once daily with water to ensure sufficient moisture content for oocyst sporulation. Unvaccinated broilers were fed diets formulated with or without an in-feed chemical anticoccidial drug, resulting in a total of 3 treatments: 1)

unmedicated and unvaccinated (**NC**), 2) in-feed chemical coccidiostat (Clinacox, Huvepharma) administration (**PC**), and 3) live oocyst vaccination (**VAC**) at day of hatch (Table 3.1). Each treatment group was represented by 10 replicate pens for each collection time point.

Measurement of Live Performance and Vaccine Cycling

Birds and feeders were weighed at 0, 12, 16, 20, 28, and 36 d post-hatch for calculation of body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**). All dead and culled birds were weighed individually and FCR calculations were adjusted to include the weight gain of dead birds. To assess vaccine cycling, the number of oocysts per gram (**OPG**) of litter samples collected from each pen was determined before bird placement and at 12, 16, 20, 28, and 36 d post-vaccination. Samples were taken from several locations within each pen and pooled into airtight plastic bags and kept refrigerated until further analysis. All sample counts were conducted within 1 week of their collection time. Samples were soaked in water overnight and the solution was homogenized by vigorous stirring. Following homogenization, 1 ml of sample was further diluted with 9 ml of saturated salt solution and pipetted into the chamber of a McMaster counting slide. Duplicate counts were made for each sample and subsequent calculations based on the following equation:

Oocysts per gram of sample = (Oocyst count × dilution × volume) / (volume of counting chamber × weight of sample), where the dilution was 10 and the volume of the counting chamber was 0.15 ml.

Digesta, Blood, and Tissue Sampling

All birds from 10 replicate pens of each treatment were humanely euthanized at 12, 16, 20, and 36 d post-hatch by CO_2 inhalation for collection of ileal digesta. Ileal contents from all birds

in each pen were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples were pooled within pen and frozen (−20°C) until analysis. At 12, 16, and 36 d post-hatch, 2 birds from the same pens used for digesta collection were randomly selected for blood and jejunal tissue collection and pH determination of duodenal lumen contents. Blood was collected immediately post-mortem via cardiac puncture into tubes containing EDTA, placed on ice, and centrifuged for 15 min at 1,300 × *g* and 4°C to separate plasma. Plasma from birds within a pen were pooled, aliquoted, and stored at -80°C until further analysis. To determine duodenal pH, a digital pH meter (Mettler-Toledo, UK) with a spear tip piercing pH electrode (Sensorex S175CD) was directly inserted into the digesta in the distal duodenal loop and the pH was recorded. The probe was rinsed with distilled water after each reading and the tip of the pH probe was stored in pH 4 solution when not in use. Jejunal tissue samples (~ 2 cm in length) were collected at the midpoint of the jejunum between the end of the duodenal loop and the Meckel's diverticulum and rinsed with PBS to remove luminal contents and placed in scintillation vials containing 10% neutral-buffered formalin.

Laboratory Analyses

Frozen digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, ether extract, starch content. Dry matter was determined according to AOAC (2006) method 934.02. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to AOAC (2006) method 920.39. Starch concentrations of feed and digesta

samples were measured using the Megazyme Total Starch Assay Kit according to instructions provided by the manufacturer (Megazyme Int. Ireland Ltd., Wicklow, Ireland). Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of dry matter, gross energy, ether extract, nitrogen, and starch were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration to TiO_2 in the diet or ileal digesta. Energy digestibility (%) values obtained from the equation above were multiplied by the gross energy content of the feed to calculate ileal digestible energy (**IDE**).

Plasma samples were analyzed to determine carotenoid and nitric oxide concentrations. All blood processing and carotenoid analysis procedures were conducted under yellow light. Plasma carotenoid concentrations were determined by spectrophotometry as previously described by Allen (1987). Plasma nitrate (NO3-) + nitrite (NO2-) concentrations were measured to determine total nitric oxide (NO) using a colorimetric assay kit (Cayman Chemical CO., Ann Arbor, MI). Prior to NO analysis, plasma samples were filtered through pre-rinsed centrifugal filters (VWR, Radnor, PA) to remove potentially interfering proteins with a molecular weight greater than 30 kilodaltons.

Jejunal tissue samples were embedded in paraffin, sectioned at 4 μm, set on a glass slide, and stained with hematoxylin and eosin. Photomicrographs of each jejunum sample were acquired using a light microscope (Nikon Eclipse) equipped with a digital camera. Imaging software (Nikon's NIS Elements Basic Research Microscope Imaging) was used for

measurement of villus height, crypt depth, and villus width under 4x magnification. For villus height, approximately 6 intact well-oriented villi per bird were randomly selected and measured. Villus height was measured from the tip of the villus to the villus-crypt junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi. The width of the villus was measured at the basal (crypt-villus junction) and apical ends (Iji et al., 2001). Apparent jejunal villus surface area was calculated using the following equation published by Iji et al. (2001):

Apparent villus surface area = (villus basal width + villus apical width) / (2 × villus height)

Statistical Analyses

Pen was considered the experimental unit with 10 replicate pens per treatment for each collection time point. Dietary treatments were arranged in randomized complete block design and the statistical model included pen location as the random blocking factor. Data within each time point were subjected to ANOVA using the MIXED procedure of SAS 9.4 and are presented as least squares means of treatment groups. Jejunal morphology data were transformed (log 10) to meet normality assumptions for the ANOVA. Statistically different treatment means were separated using a Tukey's multiple comparison test. Statistical significance was considered at $P < 0.05$.

RESULTS

Oocyst Shedding and Plasma Measurements

Litter oocyst counts of PC birds remained low throughout the experiment (**Figure 3.1**). At 12 and 16 d post hatch, OPG counts from VAC birds were significantly ($P < 0.05$) higher than those of NC and PC treatments. At 20 d post-hatch, OPG counts in both NC and VAC birds were

higher ($P < 0.05$) than those of PC birds, indicating that NC birds had become inadvertently infected. At 28 d post-hatch, OPG of litter in NC birds was significantly higher ($P < 0.05$) than both PC and VAC treatments, with no differences ($P > 0.05$) observed between PC and VAC birds. At 36 d post-hatch, OPG of litter was highest ($P < 0.05$) in NC birds, intermediate in VAC birds, and lowest in PC birds.

Plasma carotenoid concentrations and plasma nitric oxide levels are presented in Table 3.2. Plasma carotenoid concentrations in NC and PC birds were higher ($P < 0.05$) than VAC birds at both 12 and 16 d post-hatch. At 20 d post-hatch, plasma carotenoid concentrations for NC and VAC birds were lower ($P < 0.05$) than those of PC birds. At 36 d, no differences ($P > 0.05$) in plasma carotenoids were observed among the treatments. Plasma nitric oxide concentrations were unaffected ($P > 0.05$) by any of the treatments, regardless of collection time point.

Growth Performance

No differences ($P > 0.05$) in BWG, FI, or FCR were observed among birds in any of the treatment groups from 0 to 12 d or 0 to 16 d post-hatch (Table 3.3). From 0 to 20 d post-hatch, BWG and FI were lowest ($P < 0.05$) in VAC birds, intermediate in NC birds, and highest in PC birds, with no differences ($P > 0.05$) in FCR. By 28 d post-hatch, BWG in VAC birds did not differ ($P > 0.05$) from PC and NC birds, whereas NC birds had a lower ($P < 0.05$) BWG when compared with PC birds. No differences ($P > 0.05$) in FI were observed among any of the treatments at 28 d post-hatch; however, NC and VAC birds had a higher ($P < 0.05$) FCR when compared with PC birds. By 36 d post-hatch, FCR remained higher ($P < 0.05$) in NC and VAC compared with PC birds, whereas BWG of broilers and FI were not influenced ($P > 0.05$) by treatment.

Apparent Ileal Digestibility of Nutrients and IDE

Vaccinated birds had lower ($P < 0.05$) IDE and AID of nitrogen, ether extract, and starch compared with NC and PC birds at 12 d post-hatch (Table 3.4). By 16 d post-hatch, no differences ($P > 0.05$) in AID of nitrogen, starch, or IDE were observed among the treatment groups, but AID of ether extract remained lower ($P < 0.05$) in VAC birds than in NC or PC birds. At 20 d post-hatch, no differences ($P > 0.05$) in AID or IDE were observed between PC and VAC birds, however, NC birds had lower ($P < 0.05$) AID of nitrogen, ether extract, and IDE compared with PC and VAC birds. By 36 d post-hatch, no differences ($P > 0.05$) in IDE and AID of nitrogen or ether extract were observed among treatment groups. At 36 d, AID of starch remained lower ($P < 0.05$) in NC birds compared with PC birds, with no difference ($P > 0.05$) in AID of starch between PC and VAC birds.

Jejunal Morphology and Duodenal pH

No significant differences ($P > 0.05$) in intestinal jejunal morphology or duodenal pH were observed among any of the treatment groups at any time point. (Table 3.5). However, at 12 d post-hatch, there was a tendency ($P = 0.07$) for VAC birds to have deeper jejunal crypts than NC and PC birds. Furthermore, at 36 d post-hatch, there was a tendency ($P = 0.07$) for VAC birds to have reduced jejunal villus heights compared with NC and PC birds.

DISCUSSION

Coccidiosis vaccines can prevent coccidiosis outbreaks in broiler flocks but can induce damage to the intestinal epithelium, impair live performance, and potentially increase susceptibility to other enteric diseases such as necrotic enteritis. The objective of this experiment was to characterize the timing and magnitude by which coccidiosis vaccination at day of hatch

influences growth performance and nutrient utilization in floor-reared broilers during the various stages of *Eimeria* cycling. Litter oocyst counts and plasma carotenoids indicated that in-feed diclazuril administration prevented coccidial infection in PC birds, whereas increased oocyst shedding and decreased plasma carotenoid concentrations reflected cycling of vaccinal oocysts in VAC broilers. This finding aligned with previous reports that plasma carotenoids are a sensitive indicator of coccidial-induced intestinal damage in chickens (Conway et al., 1993; Holdsworth et al., 2004; Hernández-Velasco et al., 2014) and confirmed that the most commercially-important comparison of the medicated PC group and VAC remained valid throughout the experiment. However, these same measurements indicated an inadvertent infection of the NC group, which likely occurred due to the fact that all treatment groups were distributed evenly in blocks throughout a single experimental facility to minimize environmental or location-related effects. Although the infection of the NC group was unintended, this did provide another time point at which to compare the impact of infection on the responses measured which, as described below, generally aligned with the responses observed in the VAC group at 12 and 16 d post-hatch.

Coccidiosis vaccines can induce coccidiasis, a mild transient form of coccidiosis, usually occurring between 14 and 28 d post-hatch, which can impair broiler performance (Lehman et al., 2009). Coccidiosis vaccination at day of hatch did not impact broiler performance at 12 or 16 d post-hatch in the current experiment, but it did reduce FI and BWG of broilers at 20 d post-hatch, with no effects on FCR. The reductions in BWG and FI for VAC birds, compared with PC birds, had diminished by 28 d. Lehman et al. (2009) also reported vaccinated broilers had a reduction in BWG at 21 d of age, although this reduction was a result of impaired FCR and not reduced FI. Furthermore, Silva et al. (2009) similarly reported a vaccine-induced reduction in BWG at 21 d

of age but observed no differences in BWG between vaccinated and non-vaccinated birds at 36 d of age. Indeed, the goal of coccidiosis vaccination is to provide an early *Eimeria* exposure to allow sufficient time for the birds to compensate for the minor reduction in weight before the end of the grow-out period (Chapman et al., 2005). The decreased BWG in NC birds relative to PC birds at 28 d post-hatch is in agreement with the litter oocyst counts and further reflects an inadvertent infection on NC birds at this time. However, while VAC and PC birds did not differ in BWG or FI at 28 d, FCR at 28 and 36 d post-hatch remained higher for NC and VAC birds than for PC birds, possibly due to nutrient malabsorption throughout the experiment.

The greatest impacts of vaccination on nutrient and energy digestibility were observed at 12 d post-hatch, which likely corresponds with the second *Eimeria* life cycle (Hammond, 1973). Specifically, vaccination decreased IDE by 261 kcal/kg and AID of nitrogen, ether extract, and starch by 5.2, 9.7, and 3.1 percentage units, respectively, (6.3, 10.9, and 3.4% reduction, respectively) compared with PC birds at 12 d post-hatch. Reduced nitrogen digestibility leads to an increased amount of protein in the terminal ileum, where undigested protein is subjected to protein fermentation resulting in the production of toxic compounds such as biogenic amines (Sander et al., 1996; Tamim et al., 2002). While differences in nitrogen digestibility between VAC and PC birds had diminished by 16 d post-hatch, the impaired 12 d digestibility may onset early intestinal bacteria overgrowth to predispose the birds to secondary infections, such as necrotic enteritis, that typically manifests between the second and fifth week of age (Timbermont et al., 2011).

Caloric costs due to coccidiosis vaccination at 12 d post-hatch, as reflected by reduced IDE, were associated with reductions in ether extract and starch digestibility. However, no differences in starch or IDE were observed between VAC and PC birds by 16 d post-hatch. On

the other hand, ether extract digestibility remained 5.1 percentage units lower (6% reduction) in VAC birds than in PC birds at 16 post-hatch. Moreover, the severe impact of a coccidial challenge on lipid digestibility was also observed with the inadvertent infection of NC birds at 20 d post-hatch, whereby digestibility of ether extract was 20.8 percentage units lower (24% reduction) in NC birds compared to PC birds. The variation in ether extract digestibility was also higher at 20 d post-hatch than at earlier time points, and as such, the numerical reduction in ether extract digestibility of 7.3 percentage units (8% reduction) in VAC birds compared with PC birds was not statistically different. The relative impacts on starch and lipid digestibility observed in the current experiment are in agreement with findings by Amerah and Ravindran (2015), who reported broilers subjected to a mixed species challenge had an 18.8% reduction in starch and 96% reduction in lipid digestibility at 7 d post-challenge compared with non-challenged broilers. Starch provides a greater relative contribution to the overall energy content of the diet, but has a lower caloric value than that of lipids. Therefore, the impact of overall energy utilization on starch and lipid was determined by multiplying the FI per bird by the analyzed concentration of either starch or lipid, multiplied by the assumed caloric value, either 4 kcal/kg or 9.5 kcal/kg for starch and lipid, respectively. As such, the VAC-induced reductions in starch and lipid digestibility resulted in a similar caloric cost.

Carotenoids are fat-soluble components (Yonekura and Nagao, 2007), and the marked reductions in plasma carotenoids observed in the current experiment are likely associated with the observed reductions in lipid digestibility. The profound effect of coccidia on lipid digestibility can subsequently impair the absorption of other fat-soluble nutrients, including vitamin D, which can consequently impair the absorption of calcium and phosphorus, negatively impacting bone development (Morris et al., 2015; Świątkiewicz et al., 2017; Oikeh et al., 2019).

Increased digesta lipid content may also reduce the absorption of calcium via intestinal soap formation, and excess calcium in the intestinal lumen may be another predisposing factor for necrotic enteritis (Titball et al., 1999). However, since lipid digestion and absorption are relatively complex processes, it is currently unknown which of the many processes are most impacted during coccidia-exposure. Sharma and Fernando (1975) observed an accumulation of lipid globules within the duodenal villus epithelial cells of *E. acervulina* infected birds, indicating that intracellular lipid processing or transport across the basolateral cell membrane of the enterocyte may be compromised. Furthermore, Adams et al. (1996) reported coccidiosis challenged birds had an improvement in lipid digestion when supplemented with cholic acid, indicating that bile salt synthesis or secretion may be impaired during an infection.

In the current experiment, jejunum villi height was not influenced by coccidiosis vaccination at 12 d post-hatch when the greatest impacts of vaccination on nutrient and energy digestibility were observed. Although severe coccidiosis can cause morphologic damage to the intestinal mucosa, as indicated by increased crypt cell depth and shortened villi, (Fernando and McCraw, 1973; Oikeh et al., 2019), other authors have similarly reported a lack of effects of coccidiosis vaccines on intestinal morphology when administered at commercially recommended doses (Alfaro et al., 2007; Luquetti et al., 2016). There was a tendency for VAC birds to have deeper crypts in the jejunum at 12 d post-hatch in the current experiment, and this may be reflective of increased cellular proliferation to maintain villi structure during periods of increased enterocyte turnover associated with vaccine-induced coccidiasis. Indeed, Luquetti et al. (2016) reported that coccidiosis vaccination did not affect duodenum, jejunum, or ileum villi heights but did increase jejunum and ileum crypt depths of broilers at 14 d post-hatch. Although increased cellular turnover appears to be sufficient to maintain villus structure under these conditions, it

also likely increases intestinal maintenance costs for nutrients and energy (Fernando and McCraw, 1973; Teeter et al., 2008). Furthermore, rapid enterocyte turnover may reduce nutrient transporter expression and brush border enzyme activity, which would consequently reduce the digestion and absorption of nutrients (Su, 2013).

It has been suggested that *Eimeria*-induced pH reductions can cause intestinal pH to fall below the optima efficiency for digestive enzyme activity (Major and Ruff, 1978). To our knowledge, no published work had evaluated pH in a model that mimics field relevant conditions of coccidiosis vaccination. However, no differences in pH of the duodenum, where pancreatic enzymes are secreted and the majority of enzymatic digestion occurs, were observed in the current experiment. Therefore, the lack of differences observed in histology or pH in coccidiosis vaccinated broilers suggests that these factors alone are not primary contributors to the observed reductions in nutrient digestibility.

Intestinal inflammation may also contribute to the transient reductions in nutrient digestibility experienced by coccidiosis-vaccinated broilers. Nitric oxide is produced by macrophages during the inflammatory response to *Eimeria* infection via the enzyme nitric oxide synthase, and Allen (1997) reported that an *E. maxima* infection induced nitric oxide production in the mucosa of the infected intestinal area, as well as in the blood. Recently, Rochell et al. (2017) reported that a 40% reduction in dietary arginine, the key substrate for nitric oxide, did not limit the marked increase in plasma nitric oxide elicited by *E. acervulina* infection, indicating a high prioritization of arginine for nitric oxide synthesis during a coccidial challenge. In the current experiment, coccidiosis vaccination did not increase nitric oxide in the plasma, which is in agreement with the findings of Perez-Carbajal et al. (2010). Local nitric oxide production in the mucosa was not measured in the current experiment. Nonetheless, it appears that the transient

coccidiosis elicited by vaccination does not induce nitric oxide production in the periphery as do more severe challenges.

In conclusion, results reported herein indicate that coccidiosis vaccination had no significant impact on overall BWG and FI of VAC birds, although overall FCR was impaired by vaccination. Coccidiosis vaccination elicited a transient reduction in digestibility of energy and nutrients that was most apparent at 12 post-hatch, particularly for lipids, but VAC birds were able to recover from these reductions by 20 d post-hatch. These results suggest that impaired nutrient digestibility during coccidiosis vaccination may be attributed to a combination of effects, since jejunal morphology and duodenal pH were both not drastically impacted. Furthermore, the prolonged reduction of lipid digestibility in VAC broilers suggests VAC birds have an impaired ability to utilize dietary lipids throughout the various stages of vaccinal oocyst cycling. Therefore, further research is needed to determine the effects of undigested lipids on broiler gastrointestinal health, as well as practical nutrition strategies to ameliorate these effects.

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TABLES AND FIGURES

Table 3.1. Composition of experimental diets fed to broilers from 0 to 36 d post-hatch.[1]

Ingredient, % as-fed	Starter (0-14 d)	Grower (15-28 d)	Finisher (29-36 d)
Corn	57.68	61.11	62.02
Soybean meal (46.8%)	32.90	27.08	23.61
DDGS	4.00	6.00	8.00
Soybean oil	1.34	2.00	2.92
Limestone	1.25	1.22	1.17
Dicalcium phosphate	0.90	0.74	0.52
Salt	0.45	0.42	0.41
DL-methionine	0.31	0.26	0.22
L-lysine HCl	0.24	0.24	0.22
L-threonine	0.09	0.08	0.07
Trace mineral premix[2]	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10
Se premix[4] (0.06%)	0.02	0.02	0.02
Choline chloride (60%)	0.05	0.04	0.04
Santoquin	0.02	0.02	0.02
Phytase[5]	0.01	0.01	0.01
Titanium dioxide	0.50	0.50	0.50
Inert filler[6]	0.05	0.05	0.05
Calculated composition, % unless noted otherwise			
AME_n, kcal/kg	3,015	3,098	3,175
CP	22.01	20.00	19.00
Digestible lysine	1.18	1.05	0.95
Digestible TSAA	0.89	0.80	0.74
Digestible threonine	0.77	0.69	0.65
Calcium	0.90	0.84	0.76
Available P	0.45	0.42	0.38
Analyzed composition, % unless noted otherwise			
Gross energy, kcal/kg	3,991	4,027	4,059
CP	22.00	19.60	19.25
Ether extract	4.80	5.57	6.52
Starch	46.89	47.71	56.33

[1]DDGS = distillers dried grains with solubles; AME_n = nitrogen-corrected apparent metabolizable energy.

[2]Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg

[3]Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.

[4]Supplied 0.12 mg of selenium per kg of diet.

[5]Optiphos®, (Huvepharma Inc., Peachtree City, GA.) provided 250 FTU/kg of diet.

[6]Clinacox®, (Huvepharma Inc., Peachtree City, GA), provided 1ppm diclazuril to the diet at the expense of the inert filler.

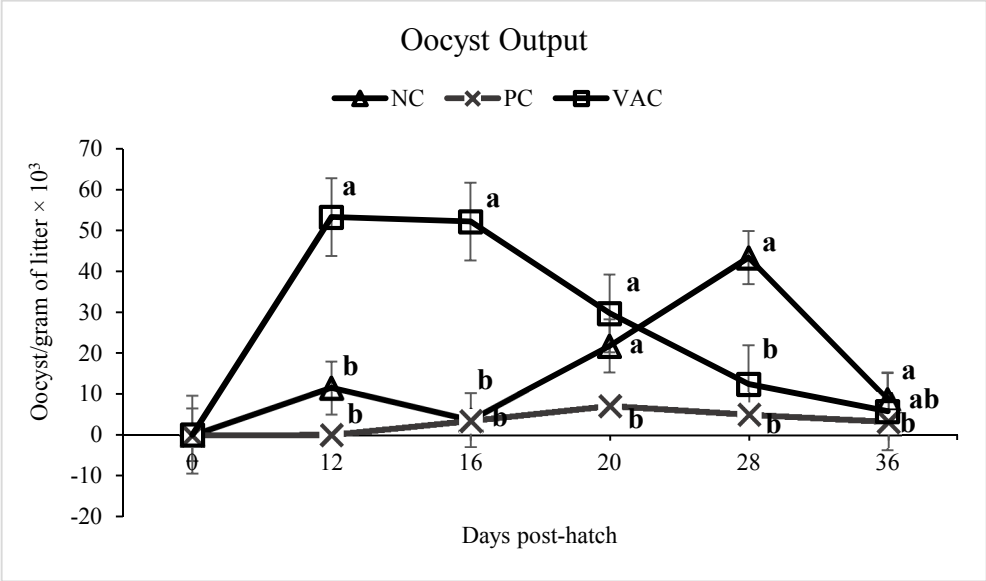

Figure 3.1.

The effects of coccidiosis vaccination on litter oocyst counts from 0 to 36 d post-hatch.

Values are LSMeans of 10 replicate pens. NC = negative control; PC = positive control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d. Within each time point, lines that do not share a common superscript are different ($P < 0.05$).

Table 3.2. Effects of coccidiosis vaccination on plasma carotenoid and nitric oxide concentrations from 12 to 36 d post-hatch.[1,2]

Item	NC	PC	VAC	SEM	*P*-value
12 d					
Plasma carotenoids, µg/mL	2.28[a]	2.38[a]	1.15[b]	0.174	0.001
Nitric oxide, µM	8.41	8.40	8.18	0.483	0.933
16 d					
Plasma carotenoids, µg/mL	2.05[a]	2.21[a]	1.30[b]	0.194	0.006
Nitric oxide, µM	9.13	8.75	8.19	0.481	0.399
20 d					
Plasma carotenoids, µg/mL	0.54[b]	1.29[a]	0.48[b]	0.134	0.001
Nitric oxide, µM	6.03	7.13	7.06	0.418	0.133
36 d					
Plasma carotenoids, µg/mL	3.37	4.24	3.14	0.315	0.050
Nitric oxide, µM	8.26	6.99	8.24	0.630	0.279

[a-b]Means within a row that do not share a common superscript are different ($P < 0.05$).

[1]Values are LSMeans of 10 replicate pens.

[2]Abbreviations: NC = negative control; PC = positive control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d.

Table 3.3. Effects of coccidiosis vaccination on growth performance of broilers from 0 to 36 d post-hatch.[1,2]

Item	NC	PC	VAC	SEM	*P*-value
0 to 12 d					
d12 BW, kg/bird	0.355	0.350	0.347	0.004	0.362
BW gain, kg/bird	0.312	0.308	0.305	0.004	0.414
Feed intake, kg/bird	0.400	0.397	0.397	0.005	0.880
FCR	1.279	1.292	1.305	0.010	0.183
0 to 16 d					
d16 BW, kg/bird	0.544	0.548	0.531	0.006	0.133
BW gain, kg/bird	0.502	0.505	0.489	0.006	0.115
Feed intake, kg/bird	0.736	0.742	0.724	0.007	0.185
FCR	1.468	1.473	1.488	0.011	0.440
0 to 20 d					
d20 BW, kg/bird	0.892[ab]	0.909[a]	0.877[b]	0.008	0.038
BW gain, kg/bird	0.849[ab]	0.867[a]	0.834[b]	0.008	0.036
Feed intake, kg/bird	1.138[ab]	1.164[a]	1.117[b]	0.009	0.006
FCR	1.341	1.345	1.341	0.011	0.961
0 to 28 d					
d28, kg/bird	1.602[b]	1.682[a]	1.647[ab]	0.016	0.007
BW gain, kg/bird	1.560[b]	1.639[a]	1.605[ab]	0.016	0.007
Feed intake, kg/bird	2.334	2.375	2.393	0.019	0.096
FCR	1.501[a]	1.454[b]	1.493[a]	0.007	0.001
0 to 36 d					
d36 BW, kg/bird	2.512	2.588	2.555	0.024	0.086
BW gain, kg/bird	2.469	2.545	2.513	0.024	0.087
Feed intake, kg/bird	3.882	3.905	3.950	0.027	0.191
FCR	1.578[a]	1.543[b]	1.576[a]	0.009	0.018

[a-b]Means within a row that do not share a common superscript are different ($P < 0.05$).

[1]Values are LSMeans of 9 or 10 replicate pens.

[2]Abbreviations: NC = negative control; PC = positive control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d.

Table 3.4. Effects of coccidiosis vaccination on the apparent ileal digestibility (%) of nutrients and ileal digestible energy (kcal/kg) in broilers to 36 d post-hatch.[1,2]

Item	NC	PC	VAC	SEM	*P*-value
12 d					
Dry matter, %	70.4[a]	71.5[a]	66.9[b]	0.58	0.001
Nitrogen, %	81.7[a]	82.8[a]	77.6[b]	0.55	0.001
Ether extract, %	88.5[a]	89.3[a]	79.6[b]	1.72	0.001
Starch, %	90.7[a]	92.0[a]	88.9[b]	0.41	0.001
IDE, kcal/kg[3]	3,337[a]	3,399[a]	3,138[b]	26	0.001
16 d					
Dry matter, %	74.9	73.3	74.0	0.52	0.086
Nitrogen, %	83.9	83.4	83.4	0.45	0.706
Ether extract, %	91.1[a]	91.0[a]	85.9[b]	1.38	0.017
Starch, %	91.0	89.7	89.7	0.76	0.380
IDE, kcal/kg[3]	3,587	3,542	3,543	24	0.317
20 d					
Dry matter, %	71.1[b]	72.6[a]	73.2[a]	0.43	0.005
Nitrogen, %	79.6[b]	82.4[a]	81.7[a]	0.46	0.004
Ether extract, %	65.1[b]	85.9[a]	78.6[a]	3.53	0.001
Starch, %	90.4[b]	91.7[a]	91.3[ab]	0.34	0.028
IDE, kcal/kg[3]	3,326[b]	3,469[a]	3,447[a]	25	0.001
36 d					
Dry matter, %	75.5[a]	75.6[a]	73.7[b]	0.41	0.005
Nitrogen, %	83.5	83.1	83.7	0.41	0.398
Ether extract, %	94.0	94.1	92.5	0.88	0.271
Starch, %	89.0[b]	90.9[a]	89.4[ab]	0.48	0.023
IDE, kcal/kg[3]	3,629	3,642	3,605	19	0.404

[a-b]Means within a row that do not share a common superscript are different ($P < 0.05$).

[1]Values are LSMeans of 10 replicate pens.

[2]Abbreviations: NC = negative control; PC = positive control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d.

[3]IDE = ileal digestible energy.

Table 3.5. Effects of coccidiosis vaccination on jejunal morphology and duodenal pH of broilers in 36 d post-hatch.[1,2]

Item	NC	PC	VAC	SEM	*P*-value[3]
12 d					
Villus height, μm	645 (2.81)	669 (2.82)	693 (2.84)	23.0 (0.015)	0.392
Crypt depth, μm	124 (2.08)	118 (2.82)	145 (2.84)	5.7 (0.028)	0.082
Villus height to crypt depth	5.81 (0.75)	6.60 (0.81)	5.38 (0.71)	0.372 (0.037)	0.159
Villus surface area, mm^2	0.10 (5.01)	0.10 (5.01)	0.10 (4.98)	0.006 (0.025)	0.772
pH	5.64	5.61	5.57	0.074	0.773
20 d					
Villus height, μm	774 (2.89)	730 (2.86)	786 (2.90)	24.1 (0.014)	0.189
Crypt depth, μm	200 (2.28)	188 (2.26)	211 (2.31)	13.4 (0.029)	0.561
Villus height to crypt depth	4.58 (0.64)	4.40 (0.62)	4.36 (0.62)	0.352 (0.033)	0.891
Villus surface area, mm^2	0.13 (5.10)	0.13 (5.11)	0.13 (5.12)	0.006 (0.021)	0.800
pH	6.11	6.08	6.12	0.028	0.628
36 d					
Villus height, μm	1,058 (3.02)	1,100 (3.04)	1,005 (3.01)	27.7 (0.012)	0.071
Crypt depth, μm	177 (2.23)	153 (2.17)	169 (2.21)	14.4 (0.035)	0.507
Villus height to crypt depth	7.10 (0.83)	8.14 (0.90)	6.96 (0.82)	0.672 (0.040)	0.306
Villus surface area, mm^2	0.19 (5.28)	0.20 (5.30)	0.19 (5.26)	0.001 (0.029)	0.653
pH	6.12	6.15	6.12	0.015	0.166

[1]Values are LSMeans of 10 replicate pens with transformed data (log_{10}) used for statistical analysis in parentheses.

[2]Abbreviations: NC = negative control; PC = positive control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d.

[3]*P*-values represent transformed data (log_{10}).

CHAPTER 4: INFLUENCE OF COCCIDIOSIS VACCINATION ON NUTRIENT UTIIIZATION OF CORN, SOYBEAN MEAL, AND DISTILLERS DRIED GRAINS WITH SOLUBLES IN BROILERS

ABSTRACT

Two experiments were conducted to determine the impact of coccidiosis vaccination on the apparent ileal digestibility (**AID**) of nutrients and ileal digestible energy (**IDE**) in commonly used feed ingredients in broilers. Eight experimental treatments based on a factorial arrangement of coccidiosis vaccination [control with in-feed diclazuril (**CTL**) or vaccinated (**VAC**)] and 4 different diets were administered to male Cobb 500 broilers in floor pens containing 12 birds per pen. For the vaccinated group, a 3x dose of a live coccidiosis vaccine was given via oral gavage on day of hatch. Experimental diets consisted of a basal diet and 3 test diets in which 30% of the basal diet was replaced with either corn, soybean meal (**SBM**), or distillers dried grains with solubles (**DDGS**) to allow for calculation of nutrient digestibility of individual ingredients by difference. Broilers were fed a common diet from 0 to 7 d and experimental diets from 7 to 12 d. On d 12, blood and ileal digesta were collected to measure plasma carotenoids and determine AID of nitrogen, ether extract, IDE (experiments 1 and 2), and amino acids (**AA**) (experiment 2). Vaccination increased ($P < 0.05$) excreta oocyst counts and decreased ($P < 0.05$) plasma carotenoids when compared with CTL birds. Interactive effects ($P < 0.05$) were observed for AID of nitrogen (experiment 1) which was reduced by vaccination in birds fed the corn diet and increased for birds fed DDGS. No differences ($P > 0.05$) in IDE were observed between VAC and CTL birds in either experiment, whereas vaccination decreased ($P < 0.05$) AID of ether extract independently of diet. Interactive effects ($P < 0.05$) were observed for AA digestibility, whereby digestibility of all AA were reduced by VAC in corn diets but generally increased AA digestibility of DDGS diets, with minimal impact on SBM diets. In conclusion, the impact of

coccidiosis vaccination on nutrient and energy digestibility varied among ingredients; however, digestibility was minimally impacted or improved with DDGS.

INTRODUCTION

Accurate feed formulation requires knowledge of nutrient digestibility for each individual ingredient within the diet. Current widespread adoption of antibiotic-free production systems for commercial poultry has increased coccidiosis vaccination as a means of coccidiosis control, and the transient coccidiasis associated with live oocyst vaccination may negatively influence nutrient digestibility during the period of peak vaccine cycling. It is likely that any deleterious effects of coccidiosis vaccination on nutrient digestibility may differ among individual feed ingredients, which would be important to recognize when formulating diets for vaccinated broilers. Indeed, Persia et al. (2006) subjected birds to an acute *E. acervulina* infection at 9 d post-hatch and found that amino acid digestibility and ME_n of diets containing fish meal were less impacted than those of diets only containing corn and soybean meal, indicating ingredient dependent effects (Persia et al., 2006).

Previous research conducted in our laboratory demonstrated that nutrient digestibility for floor-reared broiler given a coccidiosis vaccine at day of hatch was most impacted at 12 d post-hatch (Gautier et al., 2019). It has also been shown that adjustments in digestible amino acid content to account for the predicted coccidiosis-induced reductions in amino acid digestibility improved the performance of broilers challenged with a 12x dose of a coccidial vaccine (Adedokun et al., 2016). Therefore, this suggests that an opportunity may exist for dietary adjustments to be made during the starter phase to support the performance of coccidiosis vaccinated broilers, and the effectiveness of such adjustments may be improved with knowledge of the impact of vaccination on nutrient digestibility of individual feed ingredients.

Current research to quantify the impact of a coccidiosis challenge on nutrient digestibility has primarily been based on the digestibility of the complete feed rather than for individual ingredients and has been conducted in battery cages where oocyst cycling is prevented. Furthermore, these models have generally involved a single inoculation with a large number of oocysts that greatly exceeds what would ingested by the bird during typical vaccine cycling. Therefore, the objective of this experiment was to assess the impact of a coccidiosis vaccine challenge model on the nutrient utilization of corn, soybean meal (**SBM**), and distillers dried grains with solubles (**DDGS**) in floor-reared broilers. While corn and soybean meal are the primary feed ingredients for broiler diets in the United States, distillers dried grains with solubles is an alternative ingredient that is compatible for use in all vegetable based-diets, which are commonly fed to coccidiosis-vaccinated broilers. Furthermore, the compositions of these ingredients vary widely in the amount and type of carbohydrates, protein, fiber, and lipids they contain, which could possibly provide insight into the class of nutrients that have the greatest influence on the response of broilers to coccidiosis vaccination.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

General Bird Husbandry and Dietary Treatments

Seven hundred and sixty-eight male broiler chicks were obtained from a Cobb 500 female line from a commercial hatchery on day of hatch and allotted to 8 replicates pens (12 birds per pen; 0.09 m^2 per bird) of 8 treatments in a factorial arrangement of vaccination status and dietary treatment. As such, one-half (384) of the chicks were orally gavaged with a live oocyst vaccine (Coccivac®-B52; Merck Animal Health, Intervet Inc. Millsboro, DE, USA) at 3x

the manufacturers recommended dose (**VAC**), whereas the other half were not vaccinated and received in-feed diclazuril (Clinacox, Huvepharma), a chemical coccidiostat, throughout the entire experiment (**CTL**). The vaccine was delivered by oral gavage (0.25 mL/bird) using a stainless-steel gavage needle to provide uniform administration. All chicks were group-weighed and distributed to 64 floor pens on unused pine shavings. Throughout the trial, litter was sprayed daily with water to increase litter moisture to promote oocyst sporulation. Each floor pen was equipped with a hanging feeder and a nipple drinker line. Birds were provided access to feed and water *ad libitum* throughout the 12 d experiment and the lighting schedule and temperature targets were adjusted according to management guidelines published by the primary breeder (Cobb-Vantress, 2015). Birds were provided a nutritionally complete common corn-soybean meal diet until 7 d of age. Experimental diets were fed from 7 to 12 d of age and for all experimental diets choline chloride, vitamins, and minerals were kept constant to prevent deficiencies of these nutrients.

At 7 d of age, CTL and VAC birds were weighed and provided 1 of 4 experimental diets that included the same diet as fed the previous 7 days (basal) or diets in which 30% of this basal diet was replaced with either corn, SBM, or DDGS to allow for the determination of apparent ileal digestibility (**AID**) of nutrients and ileal digestible energy (**IDE**) (Experiments 1 and 2) and AID of amino acids (Experiment 2) of these individual ingredients using the difference method (Table 4.1). The same sources of test corn, SBM, and DDGS were used in both experiments and these samples were analyzed for protein, ether extract, and gross energy. The protein concentrations were 11.6, 48.1, and 31.0% for corn, SBM, and DDGS, respectively. Ether extract concentrations were 2.73, 1.60, and 8.89% and gross energy concentrations were 3,795, 4,275, and 4,529 kcal/kg for corn, SBM, and DDGS (as-is basis), respectively.

Determination of Growth Performance, Excreta Oocyst Shedding, and Plasma Carotenoids

Although growth performance was not a primary objective of this trial, birds and feeders were weighed at 0, 7, and 12 d post-hatch for calculation of body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) to assess growth performance as influenced by coccidiosis vaccination. All dead and culled birds were weighed individually and FCR calculations were adjusted to include the weight gain of dead birds.

Oocyst shedding as indicated by the number of oocysts per gram of excreta samples collected from each pen was determined before bird placement and at 7 and 12 d post-vaccination to ensure *Eimeria* infection. Samples of the unused shavings were taken before bird placement to confirm the absence of oocysts, and at 7 and 12 d, fresh excreta samples were collected from each pen using wax paper placed on the litter 12 h prior to collection. All samples were placed in airtight conical tubes and kept refrigerated until processing. Samples were soaked in water overnight and homogenized by vigorous stirring. Following homogenization, 1 ml of sample was further diluted with 9 ml of saturated salt solution and pipetted into the chamber of a McMaster counting slide. Duplicate counts were made for each sample using the following equation:

Oocysts per gram of excreta = (Oocyst count × dilution × volume) / (volume of counting chamber × weight of sample), where the dilution was 10 and the volume of the counting chamber was 0.15 ml.

At 12 d post-hatch, all birds were euthanized by CO_2 inhalation. Blood was collected from two randomly selected birds per pen via cardiac puncture and placed into tubes containing EDTA. After collection, tubes were placed on ice and subsequently centrifuged for 15 min at

1,300 × *g* and 4°C to separate plasma. Plasma from birds within a pen were pooled, aliquoted, and stored at -80°C until further analysis. All blood processing and carotenoid analysis procedures were conducted under yellow light and were determined by spectrophotometry previously described by Allen (1987).

Determination of Ingredient AID and IDE

At 12 d post-hatch, ileal contents from all birds in each pen, including the 2 birds randomly selected for the collection of blood, were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples within each pen were pooled and frozen (−20°C) until analysis. Frozen digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract content. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to AOAC (2006) method 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of dry matter, gross energy, ether extract, and nitrogen, were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration (%) to TiO_2 (%) in the diet or ileal digesta. Energy digestibility (%) values obtained from the equation above were multiplied by the gross

energy content of the feed to calculate apparent ileal digestible energy (**IDE**) in units of kcal/kg. In Experiment 2, diets and digesta were sent to a commercial laboratory (University of Missouri Agricultural Experiment Station Chemical Laboratory, Columbia, MO) for determination of total AA content (methods 982.30 E (a,b,c) and 985.28; AOAC International, 2006) for determination of AID of AA.

Upon determination of AID of nutrients and IDE for complete experimental diets, these values were calculated for the individual test ingredients using the following equation:

AID of ingredient, % = [AID (%) of test ingredient diet] - [AID (%) of basal diet × 0.66] / 0.30,

where 0.66 was the proportion of the basal diet and 0.30 was the proportion of the test ingredient. The remaining 4% of the diet was the portion kept constant to prevent nutrient deficiencies as described above.

Statistical Analysis

Treatments were comprised of a factorial arrangement of vaccination status (CTL or VAC) × 4 diet types in a completely randomized design. Pen was considered the experimental unit with 8 replicate floor pens per treatment. Data on individual ingredients were analyzed as a factorial arrangement of vaccination status (CTL or VAC) × 3 ingredients. Data were subjected to a 2-way ANOVA using the MIXED procedure of SAS 9.4 to assess the main effects of dietary treatment, vaccination status, and their interaction. Statistically different treatment means were separated using a Tukey's multiple comparison test and orthogonal contrasts between CTL and VAC birds were used to assess the impact of vaccination on birds within a dietary treatment. Statistical significance was considered at $P < 0.05$.

RESULTS

Oocyst Shedding, Plasma Carotenoids, and Growth Performance

In both experiments, no oocysts were detected in the shavings before bird placement (data not shown) and VAC birds had greater ($P < 0.05$) oocyst output at 7 d post-hatch than CTL birds (Table 4.2). In experiment 1, a diet by vaccine interaction for 12 d oocyst output was observed, whereby vaccination increased ($P < 0.05$) excreta oocyst output in birds fed the corn and SBM diets and did not differ ($P > 0.05$) for birds fed the basal and DDGS diets. In experiment 2, oocyst output was influenced by vaccination status and was higher ($P < 0.05$) in VAC birds than CTL at 12 d post-hatch. Plasma carotenoid concentrations were lower ($P < 0.05$) and tended to be lower ($P = 0.06$) in VAC birds than in CTL birds in experiments 1 and 2, respectively, with no independent or interactive diet effects.

Birds and feed were weighed in both experiments to provide a general assessment of the impact of vaccination (data not shown). At 7 d post-hatch, when all birds had been fed the common corn-soybean meal diet and experimental diets had not yet been provided, broiler performance was impacted by vaccination. In both experiments 1 and 2, VAC birds had a 9% reduction ($P < 0.05$) in BWG when compared with CTL birds. In experiment 1, the reduction in BWG of VAC birds was due to a higher ($P < 0.05$) FCR than CTL birds, with no differences ($P > 0.05$) in FI. Conversely, in experiment 2, VAC birds had a lower ($P < 0.05$) FI than CTL birds, with no differences ($P > 0.05$) in FCR.

Apparent Ileal Digestibility of Nutrients and IDE

Complete Experimental Diets: The AID of nutrients and IDE of the complete diets for both experiments 1 and 2 are presented in Table 4.3. In experiment 1, a diet by vaccine

interaction for AID of dry matter was observed, whereby vaccination reduced ($P < 0.05$) AID of dry matter in birds fed the corn diet but not those fed the basal, SBM, or DDGS diets. A diet by vaccine interaction on AID of nitrogen was also observed, where AID of nitrogen was reduced ($P < 0.05$) by vaccination in birds fed the corn diet, increased ($P < 0.05$) by vaccination in birds fed the DDGS diet, and was not influenced by vaccination ($P > 0.05$) in birds fed the basal or SBM diets. Independent effects of vaccine status ($P < 0.05$) and diet type ($P < 0.05$) were observed for AID of ether extract. Vaccinated birds had a lower AID of ether extract compared with CTL birds, and AID of ether extract was highest ($P < 0.05$) for the SBM diet (81%), and lower and similar ($P > 0.05$) among the basal (69%), corn (65%), and DDGS (73%) diets. Diet type influenced IDE ($P < 0.05$) and was highest for the corn diet, intermediate for the basal diet, and lowest for the DDGS and SBM diets. Furthermore, interactive effects between diet type and vaccination status tended ($P = 0.062$) to influence IDE, where IDE tended to be reduced by vaccination in birds fed the DDGS diet and increased by vaccination in birds fed the basal diet and did not differ ($P > 0.05$) in birds fed the corn or SBM diets.

In experiment 2, AID of dry matter was influenced by diet type ($P < 0.05$) and was highest for the basal diet (78%), intermediate for the corn (72%) and DDGS (69%) diets, and lowest for the SBM diet (65%). Furthermore, AID of dry matter tended ($P = 0.054$) to be lower in VAC birds than in CTL birds. Diet type influenced AID of nitrogen ($P < 0.05$), which was highest for the basal diet (86%) and not different ($P > 0.05$) among the corn (79%), SBM (80%), and DDGS (79%) diets. Independent effects of vaccine status ($P < 0.05$) and diet type ($P < 0.05$) were observed for AID of ether extract. Vaccinated birds had a lower AID of ether extract than CTL birds, and for diet type, AID of ether extract was lowest for the corn diet (62%) with no differences ($P > 0.05$) among the basal (72%), SBM (75%), and DDGS (75%) diets. Diet type

influenced IDE ($P < 0.05$), which was highest for the basal diet, with no differences ($P > 0.05$) observed among the other 3 diet type and IDE tended ($P = 0.054$) to be lower in VAC birds than in CTL birds.

Individual Feed Ingredients: Regarding the digestibility of individual feed ingredients, there were several cases where the digestibility values of the test ingredient diets (corn, SBM, and DDGS) were much higher than those determined for the basal diets. Therefore, when the digestibility of individual feed ingredients was determined by the difference method it led to digestibility values that were close to or exceeded 100% (Table 4.4). This indicates a potential lack of additivity due to nutrient interactions among ingredients in the basal and the test ingredient diets. In experiment 1, independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for AID of dry matter, where VAC birds had a lower AID of dry matter than CTL birds, and AID of dry matter was highest for corn (101%), intermediate for DDGS (71%), and lowest for SBM (57%). Ingredient by vaccine interactions ($P < 0.05$) for AID of nitrogen and ether extract were also observed, where both AID of nitrogen and ether extract were reduced ($P < 0.05$) by vaccination in birds fed corn, increased ($P < 0.05$) by vaccination in birds fed DDGS, and did not differ ($P > 0.05$) for birds fed SBM. Independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for IDE, where VAC birds had a reduction in IDE compared with CTL birds, and IDE was highest for corn, with no differences ($P > 0.05$) between SBM and DDGS.

In experiment 2, AID of dry matter was influenced by ingredient ($P < 0.05$), which was highest for corn (68%) and not different between SBM (52%) and DDGS (57%). Nitrogen digestibility ($P > 0.05$) was not influenced by ingredient, vaccination status, or their interaction. An ingredient by vaccine interaction for AID of ether extract was observed, whereby vaccination

reduced ($P < 0.05$) AID of ether extract in corn but not in SBM or DDGS. Similar to experiment 1, independent effects of vaccine status ($P < 0.05$) and ingredient ($P < 0.05$) were observed for IDE, where VAC birds had a reduction in IDE compared with CTL birds, and IDE was highest for corn, intermediate for DDGS, and lowest for SBM.

Apparent Ileal Amino Acid Digestibility (Experiment 2)

Complete Experimental Diets: The experimental diet amino acid digestibility values determined in experiment 2 are presented in Table 4.5. Main effects ($P < 0.05$) of diet type on arginine, histidine, lysine, methionine, threonine, aspartic acid, and glycine were observed, and digestibility values of these amino acids were generally highest for the basal diet. Interactive effects between diet type and vaccination status ($P < 0.05$) were observed for AID of isoleucine, leucine, phenylalanine, valine, alanine, cysteine, glutamic acid, proline, and serine. For each of these amino acids, this interaction was due to an increase in AID with vaccination in birds fed DDGS, with no effect ($P > 0.05$) of vaccination in birds fed the basal, corn, or SBM diets.

Individual Feed Ingredients: The individual feed ingredient amino acid digestibility values determined in experiment 2 are presented in Table 4.6. Main effects ($P < 0.05$) of ingredient on arginine, lysine, methionine, and aspartic acid were observed, and AID of these AA were generally highest for corn. Interactive effects between ingredient and vaccination status ($P < 0.05$) were observed for AID of histidine, isoleucine, leucine, phenylalanine, threonine, valine, alanine, cysteine, glutamic acid, glycine, proline, and serine. For histidine, isoleucine, leucine, phenylalanine, alanine, and glycine, AID was increased by vaccination in birds fed DDGS, with no differences ($P > 0.05$) due to vaccination in birds fed corn or SBM. For threonine, valine, glutamic acid, proline, and serine, AID was increased by vaccination in birds fed DDGS, reduced by vaccination in birds fed corn, and did not differ ($P > 0.05$) with

vaccination for birds fed SBM. The AID of cysteine was increased by vaccination in birds fed SBM and DDGS but did not differ ($P > 0.05$) for birds fed corn.

DISCUSSION

Estimates of nutrient digestibility for individual feed ingredients fed are greatly influenced by the assay method and associated diet types used, and the best approach may differ for the primary nutrient of interest. For example, it has been suggested that the use of semi-purified diets in which the test ingredient serves as the sole source of amino acids is the preferred method for generating amino acid digestibility values (Ravindran et al., 2017). Alternatively, the difference method is based on a practical diet replacement with the test ingredient of interest and digestibility values for individual test ingredients are calculated according their proportional contribution to the test diet (Kong and Adeola, 2013; Zhang and Adeola, 2017). This approach is often used for determination of ME_n of individual feed ingredients (Stefanello et al., 2016), but has also been used for determination of AA digestibility (Fan and Sauer, 1995). In order to limit the number of experimental birds and due to the potential drawbacks associated with semi-purified diets, such as reduced feed intake and enzyme secretions (Partridge et al., 1982; Shastak et al., 2014) and increased digesta passage rate (Colnago et al., 1984), which are all factors that could influence the desired development of coccidiasis induced by the live oocysts vaccine, a practical diet replacement assay was selected to simultaneously estimate the digestibility of nutrients, energy, and amino acids in the current experiment. Further, focus of this experiment was to quantify any relative reductions in nutrient and energy digestibility due to vaccination that could be applied to any chosen set of digestibility values used by formulating nutritionists, rather than to establish actual digestibility values to be implemented in feed formulation.

As with the dietary approach, the impact of coccidiosis or coccidiasis on nutrient utilization will be influenced by the challenge model applied, with important factors including the species, number, and infectivity of the oocysts administered. The vaccine model used in the current experiment attempted to reflect conditions experienced by commercial broilers vaccinated for the control of coccidiosis. Therefore, a commercially-available coccidiosis vaccine, which contained a mixture of live *Eimeria* species, was administered to floor-reared birds at day of hatch. Since immunity to *Eimeria* is species specific, it is important to vaccinate birds against all *Eimeria* species that a commercial flock may encounter, rather than just a single species (Chapman et al., 2005). Furthermore, to ensure measurable and consistent impacts of this model, birds were orally gavaged with a dose of vaccinal oocysts that was higher (3x) than the manufacturer's recommended dose. Excreta oocyst output was increased in VAC birds at 7 d post-hatch in both experiments and at 12 d post-hatch in experiment 2, confirming that oocyst cycling occurred in the vaccinated birds. The reason for the greater increase in oocyst excretion with vaccination for birds fed the corn and SBM diets compared with those fed the basal and DDGS diet in experiment 1 are unknown, as this response was not observed in experiment 2. Previous research has demonstrated that trypsin promotes the excystation of sporozoites from sporulated *Eimeria* oocysts within the intestine (Britton et al., 1964), and therefore, increased oocyst shedding observed for VAC birds fed the SBM diet may have been attributed to an increase in pancreatic trypsin secretion due to the high dietary crude protein content. However, based on this explanation, the opposite would be expected for birds fed the corn diet, which was not the case. The reduction in plasma carotenoid concentrations in VAC birds was expected, as carotenoids are inversely related to oocyst output and have proven to be a sensitive indicator of coccidial-induced intestinal damage in birds (Holdsworth et al., 2004). Further, carotenoids are

fat-soluble components (Yonekura and Nagao, 2007), and these reductions were likely associated with the observed reductions in lipid digestibility discussed below. Although there was some variation between the 2 experiments, these results collectively validate a successful vaccine challenge model in both experiments.

Due to large differences observed in oocyst shedding between the 2 experiments, the impact on nutrient digestibility will be discussed individually for each experiment. In general, the complete diet nutrient digestibility values indicated that the effects of the vaccine challenge model varied with diet composition. In experiment 1, the reduction in AID of nitrogen observed in VAC birds fed the corn diet that was not observed for birds fed the other diets may have been associated with the increased oocyst output that occurred at that time point as described above. However, the lack of a more pronounced impact on birds fed the corn diet in experiment 2 may indicate that the effects on this dietary group in experiment 1 were not related to the diet composition *per se*, but increased oocyst output due to some other factor. In experiment 1, VAC birds fed the SBM diet also had increased oocyst output, however, no vaccine-induced reduction in AID of nitrogen was observed and may be attributed to the high dietary crude protein content. Previous research has shown that increased dietary concentrations of crude protein or amino acids can be beneficial to broilers during coccidiosis by increasing the supply of amino acids needed for intestinal repair (Parker et al., 2007; Lehman et al., 2009; Lee et al., 2011; Adedokun et al., 2016; Cloft et al., 2019). Interestingly, vaccination actually increased AID of nitrogen in birds fed the DDGS diets in experiment 1, and in experiment 2, vaccination increased the AID of 4 essential and 5 nonessential AA in birds fed the DDGS diets. Therefore, the impact of DDGS in broiler diets suggests additional mechanisms other than dietary crude protein content that may be beneficial for intestinal health during an enteric infection.

Vaccine-induced reductions in ether extract digestibility, independent of diet type, were consistently observed in both experiments, with AID ether extract values of 75.3 and 68.4 and 74.3 and 67.2% for CTL and VAC birds in experiments 1 and 2, respectively. Furthermore, the average reduction of 7.0 percentage units (9.3%) in AID of ether extract between the CTL and VAC birds was generally greater than the reduction for AID of DM, nitrogen, or amino acids. Other recent trials have also indicated that *Eimeria* exposure markedly impacts lipid digestibility (Amerah and Ravindran, 2015; Gautier et al., 2019). However, the vaccine-induced reductions in AID of ether extract did not translate to reduced IDE and may possibly be attributed to a lower vaccine impact on starch digestibility, which accounts for a much larger proportion of total dietary energy. Although not determined in the current experiment, previous results from our laboratory have shown that vaccination has only a moderate impact on AID of starch compared with ether extract. In both experiments, birds fed the basal and corn diets had a lower AID of ether extract but higher IDE values when compared with birds fed the SBM and DDGS diets, further supporting that the reduction in AID of ether extract was not sufficient to markedly impact energy digestibility.

While the original objective of this experiment was to determine the digestibility of individual test ingredients (i.e., corn, SBM and DDGS), relatively high IDE of corn in experiment 1 and digestibility values greater than 100% in both experiments 1 and 2 indicate a potential violation of the necessary assumption for the difference method that no interactions exist between the digestibility values of the test feed ingredient and basal diet components (Kong and Adeola, 2013). Glencross et al. (2004) also reported digestibility values in excess of 100% for a range of plant protein ingredients when the difference method was used and suggested it could be attributed to analytical error for nutrient analyses, poor mixing of the marker in the diet,

a non-representative diet sample, or interactions among ingredients. Nonetheless, relevant conclusion from the current experiment can be still drawn regarding the impact of the vaccine challenge on nutrient digestibility of the test ingredients.

The amino acid digestibility rankings values of feed ingredients were generally similar to those reported in previous literature. The AID of lysine in SBM has been reported to range from 84 to 93% (Ravindran et al., 2005; Huang et al., 2006), slightly higher than the value of 83% obtained for CTL birds in the current experiment. The AID of lysine in corn has been reported to range from 79 to 86% (Huang et al., 2006; Adedokun et al., 2009), which falls within range for the value of 86% reported for CTL birds in the current experiment. Furthermore, the AID values of lysine are generally higher than those of threonine, which is in agreement with the results reported herein, as threonine is a major component of mucin production and continuous intestinal secretions likely attribute to low AID values (Fernandez et al., 1994). Threonine, as well as cysteine, serine, and proline, are major structural components of intestinal mucin (Montagne et al., 2004). The AID of several of these amino acids in corn, but not SBM or DDGS, were reduced by vaccination, indicating that, as expected, increased endogenous losses of these amino acids would have a proportionally higher AID value for lower protein ingredients such as corn (Lemme et al., 2004).

The overall minimal impact of coccidiosis vaccination on nutrient digestibility, and even positive effect on amino acid digestibility in some cases, for birds fed DDGS may have been due to several factors. Distillers dried grains with solubles contain a high concentration of insoluble fiber (i.e. lignin, cellulose, hemicellulose) which can decrease the viscosity of the digesta (Khajali and Slominski, 2012), increase digesta passage rate, and promote epithelial sloughing by physical contact through a process often referred to as the “scratch factor” for ruminants fed

high fiber material. This additional sloughing, while generally undesirable for nutrient utilization in nonruminant animals, may prevent the adhesion of *Eimeria* within the intestine and increase sloughing of parasitized enterocytes. Therefore, this may have increased the rate of recovery from the vaccine-induced infection for the DDGS-fed birds in the current experiment, and it has been demonstrated that nutrient utilization of broilers following peak coccidial infection is very efficient (Fernando and McCraw, 1973; Turk, 1974; Ruff and Allen, 1982). Dietary DDGS also includes yeast, which is added during the fermentation process and yeast cell wall components including mannan-oligosacchardies and β-glucans can modulate intestinal microflora, enhance immunocompetence, and increase nutrient digestibility (Ferket et al., 2002; Vohra et al., 2016). Perez et al. (2011) reported that including up to 20% DDGS in diets fed to broilers from 7 to 21 d of age, when birds were subjected to a severe *E. acervulina* infection at 10 d of age, did not positively or negatively affect the severity of an *Eimeria* infection on growth performance, but did increase bacterial diversity in the cecum, which is generally considered beneficial to gastrointestinal health.

In summary, these results indicate that the effects of the vaccine challenge model varied among diet composition and nutrient type. The vaccine challenge model had a marked impact on ether extract digestibility, with the greatest reduction observed for corn. Vaccinated birds fed SBM resulted in minimal impacts on overall nutrient digestibility, whereas vaccinated birds fed DDGS resulted in minimal impacts on ether extract digestibility, with improved nitrogen and amino acid digestibility. Though further research is needed, it appears that there is no reason to expect that DDGS of an acceptable quality would exacerbate intestinal function of broilers experiencing a mild coccidial infection.

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TABLES

Table 4.1. Composition of experimental diets fed to broilers from 0 to 12 d post-hatch.[1]

		Experimental		
Ingredient, % as-fed	Basal	Basal corn	Basal SBM	Basal DDGS
Corn	61.55	42.33	42.33	42.33
Soybean meal (46.3%)	31.75	21.85	21.85	21.85
Test corn	-	30.00	-	-
Test SBM	-	-	30.00	-
Test DDGS	-	-	-	30.00
Soybean oil	2.35	1.62	1.62	1.62
Limestone	0.99	0.99	0.99	0.99
Dicalcium phosphate	1.67	1.67	1.67	1.67
Salt	0.42	0.42	0.42	0.42
DL-methionine	0.25	0.17	0.17	0.17
L-lysine HCl	0.11	0.08	0.08	0.08
L-threonine	0.05	0.03	0.03	0.03
Trace mineral premix[2]	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10
Se premix[4] (0.06%)	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.05	0.05	0.05	0.05
Santoquin	0.02	0.02	0.02	0.02
Titanium dioxide	0.50	0.50	0.50	0.50
Inert filler[5]	0.05	0.05	0.05	0.05
Calculated composition, % unless noted otherwise				
AME_n, kcal/kg	3,088	3,140	2,858	2,949
CP	19.50	15.61	27.29	21.45
Digestible lysine	1.11	0.83	1.61	0.97
Digestible TSAA	0.83	0.66	0.93	0.82
Calcium	0.86	0.83	0.92	0.85
Available P	0.44	0.41	0.49	0.51
Analyzed composition, % unless noted otherwise[6]				
Experiment 1				
Gross energy, kcal/kg	3,938	3,885	3,952	3,954
CP	22.25	19.05	27.20	22.40
Ether extract	4.98	3.87	3.70	5.09
Experiment 2				
Gross energy, kcal/kg	3,989	3,960	4,097	4,212
CP	21.10	17.60	29.15	24.40
Ether extract	3.70	4.03	3.79	6.94

[1]SBM = soybean meal; DDGS = distillers dried grains with solubles; AME_n = nitrogen-corrected apparent metabolizable energy.

[2]Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg

[3]Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.

[4]Supplied 0.12 mg of selenium per kg of diet.

[5] For birds in the control group, Clinacox®, (Huvepharma Inc., Peachtree City, GA), provided 1ppm diclazuril to the diet at the expense of the inert filler.

Table 4.2. Effects of coccidiosis vaccination on oocyst per gram of excreta sample (OPG) and plasma carotenoid concentration (μg/mL). [1,2]

	Basal		Basal corn		Basal SBM		Basal DDGS			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Diet	Vaccination	Interaction[4]
Experiment 1												
OPG, day 7	2,182	4,736	-	-	-	-	-	-	510	N/A	0.001	N/A
OPG, day 12	756	2,724	2,275	7,786*	567	3,075*	790	2,789	697	0.001	0.001	0.040
Plasma carotenoids, day 12	1.42	0.83	1.52	1.00	1.48	0.97	1.62	0.74	0.241	0.942	0.003	0.838
Experiment 2												
OPG, day 7	1,871	40,490	-	-	-	-	-	-	3,827	N/A	0.001	N/A
OPG, day 12	2,187	21,678	4,398	22,650	2,028	22,488	5,220	21,409	4,561	0.840	0.001	0.583
Plasma carotenoids, day 12	1.90	1.46	1.91	1.46	1.66	1.01	1.75	1.46	0.353	0.712	0.060	0.961

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]Abbreviations: SBM= soybean meal; DDGS = distillers dried grains with solubles; CTL = control, birds were given an in-feed anticoccidial drug; VAC = birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of diet, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

Table 4.3. Effects of coccidiosis vaccination on the apparent ileal digestibility of nutrients (%) and energy (kcal/kg) of experimental diets in broilers at 12 d post-hatch. [1,2]

	Basal		Basal corn		Basal SBM		Basal DDGS			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Diet	Vaccination	Interaction[4]
Experiment 1												
Dry matter	64.6	67.0	75.6	72.1*	60.4	60.9	65.6	64.0	0.95	0.001	0.369	0.011
Nitrogen	74.6	77.8*	82.5	77.7*	73.4	73.4	74.1	79.8*	0.88	0.001	0.125	0.001
Ether extract	74.4	63.6	71.3	59.5	82.9	78.1	72.4	72.4	3.07	0.001	0.002	0.157
IDE[5]	3,009	3,134	3,383	3,378	2,830	2,819	2,976	2,854	47	0.001	0.918	0.062
Experiment 2												
Dry matter	79.2	76.8	73.5	70.4	65.7	64.7	69.0	68.2	1.37	0.001	0.054	0.765
Nitrogen	86.6	84.5	79.8	77.8	80.7	78.5	78.8	80.1	1.01	0.001	0.082	0.248
Ether extract	76.4	67.1	67.0	55.6	77.3	73.4	76.4	72.5	2.30	0.001	0.001	0.258
IDE[5]	3,483	3,409	3,219	3,064	3,042	2,937	3,143	3,127	73	0.001	0.077	0.779

[1]Values are LSMeans of 8 replicate pens.

[2]Abbreviations: SBM= soybean meal; DDGS = distillers dried grains with solubles; CTL = control, birds were given an in-feed anticoccidial drug; VAC = birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of diet, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

[5]IDE = ileal digestible energy.

Table 4.4. Effects of coccidiosis vaccination on the apparent ileal digestibility of nutrients (%) and energy (kcal/kg) of individual feed ingredients in broilers at 12 d post-hatch. [1,2]

	Corn		SBM		DDGS			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Ingredient	Vaccination	Interaction[4]
Experiment 1										
Dry matter	109.6	91.8	59.2	55.4	76.4	65.8	3.19	0.001	0.001	0.082
Nitrogen	111.5	88.2*	81.0	74.3	82.8	95.4*	3.05	0.001	0.025	0.001
Ether extract	83.2	58.4*	112.0	120.2	77.3	112.9*	7.88	0.001	0.310	0.001
IDE[5]	4,553	4,410	2,787	2,647	3,278	2,721	163	0.001	0.036	0.335
Experiment 2										
Dry matter	70.6	65.7	56.8	46.9	55.5	58.3	4.19	0.001	0.166	0.201
Nitrogen	77.3	74.9	78.5	74.3	72.0	79.5	3.45	0.982	0.916	0.201
Ether extract	82.5	42.7*	101.5	103.0	86.5	100.0	7.67	0.001	0.147	0.001
IDE[5]	3,255	2,748	2,785	2,132	2,817	2,764	211	0.016	0.007	0.211

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]Abbreviations: SBM= soybean meal; DDGS = distillers dried grains with solubles; CTL = control, birds were given an in-feed anticoccidial drug; VAC = birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of diet, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

[5]IDE = ileal digestible energy.

Table 4.5. Effects of coccidiosis vaccination on apparent ileal amino acid digestibility (%) of experimental diets at 12 d post-hatch (Exp. 2).[1,2]

	Basal		Basal corn		Basal SBM		Basal DDGS			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Diet	Vaccination	Interaction[4]
Essential amino acids, %												
Arginine	92.0	90.9	88.4	87.1	86.9	87.0	86.0	86.7	0.49	0.001	0.253	0.113
Histidine	89.5	89.0	83.6	82.3	82.5	82.3	81.4	83.6	0.69	0.001	0.916	0.056
Isoleucine	87.9	86.5	81.9	80.3	80.3	79.8	79.5	81.9*	0.80	0.001	0.597	0.048
Leucine	88.6	87.1	83.5	81.8	80.3	79.5	81.1	84.3*	0.78	0.001	0.690	0.006
Lysine	90.0	89.0	84.5	82.9	83.5	83.4	81.6	81.9	0.71	0.001	0.191	0.511
Methionine	94.1	93.3	89.3	88.5	87.1	87.5	88.0	88.9	0.51	0.001	0.795	0.229
Phenylalanine	88.3	86.9	82.8	80.8	80.1	79.3	80.5	83.1*	0.80	0.001	0.453	0.017
Threonine	83.8	83.4	75.6	73.4	75.1	74.9	74.5	76.7	0.87	0.001	0.792	0.075
Valine	86.1	85.6	79.4	77.3	78.1	77.8	76.9	79.6*	0.84	0.001	0.875	0.032
Nonessential amino acids, %												
Alanine	88.1	87.7	82.9	81.1	79.9	79.4	80.3	83.1*	0.70	0.001	0.902	0.007
Aspartic acid	86.9	86.1	81.6	79.8	79.1	78.6	78.0	79.9	0.81	0.001	0.560	0.118
Cysteine	80.9	80.3	73.4	71.5	62.9	65.0	71.1	75.4*	1.14	0.001	0.202	0.029
Glutamic acid	91.6	90.6	87.9	86.6	85.8	85.0	84.9	86.7*	0.53	0.001	0.444	0.021
Glycine	85.5	84.9	77.6	76.1	76.0	76.3	75.0	77.6	0.83	0.001	0.762	0.074
Proline	88.1	86.6	83.0	81.3	79.1	79.3	80.1	83.7*	0.74	0.001	0.815	0.001
Serine	86.6	85.3	80.1	78.1	77.9	77.9	77.8	80.6*	0.82	0.001	0.801	0.016

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]Abbreviations: SBM= soybean meal; DDGS = distillers dried grains with solubles; CTL = control, birds were given an in-feed anticoccidial drug; VAC = birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of diet, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

Table 4.6. Effects of coccidiosis vaccination on apparent ileal amino acid digestibility (%) of individual feed ingredients at 12 d post-hatch (Exp. 2).[1,2]

	Corn		SBM		DDGS			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Diet	Vaccination	Interaction[4]
Essential amino acids, %										
Arginine	93.9	90.0	89.6	89.6	86.1	88.9	1.59	0.020	0.776	0.102
Histidine	83.0	78.8	78.7	80.7	75.2	81.7*	2.15	0.493	0.395	0.041
Isoleucine	82.5	76.7	77.6	78.1	74.4	82.2*	2.29	0.696	0.637	0.014
Leucine	86.7	80.9	75.3	75.4	78.4	89.5*	2.20	0.001	0.297	0.001
Lysine	85.7	80.8	83.1	84.4	76.1	77.6	2.07	0.002	0.662	0.187
Methionine	91.7	89.1	84.9	87.4	87.9	90.4	1.73	0.043	0.540	0.219
Phenylalanine	84.3	78.3	76.1	76.4	77.5	86.3*	2.27	0.026	0.564	0.006
Threonine	68.7	60.6*	67.0	69.0	64.7	72.2*	2.56	0.241	0.812	0.009
Valine	76.0	68.8*	72.0	73.6	68.5	76.6*	2.48	0.986	0.665	0.009
Nonessential amino acids, %										
Alanine	83.3	77.3	72.9	73.5	74.4	84.6*	2.29	0.004	0.383	0.003
Aspartic acid	82.2	76.3	74.3	75.5	70.5	76.7	2.25	0.030	0.780	0.057
Cysteine	67.7	61.4	32.9	43.1*	60.7	74.5*	3.36	0.001	0.031	0.007
Glutamic acid	93.8	89.5*	85.9	85.5	82.7	89.4*	1.59	0.004	0.577	0.003
Glycine	72.5	67.3	67.1	70.0	63.6	71.5*	2.57	0.630	0.356	0.035
Proline	85.6	80.2*	73.6	76.4	76.5	87.8*	2.02	0.001	0.076	0.001
Serine	79.8	72.6*	72.4	74.9	71.9	81.1*	2.25	0.364	0.400	0.002

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]Abbreviations: SBM= soybean meal; DDGS = distillers dried grains with solubles; CTL = control, birds were given an in-feed anticoccidial drug; VAC = birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of diet, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

CHAPTER 5: INFLUENCE OF COCCIDIOSIS VACCINE CHALLENGE ON ILEAL ENDOGENOUS FATTY ACID FLOW AND FATTY ACID DIGESTIBILITY OF DIETS CONTAINING SOYBEAN OIL AND POULTRY FAT IN BROILERS

ABSTRACT

An experiment was conducted to determine the impact of coccidiosis vaccination on the apparent ileal digestibility (**AID**) of ether extract (**EE**) and fatty acids (**FA**) from soybean oil and poultry fat diets and a fat-free diet was used to determine endogenous EE and FA losses and standardized ileal digestibility (**SID**) during vaccination. Four experimental treatments based on a factorial arrangement of vaccine status [control group in-feed diclazuril (**CTL**) or vaccine (**VAC**)] and 2 experimental diets were administered to 384 male Cobb 500 broilers in floor pens containing 12 birds per pen. For the vaccinated group, a 3x dose of a live coccidiosis vaccine was given via oral gavage on day of hatch. Experimental diets consisted a nutritionally complete diet supplemented with either soybean oil or poultry fat and fed to broilers from 0 to 12 d. For the determination of endogenous losses, CTL and VAC birds (192 birds total) were fed a common diet from 0 to 9 d and a fat free diet from 9 to 12 d. On d 12, ileal digesta was collected to determine AID of EE and FA and endogenous losses of EE and FA. Vaccination decreased ($P < 0.05$) AID of stearic acid and AID and SID of EE and oleic acid, whereas no differences ($P > 0.05$) in AID or SID of linoleic acid or total fatty acids were observed between CTL and VAC birds. Interactive effects ($P < 0.05$) were observed for AID and SID of palmitic acid which was reduced by vaccination in the soybean oil diet with no impact on the poultry fat diet. No differences ($P > 0.05$) in endogenous losses were observed between CTL and VAC birds; however, upon correcting for endogenous losses, the vaccine-induced impact in SID of EE and FA were lower than vaccine-impact reported in AID. In conclusion, endogenous FA losses are largely responsible for the reduction in lipid digestibility during coccidiosis vaccination.

INTRODUCTION

The supply of energy represents a major cost in feed formulation and supplemental lipids are the most concentrated energy sources used in broiler diets. In addition to having a high caloric value, lipids also aid in the absorption of fat-soluble vitamins (A, D, E, and K), supply essential fatty acids, and slow the feed passage rate, thus improving the digestion and absorption for all nutrients present in the diet (Mateos and Sell, 1980). Soybean oil and poultry fat are commonly used lipid sources in broiler diets due to their high digestibility and ideal fatty acid profile, whereby soybean oil is compatible for use in all vegetable based-diets and poultry fat is often utilized in conventional diets. However, bird specific factors, such as intestinal health status can influence to the digestion and absorption of dietary lipids.

Coccidiosis, a costly enteric disease, has been shown to negatively impact nutrient digestibility in broilers, with lipid digestibility being particularly affected. Indeed, Amerah and Ravindran (2015) reported that *Eimeria* infected broilers had a 18.8% reduction in starch and a 96% reduction in lipid digestibility compared with non-challenged birds at 21 d. In recent years, widespread adoption of antibiotic-free production systems, where the use of anticoccidial ionophores are not permitted, has increased live coccidiosis vaccination as a means of coccidiosis control but is often associated with impaired broiler performance and nutrient digestibility (Lehman et al., 2009; Gautier et al., 2019). Previous research conducted in our laboratory revealed that nutrient digestibility for floor-reared broiler given a coccidiosis vaccine at day of hatch was most impacted at 12 d post-hatch, whereby lipid digestibility was most severely impacted even when broilers were fed nutritionally complete diets that contained high-quality soybean oil as the lipid source (Gautier et al., 2019). Therefore, knowledge of the impact

of vaccination on different lipid sources can lead to informed dietary adjustments during the starter phase to support lipid digestibility in coccidiosis vaccinated broilers.

Endogenous nutrient losses are derived from digestive enzymes, mucin, and sloughed intestinal epithelial cells, and coccidiosis can influence the amount of endogenous nutrient losses in broilers (Fernando and McCraw, 1973; Collier et al., 2008; Amerah and Ravindran, 2015, Adedokun et al., 2016). Endogenous nutrient secretions are largely comprised of amino acids, and therefore previous research has frequently estimated the ileal endogenous losses of amino acids (Ravindran and Bryden, 1999; Lemme et al., 2004), but endogenous fatty acid and ether extract losses are also primarily derived from sloughed intestinal epithelial cells, as well as bile (Clément, 1980). Only a few studies have determined the ileal endogenous fatty acid losses in poultry (Ajuyah et al., 1996; Tancharoenrat et al., 2014) and to our knowledge, none have investigated the effect of *Eimeria* infection on endogenous fatty acid losses. This information would provide insight into the extent by which the reduction in apparent lipid digestibility can be attributed to increased endogenous fatty acid flow during coccidiosis, as well as the potential source of these fatty acids. Therefore, a fat-free diet was fed to determine the impact on of a coccidiosis vaccination model on ileal endogenous fatty acid flow and the values obtained were used to standardize the ether extract and fatty acid digestibility values of birds fed soybean oil or poultry fat diets.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

Coccidiosis Vaccination and General Bird Husbandry

A total of 576 male broiler chicks were obtained from a Cobb 500 female line from a commercial hatchery on day of hatch and allotted to 8 replicates pens (12 birds per pen; 0.09 m^2 per bird) in a factorial arrangement of vaccination status and dietary treatment. As such, one-half (288) of the chicks was orally gavaged with a live oocyst vaccine (Coccivac®-B52; Merck Animal Health, Intervet Inc. Millsboro, DE, USA) at 3x the manufacturers recommended dose (**VAC**), whereas the other half were not vaccinated and received in-feed diclazuril (Clinacox, Huvepharma), a chemical coccidiostat, throughout the entire experiment (**CTL**). The vaccine was delivered by oral gavage (0.25 mL/bird) using a stainless-steel gavage needle to provide uniform administration. All chicks were group-weighed and distributed to 48 floor pens on unused pine shavings. Throughout the trial, litter was sprayed daily with water to increase litter moisture to promote oocyst sporulation. Each floor pen was equipped with a hanging feeder and a nipple drinker line. Birds were provided access to feed and water *ad libitum* throughout the 12 d experiment and the lighting schedule and temperature targets were adjusted according to management guidelines published by the primary breeder (Cobb-Vantress, 2015).

Digestibility of Diets Containing Soybean Oil and Poultry Fat

Upon arrival, CTL and VAC birds (384 male broiler chicks total) were weighed and provided 1 of 2 experimental diets that consisted of a nutritionally complete corn soybean meal-based diet, where either soybean oil or poultry fat was added as the lipid source (Table 5.1). Both soybean oil and poultry fat were added at the same inclusion level (2.35%). Furthermore, the ME value of soybean oil used in dietary formulation was 8,800 kcal/kg and the ME value used for poultry fat was 8,200 kcal/kg, and therefore experimental diets were not isocaloric. Experimental diets were fed from 0 to 12 d post-hatch and all diets were formulated to meet or exceed published nutrient recommendations (Cobb-Vantress, 2015).

Although growth performance was not a primary objective of this trial, birds and feeders were weighed at 0 and 12 d post-hatch, for calculation of body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) to assess growth performance as influenced by coccidiosis vaccination. All dead and culled birds were weighed individually and FCR calculations were adjusted to include the weight gain of dead birds. The number of oocysts per gram of excreta samples collected from each pen was determined before bird placement and at 7 and 12 d post-vaccination to ensure *Eimeria* infection. Fresh excreta samples were collected from each pen using wax paper placed on the litter 12 h prior to collection. Additionally, unused litter samples were taken before placement to confirm the absence of oocysts. All samples were placed in airtight conical tubes and kept refrigerated until processing. Samples were soaked in water overnight and homogenized by vigorous stirring. Following homogenization, 1 ml of sample was further diluted with 9 ml of saturated salt solution and pipetted into the chamber of a McMaster counting slide. Duplicate counts were made for each sample using the following equation:

Oocysts per gram of sample = (Oocyst count × dilution × volume) / (volume of counting chamber × weight of sample), where the dilution was 10 and the volume of the counting chamber was 0.15 ml.

At 12 d post-hatch, all birds were humanely euthanized by CO_2 inhalation. Ileal contents from all birds in each pen were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples within each pen were pooled and frozen (−20°C) until analysis.

Ileal Endogenous Losses of Ether Extract and Fatty Acids

Upon arrival, CTL and VAC birds (192 male broiler chicks total) were weighed and provided of a nutritionally complete corn soybean meal-based common diet, with soybean oil as

the lipid source, from 0 to 9 d post-hatch (Table 1). A fat-free purified diet was formulated and introduced at 9 d post-hatch and fed *ad libitum* till 12 d post-hatch (Table 5.2) to allow for the determination of ileal endogenous ether extract losses, endogenous fatty acid (**FA**) composition, and to use these ileal endogenous losses to standardize the apparent ileal digestibility values of the soybean oil and poultry fat diets. At 12 d post-hatch, all birds were humanely euthanized by CO_2 inhalation and immediately dissected for ileal digesta collection. Ileal contents from all birds in each pen were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples within each pen were pooled and frozen (−20°C) until analysis.

Laboratory Analyses

Frozen digesta samples were lyophilized and ground using an electric coffee grinder. Diets and digesta were analyzed for dry matter, titanium, ether extract, and FA content. Diets were also analyzed for nitrogen and gross energy content. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to AOAC (2006) method 920.39. The fatty acid composition was also determined in ileal digesta and diet samples. Direct transesterification was performed by incubating dried samples in 2.0 mL of 0.2M methanolic potassium hydroxide in screw-capped tubes at 50°C for 30 minutes. Samples were vortexed 2 to 3 times per minute until samples were dissolved (Murrieta et al., 2003). After tubes had cooled to room temperature, 1 mL of saturated sodium chloride was added to each tube. A 1-mL hexane solution, which contained an internal standard [glyceryl tridecanoic acid (13:0)] was then added to each tube, and the hexane was evaporated before tubes were vortexed and subsequently centrifuged for 5 minutes at 1,100 × g

and 20°C to separate the methyl esters. Separation was achieved by injecting 1 μL of the hexane layer which contained the FA methyl esters into gas chromatography vials which contained a 1.0-mm bed of anhydrous sodium sulfate. Fatty acids were determined using gas chromatography (Model HP 5890 Series II GC, with an HP-7673 automatic injector and HP-3365 software; Hewlett-Packard, Avondale, PA) equipped with a 100-m capillary column (0.25-mm i.d.; Model 2560 fused-silica capillary column; Supelco Inc., Bellefonte, PA) and helium as the carrier gas at 1.0 mL/minute (1:50 split ratio) and a flame ionization detector. Oven temperature was maintained at 150°C for 5 minutes, raised to 195°C at a rate of 4°C/minute for 15 minutes, and then increased to 235°C at a rate of 2.5°C/minute for 15 minutes, whereas injector and detector temperatures were maintained at 250°C. Identification of peaks was accomplished by using purified standards obtained from Nu-Chek Prep (Elysian, MN), Matreya (Pleasant Gap, PA), and Supelco Inc. (Bellefonte, PA). Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of dry matter, ether extract, and individual FA were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration (%) to TiO_2 (%) in the diet or ileal digesta.

The endogenous ether extract losses and individual FA at the distal half of the ileum were calculated using the following equation:

$$\text{Endogenous losses or FA, mg/kg of DM intake} = [(TiO_{2diet} / TiO_{2digesta}) \times \text{fat or FA concentration}_{digesta}]$$

Upon determination of the endogenous losses, the apparent ileal digestibility values were standardized, where the standard ileal digestibility (**SID**) of ether extract and FA were calculated using the following equation:

$$\text{SID, \%} = \text{AID} + [(\text{Endogenous loss / lipid concentration}_{\text{diet}}) \times 100]$$

Statistical Analyses

Treatments for determining fatty acid digestibility were comprised of a factorial arrangement of vaccination status (CTL or VAC) × 2 lipid sources in a completely randomized design. Data were subjected to a 2-way ANOVA using the MIXED procedure of SAS 9.4 to assess the main effects of lipid source, vaccination status, and their interaction. Statistically different treatment means were separated using a Tukey's multiple comparison test and orthogonal contrasts between CTL and VAC birds were used to assess the impact of vaccination on birds within a dietary treatment. Data obtained from birds fed the fat-free diet were subjected to ANOVA using the MIXED procedure of SAS 9.4 and are presented as least squares means of vaccination status (CTL or VAC). In both analyses, pen was considered the experimental unit with 8 replicate floor pens per treatment and statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Digestibility of Diets Containing Soybean Oil and Poultry Fat

To ensure measurable impacts of vaccination, birds were orally gavaged with a dose of vaccinal oocysts that was higher (3x) than the vaccine manufacturer's recommended dose in this model. No oocysts were detected in the litter before bird placement and VAC birds had greater ($P < 0.05$) excreta oocyst output at 7 and 12 d post-hatch than CTL birds, with no independent or interactive lipid source effects, confirming that oocyst cycling occurred in the vaccinated birds

(data not shown). Vaccination decreased ($P < 0.05$) FI and BWG by 10 and 16%, and increased ($P < 0.05$) FCR by 2 points, respectively, compared with CTL birds (data not shown). Therefore, the increase in oocysts and reduction in performance collectively indicate a successful vaccine model was achieved.

The unsaturated to saturated FA ratios in the soybean oil and poultry fat diets were 4.05 and 2.65, respectively (Table 5.3). Palmitic and stearic acids were the major saturated FA in both the soybean oil and poultry fat diets, whereas oleic and linoleic acids were the major unsaturated FA. The FA composition of individual soybean oil and poultry fat were not analyzed in the current experiment but the FA composition of the experimental diets were generally as expected based on previous reports of the FA composition of soybean oil and poultry fat (Sauvant et al., 2004; Tancharoenrat et al., 2014). Additionally, FI tended ($P = 0.06$) to be higher in birds fed the poultry fat diet than in birds fed the soybean oil diet, with no independent or interactive effects ($P > 0.05$) of lipid source observed on BWG or FCR.

The AID of ether extract and the 4 major FA (palmitic, stearic, oleic, and linoleic acids) found in the soybean oil and poultry fat diets are presented in Table 5.4. Vaccination decreased AID of ether extract ($P < 0.05$), with no independent or interactive lipid source effects. A lipid source by vaccine interaction for AID of palmitic acid was observed, whereby vaccination reduced ($P < 0.05$) AID of palmitic acid in birds fed the soybean oil diet but not ($P > 0.05$) in those fed the poultry fat diet. Independent effects of vaccine status ($P < 0.05$) and lipid source ($P \leq 0.05$) were observed for AID of stearic acid, whereby AID of stearic acid was lower in VAC birds than in CTL birds and higher for birds fed the poultry fat diet than for those fed the soybean oil diet. The AID of oleic acid was lower ($P < 0.05$) in VAC birds than in CTL birds,

with no independent or interactive effects of lipid source, whereas neither lipid source nor vaccine status influenced ($P > 0.05$) AID of linoleic acid or AID of total FA.

In general, FA digestibility was typically not influenced by lipid source and is likely attributed to the similar FA profile between soybean oil and poultry fat. Tancharoenrat et al. (2013) also reported that ether extract digestibility values were similar between soybean oil and poultry fat diets when total tract digestibility was determined. The vaccine-induced reduction in AID of ether extract observed in the current experiment is in agreement with findings of other trials that have indicated that *Eimeria* exposure severely impacts lipid digestibility (Amerah and Ravindran, 2015; Gautier et al., 2019). Additionally, Adams et al. (1996) published that the replacement of animal fat with coconut oil resulted in improved lipid digestibility during an *E. acervulina* infection, whereas, the replacement of animal fat with soybean oil did not improve lipid digestibility. This was likely attributed to differences in fatty acid chain length among sources, whereby medium chain fatty acids such as lauric and myristic acids, which coconut oil is largely comprised of, are more efficiently absorbed than longer chain fatty acids (Adams et al., 1996). In the current experiment, impaired ether extract digestibility did not translate to a reduction in total fatty acid digestibility, which may be attributed to the high concentration of linoleic acid in both diets, which was also not impacted by vaccination.

Lipid digestion relies heavily on bile salts for efficient lipid emulsification to increase the surface area of lipid droplets for access by enzymatic lipase (Polin et al., 1980). Adams et al. (1996) reported coccidiosis challenged birds supplemented with cholic acid had an improvement in lipid digestibility at d 21, indicating that bile salt synthesis or secretion may be impaired. However, a reduction in bile salt secretion would likely affect the solubilization of saturated FA more than that of unsaturated FA (Garrett and Young, 1975), and because oleic acid was also

negatively impacted by vaccination in the current experiment, this suggests additional mechanisms other than just solely impaired bile salt secretion or synthesis during *Eimeria* exposure. Furthermore, the vaccine-induced reduction of oleic acid may have partially attributed to the lower digestibility of stearic acid in VAC birds, as saturated FA also require an adequate presence of unsaturated FA for efficient lipid emulsification. Unsaturated FA function as natural emulsifiers and form mixed micelles with saturated fatty acids, thus improving the emulsification and absorption of saturated FA (Polin et al., 1980). However, despite the soybean oil diet having a higher concentration of unsaturated FA, the AID of palmitic acid was reduced by vaccination in birds fed the soybean oil diet but was not impacted by vaccination in birds fed the poultry fat diet. This further suggests that the absorption of FA may be more limiting than the digestion of FA during an *Eimeria* exposure. Birds infected with *E. acervulina* are believed to have impaired intracellular lipid processing or transport across the enterocyte cell membrane, as supported by an observed accumulation of lipids within duodenal villus epithelial cells (Sharma and Fernando, 1975). Therefore, intestinal villus cells infected by *E. acervulina* may prevent lipids that were absorbed by enterocytes from being incorporated into portomicrons to be secreted into circulation for utilization.

Ileal Endogenous Losses of Ether Extract and Fatty Acids

Apparent ileal digestibility does not account for endogenous nutrients that are secreted into the lumen. Coccidiosis can result in increased mucogenesis and rapid turnover of and sloughing of intestinal cells, which will likely increase endogenous amino acid losses (Fernando and McCraw, 1973; Collier et al., 2008; Amerah and Ravindran, 2015). However, the primary sources of endogenous fatty acid and ether extract losses are also derived from sloughed intestinal epithelial cells, as well as bile (Clément, 1980). Ileal endogenous losses of ether extract

were determined to be 2,688 and 4,136 mg/kg of DM intake for the CTL and VAC birds, respectively (Table 5.5). However, this 54% increase was determined to not be statistically different ($P > 0.05$) due to the large variability associated with this measurement, which likely stemmed from the low sample size of endogenous fatty acid losses. For both the CTL and VAC birds, the major saturated FA in ileal endogenous losses were palmitic and stearic acids, whereas the main unsaturated FA were oleic and linoleic acids. Similarly, for both the CTL and VAC birds the endogenous FA profile showed the major saturated FA were palmitic and stearic acids and the major unsaturated FA were oleic and linoleic acids. Ajuyah et al. (1996) and Tancharoenrat et al. (2014) also reported that palmitic, stearic, oleic, and linoleic acids were the major endogenous FA when birds were fed either a semi-purified diet containing less than 0.1% lipid or a fat-free diet, with Tancharoenrat et al. (2014) also observing a high content of arachidonic acid. Based off the endogenous FA profile in the current experiment, only 14 and 11% of the endogenous ether extract was of FA origin for the CTL and VAC birds, respectively, whereby the remainder likely consisted of non-FA sources such as cholesterol and the phosphate and glycerol head of the phospholipids in both bile and desquamated intestinal epithelial cells (Clément, 1980; Ajuyah et al., 1996; Tuchweber et al., 1999). Furthermore, Tancharoenrat et al. (2014) observed that the endogenous FA profile corresponded closely to the FA profile of bile in birds at 26 post-hatch whereby the unsaturated FA profile of bile was largely comprised of linoleic and arachidonic acids and therefore, the lack of arachidonic acid detection in the endogenous FA pool herein may be due to limited arachidonic acid synthesis, lower bile secretion, or both, in the younger birds used in this experiment compared with those used by Tancharoenrat et al. (2014).

The SID values were calculated for the digestibility of ether extract and individual FA by correcting the AID values for endogenous losses (Table 5.6). Vaccinated birds had a 5% reduction ($P < 0.05$) in SID of ether extract when compared with CTL birds, which is much lower than the vaccine-induced reduction of 12% reported for AID of ether extract. The vaccine-induced reduction ($P < 0.05$) of palmitic acid in birds fed the soybean oil diet persisted for SID. Furthermore, vaccination did not impact ($P > 0.05$) SID of stearic acid, whereas before correcting for endogenous losses, vaccinated birds had a 4% reduction ($P < 0.05$) in AID of stearic acid when compared with CTL birds. On the other hand, the 6% reduction in SID of oleic acid ($P < 0.05$) between CTL and VAC birds was only slightly less than the observed 7% reduction in AID of oleic acid between CTL and VAC birds. Additionally, vaccine status did not influence ($P > 0.05$) SID of linoleic acid or SID of total FA.

Overall, the findings reported herein help further understand the digestion of FA in coccidiosis vaccinated broilers and provide information on the unavoidable endogenous losses of ether extract and FA that occur throughout the digestive process. Furthermore, these results indicate that the vaccine challenge model negatively impacted the digestibility of ether extract and most FA, regardless of the lipid source used in the diet. Though additional research is needed, these results suggest that endogenous losses from non-fatty acid sources are largely responsible for the reduction in lipid digestibility.

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TABLES

Table 5.1. Composition of experimental diets fed to broilers from 0 to 12 d post-hatch.[1]

Ingredient, % as-fed	Soybean oil	Poultry fat
Corn	61.55	61.55
Soybean meal (45%)	31.78	31.78
Lipid inclusion[2]	2.35	2.35
Limestone	0.99	0.99
Dicalcium phosphate	1.67	1.67
Salt	0.42	0.42
DL-methionine	0.25	0.25
L-lysine HCl	0.11	0.11
L-threonine	0.05	0.05
Trace mineral premix[3]	0.10	0.10
Vitamin premix[4]	0.10	0.10
Se premix[5] (0.06%)	0.02	0.02
Choline chloride (60%)	0.05	0.05
Santoquin	0.02	0.02
Titanium dioxide	0.50	0.50
Inert filler[6]	0.05	0.05
Calculated composition, % unless noted otherwise		
AME_n, kcal/kg	3,088	3,074
CP	19.88	19.87
Digestible lysine	1.11	1.11
Digestible TSAA	0.83	0.83
Digestible threonine	0.73	0.73
Calcium	0.86	0.86
Available P	0.44	0.44
Analyzed composition, % unless noted otherwise		
Gross energy, kcal/kg	3,989	4,039
CP	21.10	21.30
Ether extract	3.70	4.29

[1]AME_n = nitrogen-corrected apparent metabolizable energy.
[2]Soybean oil or poultry fat was added.
[3]Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg
[4]Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.
[5]Supplied 0.12 mg of selenium per kg of diet.
[6]Clinacox®, (Huvepharma Inc., Peachtree City, GA), provided 1ppm diclazuril to the diet at the expense of the inert filler.

Table 5.2. Composition of the fat-free purified diet fed to broilers from 9 to 12 d post-hatch.[1]

Ingredient	%, as-fed
Dextrose	66.50
Casein	22.00
Solka-Floc[2]	4.00
Dicalcium phosphate	2.20
Sodium bicarbonate	0.50
Limestone	0.70
Potassium sulfate	1.00
Salt	0.23
DL-methionine	0.55
L-arginine	0.95
Trace mineral premix[3]	0.10
Vitamin premix[4]	0.10
Se premix[5] (0.06%)	0.02
Choline chloride (60%)	0.10
Santoquin	0.50
Titanium dioxide	0.50
Inert filler[6]	0.05
Calculated composition, % unless noted otherwise	
AME_n, kcal/kg	3,327
CP	22.74

[1]AME_n = nitrogen-corrected apparent metabolizable energy.
[2]Purified cellulose (International Fiber Corp., North Tonawanda, NY).
[3]Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg
[4]Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.
[5]Supplied 0.12 mg of selenium per kg of diet.
[6]Clinacox®, (Huvepharma Inc., Peachtree City, GA), provided 1ppm diclazuril to the diet at the expense of the inert filler.

Table 5.3. Analyzed fatty acid composition of experimental diets (g/kg, as-fed basis).

Item	Soybean oil	Poultry fat
Saturated fatty acid		
C14:0 Myristic	0.00	0.05
C16:0 Palmitic	2.52	3.89
C18:0 Stearic	0.62	0.90
C20:0 Arachidic	0.07	0.05
C22:0 Behenic	0.06	0.00
Unsaturated fatty acid		
C16:1 Palmitoleic	0.02	0.29
C18:1n-7 Vaccenic	0.30	0.34
C18:1 Oleic	3.92	4.73
C18:2 Linoleic	8.29	7.07
C18:3 Linolenic	0.69	0.50
C20:1 Elcosenoic	0.03	0.00
Total fatty acids	16.34	17.92
Unsaturated to saturated fatty acid ratio	4.05	2.65

Table 5.4. Apparent ileal digestibility (%) of ether extract and fatty acids at 12 d post-hatch in broilers fed diets supplemented with soybean oil or poultry fat.[1]

Treatment[2]	Ether Extract	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Total fatty acids
Interaction means						
Soybean oil + CTL	76.4	86.4	85.5	87.8	95.3	88.8
Soybean oil + VAC	67.1	79.9	80.2	79.0	93.1	90.3
Poultry fat + CTL	76.2	85.6	85.7	81.5	95.0	88.7
Poultry fat + VAC	67.8	85.1	84.4	78.6	92.1	89.1
SEM	2.89	1.56	1.27	2.59	1.70	1.55
Main effect of lipid source						
Soybean oil	71.8	83.2	82.8	83.8	94.2	89.6
Poultry fat	72.0	85.3	85.1	80.1	93.6	88.9
SEM	2.00	1.02	0.82	1.63	1.06	1.02
Main effect of vaccine						
CTL	76.3	86.0	85.6	84.6	95.1	88.7
VAC	67.4	82.5	82.3	78.8	92.6	89.7
SEM	2.04	1.00	0.80	1.67	1.06	1.05
P-values[3]						
Lipid source	0.934	0.118	0.051	0.134	0.658	0.641
Vaccination	0.004	0.015	0.006	0.013	0.080	0.520
Lipid source × vaccination	0.881	0.037	0.073	0.183	0.808	0.704

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of lipid source, vaccination, and their interaction.

Table 5.5. Ileal endogenous flow of ether extract and fatty acids, and the fatty acid profile of endogenous fat in broiler chickens.[1,2]

Treatment[3]	Ether Extract	C14:0 Myristic	C16:0 Palmitic	C18:0 Stearic	C16:1 Palmitoleic	C18:1 Oleic	C18:2 Linoleic	Total fatty acids
Ileal flow (mg/kg of DM intake)								
CTL	2,688	0.7	110.2	90.7	0.8	105.7	58.7	367
VAC	4,136	1.5	97.9	101.8	0.5	128.1	116.8	447
SEM	63.52	1.05	23.64	24.55	0.68	35.48	28.26	113.66
P-value	0.112	0.591	0.701	0.736	0.696	0.641	0.148	0.583
Profile of endogenous fat (g/kg of fat)								
CTL	-	0.3	41.0	33.7	0.3	39.3	21.8	136.5
VAC	-	0.4	23.7	24.6	0.1	31.0	28.2	108.1
SEM	-	0.37	9.65	10.25	0.30	14.78	10.73	43.17
P-value	-	0.817	0.103	0.395	0.517	0.584	0.565	0.524

[1]No other fatty acids were detected.

[2]Each value represents the mean of 5 or 6 replicates.

[3]CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a 3× dose of vaccine on 0 d.

Table 5.6. Standardized ileal digestibility (%) of ether extract and fatty acids at 12 d post-hatch in broilers fed diets supplemented with soybean oil or poultry fat.[1]

Treatment[2]	Ether Extract	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Total fatty acids
Interaction means						
Soybean oil + CTL	82.4	90.5	98.9	90.4	95.9	90.9
Soybean oil + VAC	78.1	82.6	96.8	82.4	94.6	93.3
Poultry fat + CTL	83.3	88.6	95.5	83.8	95.0	90.8
Poultry fat + VAC	77.2	87.5	95.4	81.2	93.7	90.0
SEM	2.71	1.18	1.32	2.59	1.63	1.35
Main effect of lipid source						
Soybean oil	80.3	86.6	97.9	86.4	95.3	92.1
Poultry fat	80.3	88.0	95.8	82.5	94.4	90.4
SEM	1.77	1.16	0.83	1.63	1.02	0.92
Main effect of vaccine						
CTL	82.9	89.5	97.2	87.1	95.5	90.9
VAC	77.7	85.0	96.1	81.8	94.2	91.6
SEM	1.77	1.16	0.81	1.67	1.05	0.92
P-values[3]						
Lipid source	0.990	0.353	0.035	0.086	0.514	0.199
Vaccination	0.037	0.007	0.282	0.021	0.342	0.557
Lipid source × vaccination	0.699	0.036	0.353	0.222	0.994	0.217

[1]Values are LSMeans of 7 or 8 replicate pens.

[2]CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a 3× dose of vaccine on 0 d.

[3]Overall ANOVA *P*-values for the effects of lipid source, vaccination, and their interaction.

CHAPTER 6: INFLUENCE OF STARTER DIET ENERGY CONCENTRATION ON NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE AND PROCESSING CHARACTERISTICS OF COCCIDIOSIS VACCINATED BROILERS

ABSTRACT

An experiment was conducted to determine if live performance, processing characteristics, and apparent ileal digestibility (**AID**) of nutrients and energy (**IDE**) in coccidiosis vaccinated broilers can be improved by feeding diets that account for vaccine-induced reductions in lipid digestibility. Six treatments based on a factorial arrangement of vaccine status [control with in-feed diclazuril (**CTL**) or vaccinated (**VAC**)] and 3 starter diets [3,008 (standard), 3,058 (moderate), and 3,108 (high) kcal/kg apparent ME_n] with varying soybean oil concentrations were administered to male Cobb 500 broilers in floor pens (12 birds per pen). A 1x dose of a live coccidiosis vaccine was given to VAC birds via oral gavage on day of hatch. Birds received experimental starter diets from 0 to 18 d and common grower (18 to 31 d) and finisher (31 to 43 d) diets. Body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined at 11, 18, 31, and 43 d. On d 11, blood and ileal digesta were collected from birds in 7 replicate pens to measure plasma carotenoids and determine nutrient AID and IDE. At d 11, VAC increased ($P < 0.05$) excreta oocyst counts and decreased ($P < 0.05$) plasma carotenoids, nutrient AID, and IDE in all diets compared with CTL birds. From 0 to 18 and 0 to 31 d, VAC decreased ($P < 0.05$) BWG. For the same periods, VAC increased (energy x VAC, $P < 0.05$) FCR of birds fed moderate and high energy but not those fed standard energy diet. From 0 to 43 d, VAC only increased FCR of birds fed the moderate energy starter diet (energy x VAC, $P < 0.05$). Carcass yields were lower ($P < 0.05$) for VAC birds than for CTL birds and interactive effects ($P < 0.05$) were observed for wing yield. Overall, coccidiosis vaccinated birds had a reduction in AID and IDE and increasing starter diet energy levels through soy oil supplementation impaired FCR of VAC birds.

INTRODUCTION

Consumer demand for poultry products originating from birds reared without antibiotics has increased the use of vaccines to replace ionophore anticoccidial drugs for the control of coccidiosis. As such, vaccines are increasingly used in rotational or "bioshuttle" programs with chemical anticoccidial drugs, which are often permitted in these systems but are susceptible to resistance. Although live oocyst vaccination is effective in promoting immunity and protection against clinical coccidiosis, it can induce a mild transient form of coccidiosis, referred to as "coccidiasis", that usually occurs between 14 and 28 d post-hatch and can impair broiler performance (Lehman et al., 2009). Impaired feed efficiency during vaccine cycling is presumably due in part to nutrient malabsorption associated with intestinal damage and inflammation attributed to the sub-clinical, vaccine-induced infection (Lehman et al., 2009; Lee et al., 2011; Adedokun et al., 2016),

Previous work conducted in our laboratory longitudinally characterized nutrient digestibility in floor-reared broilers given a coccidiosis vaccine at day of hatch and observed the greatest impact on nutrient digestibility at 12 d post-hatch, with the duration and magnitude of this impact greatest for lipid digestibility (Gautier et al., 2019). Intestinal damage associated with an *Eimeria* infection can increase epithelial cell turnover and induce an inflammatory immune response, both which are both energetically costly and occur at the expense of broiler performance (Choct, 2009; Persia et al., 2006; Teeter et al., 2008). In addition to directly influencing energy utilization, impaired lipid digestibility can also reduce the absorption of fat-soluble nutrients, including vitamin D, which will in return reduce the absorption of calcium and phosphorus.

Providing an increased concentration or a more available source of nutrients for which digestibility is impaired is a potential strategy to support the performance of broilers during coccidial vaccine cycling (Adedokun et al., 2016). Indeed, previous research reported that increased dietary digestible amino acid content improved the performance of coccidiosis vaccinated broilers (Adedokun et al., 2016; Cloft et al., 2019), but responses of vaccinated broilers to dietary energy has not been investigated. Korver et al. (1998) reported that increasing dietary metabolizable energy from 2,714 to 3,303 kcal/kg by increasing corn oil supplementation improved the performance of lipopolysaccharide-challenged broilers, whereas increased tallow supplementation did not. Therefore, the potential benefits of feeding increased energy diets to broilers may be influenced by the source of dietary energy. As such, the objective of this experiment was to determine if live performance and processing characteristics of coccidiosis vaccinated broilers can be improved by feeding increased dietary energy density diets, achieved through increased soybean oil supplementation, during the starter phase to account for the expected vaccine-induced reductions in energy digestibility during this period.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

General Bird Husbandry and Dietary Treatments

A total of 1,368 male broiler chicks from a Cobb 500 female line were obtained from a commercial hatchery on day of hatch. Upon arrival, one-half (684) of the chicks was orally gavaged with the manufacturer's recommended dose of a live oocyst vaccine (Coccivac®-B52; Merck Animal Health, Intervet Inc. Millsboro, DE, USA) (**VAC**), whereas the other half was not vaccinated and received in-feed diclazuril (Clinacox, Huvepharma), a chemical coccidiostat,

throughout the entire experiment (**CTL**). The vaccine was delivered by oral gavage (0.25 mL/bird) using a stainless-steel gavage needle to provide uniform administration. All chicks were group-weighed and distributed to 114 floor pens on unused pine shavings. Throughout the trial, litter was sprayed daily with water to increase litter moisture to promote oocyst sporulation. Each floor pen was equipped with a hanging feeder and a nipple drinker line and contained 12 birds (0.09 m^2 per bird). Birds were provided access to feed and water *ad libitum* throughout the 43 d experiment and the lighting schedule and temperature targets were adjusted according to management guidelines provided by the primary breeder (Cobb-Vantress, 2015).

From 0 to 18 d of age, CTL and VAC birds were provided 1 of 3 isonitrogenous experimental starter diets that contained 2.10%, 2.67%, or 3.24% soybean oil (ME value of soybean oil used in dietary formulation was 8,800 kcal/kg) included at the expense of cellulose and had calculated AME_n contents of 3,008 (standard), 3,058, (moderate) and 3,108 (high) kcal/kg, respectively (Table 6.1). Throughout the remainder of the trial, CTL and VAC birds were fed common grower (18 to 31 d) and finisher (31 to 43 d) diets. Corn, soybean meal, and distillers dried grains with solubles were used as the primary ingredients and all diets were formulated to meet or exceed published nutrient recommendations (Cobb-Vantress, 2015).

Determination of Growth Performance, Excreta Oocyst Shedding, and Plasma Carotenoids

Birds and feeders were weighed at 0, 11, 18, 32, and 43 d post-hatch for calculation of body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) to assess growth performance. All dead and culled birds were weighed individually and FCR calculations were adjusted to include the weight gain of dead birds. From 0 to 11 d post-hatch, performance parameters were represented by 19 replicate pens for each treatment. At 11 d post-hatch, after all pens had been weighed, 7 replicate pens were selected for the collection of blood and ileal

digesta. The remaining 12 replicate pens were used for performance measurements throughout the remainder of the trial.

Oocyst shedding, as indicated by the number of oocysts per gram of excreta samples collected from each pen, was determined before bird placement and at 11 d post-vaccination to confirm vaccine cycling. Samples of unused litter shavings were taken before placement, whereas fresh excreta samples at 11 d were collected from each pen using wax paper placed on the litter 12 h prior to collection. All samples were placed in airtight conical tubes and kept refrigerated until processing. Samples were soaked in water overnight and homogenized by vigorous stirring. Following homogenization, 1 ml of sample was further diluted with 9 ml of saturated salt solution and pipetted into the chamber of a McMaster counting slide. Duplicate counts were made for each sample using the following equation:

Oocysts per gram of excreta = (Oocyst count × dilution × volume) / (volume of counting chamber × weight of sample), where the dilution was 10 and the volume of the counting chamber was 0.15 ml.

At 11 d post-hatch, all birds from 7 replicate pens were euthanized by CO_2 inhalation. Blood was collected from two randomly selected birds per pen via cardiac puncture and placed into tubes containing EDTA. After collection, tubes were placed on ice and subsequently centrifuged for 15 min at 1,300 × *g* and 4°C to separate plasma. Plasma from birds within a pen were pooled, aliquoted, and stored at -80°C until further analysis. All blood processing and carotenoid analysis procedures were conducted under yellow light and were determined by spectrophotometry previously described by Allen (1987).

Determination of AID and IDE

At 11 d post-hatch, contents of the distal half of the ileum from all birds in the 7 replicate pens, including the 2 birds randomly selected for the collection of blood, were collected by gently flushing with deionized water. Digesta samples within each pen were pooled and frozen (−20°C) until analysis. Frozen digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract content. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to AOAC (2006) method 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (**AID**) of dry matter, gross energy, ether extract, and nitrogen, were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration (%) to TiO_2 (%) in the diet or ileal digesta. Energy digestibility (%) values obtained from the above equation were multiplied by the gross energy content of the feed to calculate apparent ileal digestible energy (**IDE**) in units of kcal/kg.

Processing Characteristics

At 43 d post-hatch, all birds from 8 replicate pens were selected for processing after 12 h of feed withdrawal. Birds were transported in coops to the University of Arkansas Pilot

Processing Plant. Individual live bird weights were recorded immediately before live-hanging. Birds were humanely euthanized, processed, and eviscerated. Hot carcass and abdominal fat pad weights were collected, and carcasses were chilled for at least 2 h before deboning. All carcasses from each pen were deboned for collection of parts weights and yields, with yield calculated as a percentage of pre-slaughter live body weight. Processing outcomes included weights and yields of the following: hot and chilled carcass, hot abdominal fat pad, pectoralis major (breast), pectoralis minor (tenders), leg quarters (thigh and drum), and wings.

Statistical Analysis

Treatments were comprised of a factorial arrangement of vaccination status (CTL or VAC) × 3 starter dietary energy levels in a completely randomized block design. Pen was considered the experiment unit for all measurements. Data within each time point were subjected to a 2-way ANOVA using the MIXED procedure of SAS 9.4 to assess the main effects of dietary energy level, vaccination status, and their interaction. Statistically different treatment means were separated using a Tukey's multiple comparison test and orthogonal contrasts between CTL and VAC birds were used to assess the impact of vaccination on birds within a dietary treatment. Statistical significance was considered at $P < 0.05$.

RESULTS

Oocyst Shedding and Plasma Carotenoids

No oocysts were detected in the shavings before bird placement (data not shown). At 11 d post-hatch, VAC birds had higher ($P < 0.05$) excreta oocyst output and lower ($P < 0.05$) plasma carotenoid concentrations than CTL birds, with no independent or interactive effects of energy level (Table 6.2).

Growth Performance and Apparent Ileal Digestibility of Nutrients and IDE at 11 d post-hatch

Growth performance from 0 to 11 d post-hatch and nutrient and energy digestibility data are presented in Table 6.3. At 11 d, VAC birds had lower ($P < 0.05$) BWG and higher ($P < 0.05$) FCR than CTL birds, with no differences ($P > 0.05$) in FI. Independent effects of vaccine status ($P < 0.05$) and energy level ($P < 0.05$) were observed for AID of nutrients and IDE at 11 d post-hatch. Vaccinated birds had a lower ($P < 0.05$) AID of dry matter, nitrogen, ether extract, and energy and IDE compared with CTL birds. The AID of nitrogen was influenced by energy level ($P < 0.05$) and was highest for the high energy diet (81%), intermediate for the moderate energy diet (80%), and lowest for the standard energy diet (78%). Energy level also influenced AID of ether extract ($P < 0.05$) and was highest for the standard energy diet (91%), intermediate for the moderate energy diet (88%), and lowest for the high energy diet (86%). Furthermore, energy level also influenced IDE ($P < 0.05$) and was highest for the high energy diet (3,426 kcal/kg), intermediate for the moderate energy diet (3,384 kcal/kg), and lowest for the standard energy diet (3,279 kcal/kg). Dietary energy level did not influence ($P > 0.05$) AID of dry matter or energy.

Growth Performance to 43 d post-hatch and Processing Characteristics

At 18 d post-hatch, vaccination status influenced BWG and FI, whereby VAC birds had a lower ($P < 0.05$) BWG and FI than CTL birds, with no independent or interactive effects of energy level (Table 6.4). Additionally, an energy level by vaccine interaction for FCR was observed, where FCR was increased ($P < 0.05$) by vaccination in birds fed the moderate and high energy diets and was not influenced by vaccination ($P > 0.05$) in birds fed the standard energy diet. After 18 d post-hatch, birds were fed a common grower diet till 31 d post-hatch and a common finisher diet was fed from 31 to 43 d post-hatch. At 31 d post-hatch, BWG ($P < 0.05$) was lower for VAC birds than for CTL birds, with no dietary effects. There were no treatment

effects on FI ($P > 0.05$), but similar to the starter period, there was an energy level by vaccine interaction was observed for FCR ($P < 0.05$) whereby vaccination increased FCR in birds fed the moderate and high energy diets but not those ($P > 0.05$) fed the standard energy diet. Similarly, at 43 d post-hatch, vaccination status or energy level did not impact broiler BWG or FI ($P > 0.05$). However, a diet by vaccine interaction for FCR persisted whereby FCR was increased ($P < 0.05$) by vaccination in birds fed the moderate energy diet and was not influenced by vaccination ($P > 0.05$) in birds fed the standard or high energy diets.

At 44 d post-hatch, hot and chilled carcass yields and wing yields were lower ($P < 0.05$) for VAC birds than for CTL birds, with no effects of energy level (Table 6.5). A diet by vaccine interaction for wing weight was observed, whereby vaccination reduced ($P < 0.05$) the wing weight in birds fed the moderate and high energy diets but not those ($P > 0.05$) fed the standard energy diet. No other processing measurement was influenced by energy level or vaccination status ($P > 0.05$).

DISCUSSION

Previous experiments conducted within our laboratory to quantify reductions in IDE due to coccidiosis vaccination informed the selected increases in dietary energy concentration utilized for the current experiment (Gautier et al., 2019). Increased excreta oocyst output and decreased plasma carotenoids in VAC birds confirmed that oocyst cycling occurred in the vaccinated birds and that a successful vaccination model was achieved. Plasma carotenoids are inversely related to oocyst output and appear to be a sensitive indicator of coccidial-induced intestinal damage in birds (Hernández-Velasco et al., 2014). Coccidial-induced intestinal damage is often associated with nutrient malabsorption and the marked reduction in carotenoids, which are fat-soluble components (Yonekura and Nagao, 2007), are likely associated with the observed

reductions in lipid digestibility. However, carotenoid concentrations were not influenced by the dietary energy level, which was obtained from feeding increased concentrations of soybean oil, whereas lipid digestibility was.

Coccidiosis vaccination at day of hatch reduced BWG and impaired FCR of broilers at 11 d post-hatch, with no effects on FI. This impaired FCR was likely due to nutrient malabsorption, as vaccination decreased IDE by 303 kcal/kg and AID of dry matter, nitrogen, ether extract, and energy by 5.7, 4.5, 6.2, and 6.0 percentage units, respectively when compared with CTL birds at 11 d post-hatch. The vaccine-induced reduction in IDE was partially attributed to the reduced AID of ether extract, which reflects caloric costs due to coccidiosis vaccination. The vaccine-induced impact on lipid digestibility observed in the current experiment is in agreement with findings of other trials that have indicated that *Eimeria* exposure severely impacts lipid digestibility (Amerah and Ravindran, 2015; Gautier et al., 2019). However, lipid digestion and absorption are relatively complex processes and reductions in lipid digestibility during coccidia exposure are not well understood. Impaired lipid digestibility can certainly impair the absorption of other fat-soluble vitamins, particularly vitamin D, which can subsequently impair the absorption of calcium and phosphorus and influence bone development (Oikeh et al., 2019). Furthermore, increased digesta lipid content resulting from lipid malabsorption may decrease calcium utilization via the formation of insoluble intestinal soaps, which render both the lipid and the mineral unavailable to the bird (Tancharoenrat and Ravindran, 2014). Therefore, the potential for coccidiosis and excess dietary calcium to predispose broilers to necrotic enteritis may be associated with impaired lipid utilization (Titball et al., 1999).

As the energy density of starter diets were increased with soybean oil supplementation, the AID of nitrogen and IDE also increased. These results were expected, as previous research

has shown that supplemental lipids can reduce the digesta passage rate through the intestinal tract, allowing more time for the digestion and absorption of all nutrients present in the diet, including protein and starch (Mateos and Sell, 1980; Latshaw, 2008). However, a reduction in AID of ether extract, regardless of vaccination status, occurred as soybean oil supplementation increased. Therefore, birds at 11 d post-hatch may have a limited ability to efficiently digest or absorbed lipids and the additional supplemental lipids were not able to be utilized by the bird.

At 18 d post-hatch, coccidiosis vaccination reduced FI and BWG of broilers and while the reduction in FI for VAC birds, compared with CTL birds, had diminished by 31 d, VAC birds continued to have a lower BWG than CTL birds. Although, the reduction in BWG for VAC birds, compared with CTL birds, had diminished by 43 d post-hatch. Previous literature has also reported broilers administered an *Eimeria* vaccine at day of hatch had a 3 to 4% reduction in BWG at 17 and 21 d post-hatch, with no differences in BWG observed at 28 d post-hatch when compared with non-vaccinated broilers (Parker et al., 2007; Silva et al., 2009; Gautier et al., 2019). Therefore, coccidiosis vaccination provided an early *Eimeria* exposure which allowed birds to compensate for the minor reduction in weight before the end of the grow-out period. Similar to the finisher live weights, vaccination did not impact hot or chilled carcass weights at 44 d post-hatch in the current experiment. The reduction in wing weight for VAC birds fed moderate and high energy diets may be due to differences in the nutrient composition of the wing compared with the nutrient composition of the other processing parts, which were not impacted by energy level or vaccination status (Cloft et al., 2019). Starter diet energy levels did not influence any processing characteristics. Similarly, Birk et al. (2016) reported no differences in processing yields at 50 d of age when broilers were fed pre-starter diets that contained increased energy densities achieved by increased lipid inclusions at 1.3, 6, or 8% of the diet.

In the current experiment, increasing the dietary energy density by soybean oil supplementation in the starter period negatively impacted the FCR of VAC birds, not only during the starter period, but also throughout the grower and finisher periods. Vaccinated birds fed the moderate and high energy diets during the starter period had impaired FCR at 18 and 31 d post-hatch and while no differences in FCR were observed between CTL and VAC birds fed the high energy diet at 43 d post-hatch, the FCR of VAC birds fed the moderate energy diet remained impaired. However, since increased starter energy levels did not differentially impair nutrient digestibility of coccidiosis vaccinated birds, as no energy level by vaccine interaction was observed, this suggests an additional mechanism beyond impaired 11 d nutrient digestibility that caused the persistent energy level by vaccine interaction for FCR.

The negative impact of coccidiosis vaccination on FCR for birds fed the moderate and high energy levels may have been due to several factors. Benson et al. (1993) reported that BWG and FCR of lipopolysaccharide-injected birds responded positively when dietary energy was increased from 2,800 to 3,200 kcal ME/kg using cornstarch, whereas increasing dietary energy from 2,800 to 3,200 kcal ME/kg with corn oil resulted in a greater growth depression following lipopolysaccharide injection. Furthermore, following LPS administration, increased utilization of glucose by peripheral tissues and enhanced rates of gluconeogenesis from lactate and glucogenic amino acids have been reported (Spitzer and Spitzer, 1983). During periods of stress attributed to pathogen enteric infections, the demand for glucose *per se* a fuel source to enterocytes may be increased, as it has been recognized that the demand for glutamine, a glucogenic amino acid, increases during infection, to be utilized an energy source for immune cells and enterocytes (Mussini et al., 2012). Additionally, serum lipid levels have been reported to increase following an infection or inflammatory response, likely attributed to increased lipolysis and decreased

lipoprotein lipase activity, and therefore uptake and utilization of fatty acids by tissues may be reduced during an inflammatory response (Blackburn, 1977; Grunfeld and Feingold, 1992; van Heugen et al., 1996). Consumption of high fat diets in humans has also been shown to increase intestinal permeability via a reduction in mucin production and downregulation of tight junction proteins (Duan et al., 2018; Rohr et al., 2019). Therefore, the intestinal damage and inflammation associated with the sub-clinical, vaccine-induced infection in the current experiment may have been exacerbated when birds were fed increased supplementation of soybean oil. As such, an increase in energy density by carbohydrate or amino acid supplementation, and not lipid, may be important for maintaining the performance of coccidiosis vaccinated broilers.

In conclusion, coccidiosis vaccination did not compromise overall BWG, FI, or most of the processing weights. However, the results obtained from this study indicate that feeding increased energy density diets through greater soybean oil supplementation during the starter period can cause a detrimental effect to feed efficiency of vaccinated broilers that persists beyond the period of vaccine cycling. As such, further research is warranted to understand the mechanisms of vaccine-induced lipid malabsorption and its impacts on broiler gastrointestinal health beyond reduced energy availability and lipid-soluble vitamin absorption.

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TABLES

Table 6.1. Composition of diets fed to broilers from 0 to 43 d post-hatch.[1]

	Experimental (0 -18 d)				
Ingredient, % as-fed	Standard	Moderate	High	Grower	Finisher
Corn	53.94	53.94	53.94	58.46	60.45
Soybean meal (45%)	34.81	34.81	34.81	30.12	27.84
DDGS	4.00	4.00	4.00	6.00	6.00
Soybean oil	2.10	2.67	3.24	2.29	2.97
Limestone	1.09	1.09	1.09	1.09	1.03
Dicalcium phosphate	1.03	1.03	1.03	0.83	0.63
Salt	0.34	0.34	0.34	0.33	0.31
DL-methionine	0.31	0.31	0.31	0.25	0.21
L-lysine HCl	0.23	0.23	0.23	0.18	0.10
L-threonine	0.09	0.09	0.09	0.06	0.08
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10
Se premix [4] (0.06%)	0.02	0.02	0.02	0.02	0.02
Choline chloride (60%)	0.10	0.10	0.10	0.05	0.05
Santoquin	0.02	0.02	0.02	0.02	0.02
Phytase[5]	0.01	0.01	0.01	0.01	0.01
Xylanase[6]	0.03	0.03	0.03	0.03	0.03
Titanium dioxide	0.50	0.50	0.50	-	-
Inert filler[7]	1.19	0.62	0.05	0.05	0.05
Calculated composition, % unless noted otherwise					
AME_n, kcal/kg	3,008	3,058	3,108	3,108	3,175
CP	22.00	22.00	22.00	20.00	19.00
Digestible lysine	1.18	1.18	1.18	1.05	0.95
Digestible TSAA	0.89	0.89	0.89	0.80	0.74
Digestible threonine	0.77	0.77	0.77	0.69	0.65
Calcium	0.90	0.90	0.90	0.84	0.76
Available P	0.45	0.45	0.45	0.42	0.38
Analyzed composition, % unless noted otherwise[8]					
Gross energy, kcal/kg	4,027	4,082	4,062	4,038	4,097
CP	23.15	23.65	22.95	21.55	20.55
Ether extract	5.00	5.41	6.00	4.71	5.35

[1]DDGS = distillers dried grains with solubles; AME_n = nitrogen-corrected apparent metabolizable energy.

[2]Supplied the following per kg of diet: manganese, 100 mg; zinc, 100 mg; copper, 10.0 mg; iodine, 1.0 mg; iron, 50 mg; magnesium, 27 mg

[3]Supplied the following per kg of diet: vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine, 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3.5 mg; biotin, 0.33 mg.

[4]Supplied 0.12 mg of selenium per kg of diet.

[5]Optiphos®, (Huvepharma Inc., Peachtree City, GA), provided 250 FTU/kg of diet.

[6]Hostazym® X 250, (Huvepharma Inc., Peachtree City, GA) provided 250 FTU/kg of diet.

[7]Solcka-Floc (Cellulose International Fiber Corporation, North Tonawanda, NY) was used as the inert filler to make space for Clinacox® (Huvepharma Inc., Peachtree City, GA) to be fed to the unvaccinated birds and for the addition of soybean oil.

Table 6.2. Effects of coccidiosis vaccination and starter diet energy concentrations on oocyst per gram of excreta sample (OPG) and plasma carotenoid concentration (μg/mL) at 11 d post-hatch. [1,2]

	Standard		Moderate		High			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Energy	Vaccination	Interaction
OPG	268	8,113	1,416	9,826	567	14,746	1,791	0.137	0.001	0.134
Plasma carotenoids	1.96	1.17	1.69	1.05	1.86	1.05	0.191	0.557	0.001	0.876

[1]Values are LSMeans of 18 or 19 replicate pens for OPG and 7 replicate pens for plasma carotenoids.
[2]Abbreviations: CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d. Standard = 3,008 kcal/kg; moderate = 3,058 kcal/kg; high = 3,108 kcal/kg of nitrogen-corrected apparent metabolizable energy.
[3]Overall ANOVA *P*-values for the effects of energy, vaccination, and their interaction.

Table 6.3. Effects of coccidiosis vaccination and starter diet energy concentrations on broiler growth performance and apparent ileal digestibility of nutrients and energy in broilers at 11 d post-hatch.[1,2]

	Standard		Moderate		High			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Energy	Vaccination	Interaction[4]
Performance from 0 to 11 d post-hatch										
Body weight gain, kg/bird	0.288	0.281	0.295	0.277	0.283	0.276	0.004	0.277	0.002	0.316
Feed intake, kg/bird	0.349	0.340	0.353	0.339	0.334	0.341	0.005	0.127	0.182	0.061
FCR	1.219	1.232	1.200	1.247	1.189	1.244	0.014	0.817	0.001	0.270
Apparent ileal digestibility at 11 d post-hatch										
Dry matter, %	69.1	63.4	70.4	64.7	71.4	65.8	1.10	0.077	0.001	0.999
Nitrogen, %	80.3	76.2	82.0	77.2	83.4	79.1	0.88	0.003	0.001	0.767
Ether extract, %	92.3	89.2	90.6	84.7	90.9	81.3	1.86	0.024	0.001	0.142
Energy, %	72.3	66.6	73.5	67.5	74.8	68.4	1.14	0.116	0.001	0.955
IDE[5], kcal/kg	3,432	3,125	3,530	3,239	3,581	3,271	54	0.014	0.001	0.980

[1]Values are LSMeans of 19 replicate pens for broiler performance and 6 or 7 replicate pens for nutrient digestibility.

[2]Abbreviations: CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d. Standard = 3,008 kcal/kg; moderate = 3,058 kcal/kg; high = 3,108 kcal/kg of nitrogen-corrected apparent metabolizable energy.

[3]Overall ANOVA *P*-values for the effects of energy, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

[5]IDE = ileal digestible energy.

Table 6.4. Effects of coccidiosis vaccination and starter diet energy concentrations on broiler growth performance.[1,2]

	Standard		Moderate		High			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Energy	Vaccination	Interaction[4]
0 to 18 d post-hatch										
Body weight gain, kg/bird	0.700	0.681	0.711	0.659	0.703	0.667	0.009	0.740	0.001	0.206
Feed intake, kg/bird	0.899	0.879	0.899	0.859	0.881	0.881	0.012	0.671	0.044	0.233
FCR	1.290	1.316	1.279	1.328*	1.264	1.340*	0.008	0.981	0.001	0.006
0 to 31 d post-hatch										
Body weight gain, kg/bird	2.096	2.094	2.115	2.046	2.112	2.049	0.021	0.703	0.007	0.178
Feed intake, kg/bird	2.972	2.956	2.963	2.919	2.975	2.965	0.029	0.579	0.330	0.814
FCR	1.445	1.443	1.415	1.479*	1.416	1.459*	0.009	0.507	0.001	0.001
0 to 43 d post-hatch										
Body weight gain, kg/bird	3.419	3.439	3.424	3.384	3.429	3.392	0.028	0.628	0.377	0.447
Feed intake, kg/bird	5.392	5.406	5.363	5.350	5.409	5.395	0.434	0.505	0.892	0.936
FCR	1.603	1.595	1.579	1.627*	1.584	1.602	0.008	0.381	0.001	0.002

[1]Values are LSMeans of 12 replicate pens.

[2]Abbreviations: CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d. Standard = 3,008 kcal/kg; moderate = 3,058 kcal/kg; high = 3,108 kcal/kg of nitrogen-corrected apparent metabolizable energy.

[3]Overall ANOVA *P*-values for the effects of energy, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

Table 6.5. Effects of coccidiosis vaccination and starter diet energy concentrations on broilers processed at 44 d post-hatch.[1,2]

	Standard		Moderate		High			*P*-values[3]		
Item	CTL	VAC	CTL	VAC	CTL	VAC	SEM	Energy	Vaccination	Interaction[4]
Hot Carcass										
Weight, kg	2.541	2.530	2.533	2.506	2.557	2.520	0.029	0.777	0.301	0.900
Yield, %	75.12	74.73	75.26	75.05	75.40	74.87	0.175	0.351	0.012	0.672
Fat Pad										
Weight, kg	0.034	0.032	0.034	0.035	0.037	0.034	0.002	0.203	0.404	0.360
Yield, %	1.00	0.95	0.99	1.05	1.08	1.02	0.043	0.160	0.533	0.210
Chilled Carcass										
Weight, kg	2.575	2.565	2.569	2.538	2.592	2.555	0.029	0.766	0.284	0.895
Yield, %	76.13	75.75	76.34	76.01	76.43	75.94	0.176	0.312	0.008	0.894
Breast										
Weight, kg	0.683	0.680	0.684	0.673	0.688	0.680	0.010	0.757	0.202	0.965
Yield, %	20.46	20.04	20.31	20.12	20.23	20.15	0.150	0.925	0.063	0.510
Tender										
Weight, kg	0.132	0.134	0.130	0.130	0.133	0.132	0.002	0.196	0.937	0.624
Yield, %	3.91	3.91	3.86	3.89	3.90	3.92	0.030	0.455	0.495	0.848
Leg Quarters										
Weight, kg	0.785	0.788	0.779	0.781	0.789	0.792	0.010	0.517	0.740	0.999
Yield, %	23.14	23.26	23.15	23.41	23.30	23.54	0.133	0.248	0.053	0.829
Wings										
Weight, kg	0.269	0.270	0.273	0.263*	0.277	0.265*	0.002	0.489	0.001	0.023
Yield, %	7.96	7.97	8.12	7.89	8.08	7.89	0.617	0.797	0.008	0.094

[1]Values are LSMeans of 8 replicate pens.

[2]Abbreviations: CTL = control, birds were given an in-feed anticoccidial drug; VAC = vaccinated, birds were given a commercial dose of vaccine on 0 d. Standard = 3,008 kcal/kg; moderate = 3,058 kcal/kg; high = 3,108 kcal/kg of nitrogen-corrected apparent metabolizable energy.

[3]Overall ANOVA *P*-values for the effects of energy, vaccination, and their interaction.

[4]In the case of an interaction, an asterisk (*) denotes statistical significance ($P < 0.05$) of change due to vaccination within a diet type.

CHAPTER 7: GENERAL CONCLUSIONS

The overall focus of these studies were to evaluate the interrelationships among coccidiosis vaccination and nutrient utilization in floor-reared broiler chickens. In experiment 1, coccidiosis vaccination had no significant impact on overall body weight gain and feed intake of vaccinated birds, although overall FCR was impaired by vaccination. Coccidiosis vaccination elicited a transient reduction in digestibility of energy and nutrients that was most apparent at 12 post-hatch, particularly for lipids, but vaccinated birds were able to recover from these reductions by 20 d post-hatch. Jejunal morphology and duodenal pH were not impacted by vaccination and therefore impaired nutrient digestibility during coccidiosis vaccination may be attributed to intestinal inflammation, rather than intestinal damage. Additionally, the lack of response in plasma nitric oxide, a hallmark systemic inflammatory marker of acute coccidiosis, suggest that the inflammation is likely more localized with vaccination. Experiment 2 indicated that the effects of the vaccine challenge model varied among diet composition. Nutrient digestibility was negatively impacted in vaccinated birds fed corn and SBM, whereas nutrient digestibility was minimally impacted and in some cases, even positive effects on amino acid digestibility were observed in vaccinated birds fed DDGS. The high concentration of insoluble fiber in DDGS may have increased the rate of recovery from the vaccine-induced infection for the DDGS-fed birds. Therefore, during a mild coccidial infection, DDGS may be beneficial to intestinal function in broilers. Regarding experiment 3, the vaccine challenge model negatively impacted the digestibility of ether extract and most FA, regardless of the lipid source used in the diet. However, it is important to note that endogenous fatty acid losses appeared to be primarily responsible for the reduction in lipid digestibility. In experiment 4, coccidiosis vaccination reduced nutrient digestibility in all diets [3,008 (standard), 3,058 (moderate), and 3,108 (high) kcal/kg apparent ME_n], while overall body weight gain, feed intake, and most processing weights

were not influenced by coccidiosis vaccination. However, birds fed higher energy density diets through increased soybean oil supplementation in the starter period resulted in impaired feed efficiency in coccidiosis vaccinated broilers that persists beyond the period of vaccine cycling. Therefore, intestinal damage and inflammation associated with the sub-clinical, vaccine-induced infection may have impaired the uptake and utilization of lipids, which was further exacerbated when birds were fed increased soybean oil supplementation.

Collectively, data from these experiments indicate coccidiosis vaccination impaired nutrient digestibility during the starter period, particularly for lipids. Interestingly, morphological damage appeared to have no bearing on the reduction in nutrient digestibility during coccidiosis vaccination and instead the impaired nutrient digestibility was likely attributed to intestinal inflammation. Additionally, the inclusion of DDGS may be beneficial to broilers commercially vaccinated for the control of coccidiosis. Further research should be conducted to determine whether an increase in energy density by carbohydrate or glucogenic amino acid supplementation, instead of lipid supplementation, would be beneficial for maintaining the performance of coccidiosis vaccinated broilers.