THE EFFECT OF DIETARY PHYTIC ACID CONCENTRATION AND PHYTASE SUPPLEMENTATION ON PERFORMANCE, BONE ASH, AND INTESTINAL HEALTH OF BROILERS VACCINATED WITH A LIVE COCCIDIAL OOCYST VACCINE

Regina N. Lehman

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

Animal and Poultry Sciences

Audrey P. McElroy, Chair
Aaron J. Cowieson
Rami A. Dalloul
F. William Pierson

November 3, 2011
Blacksburg, Virginia

Keywords: phytic acid, phytase, coccidiosis vaccination, intestine, broiler

The effect of dietary phytic acid concentration and phytase supplementation on performance, bone ash, and intestinal health of broilers vaccinated with a live coccidial oocyst vaccine

Regina N. Lehman

ABSTRACT

The role of nutrition in providing optimal broiler growth and intestinal health is essential, especially during stress or disease challenge. Feed enzymes are useful for improving performance of poultry, particularly when nutrition, management, or health status is not favorable. The objective of the following experiments was to evaluate the effect of dietary phytic acid (PA) and phytase on the performance and intestinal health of birds that were vaccinated with a live coccidial oocyst vaccine. For each experiment, half of the chicks were spray-vaccinated at day-of-hatch with Coccivac®-B and grown out in floor pens with ad libitum access to diets formulated to meet Cobb nutrient recommendations. In the first experiment, birds were given one of three diets that included different levels of a PA solution to obtain dietary PA levels of 0.74, 0.87, and 1.12% for low, medium, and high PA diets, respectively. In the second experiment, two levels of PA were included to obtain dietary PA levels of 0.75 and 1.05% for low and high PA diets, respectively. In addition, phytase was added over the top to half of the diets at 1000 FTU/kg, resulting in four diets: low PA without phytase, low PA with phytase, high PA without phytase, and high PA with phytase. Live performance parameters including body weight, body weight gain, feed intake, feed conversion, and mortality were measured as well as tibia ash (experiment 2) and indicators of small intestinal health including morphology, apparent ileal amino acid digestibility (IAAD), and pH (experiment 2). The results presented here indicated that giving broilers vaccinated against coccidiosis a medium level of PA was detrimental to feed intake, body weight gain, and it induced necrotic enteritis ($P \leq 0.05$). Adding

phytase on top of nutritionally adequate diets did not improve performance ($P \geq 0.05$), but did improve ($P \leq 0.05$) apparent IAAD and morphology of the small intestine, especially in younger birds. In addition, it has been determined that important considerations in diet formulation also can include the phytate: protein as well as calcium: total phosphorus ratios, as these may critically affect how phytate impacts bird health and performance.

ACKNOWLEDGEMENTS

Thanks to God through whom all things are possible.

I would like to thank Dr. Audrey McElroy for the opportunity to do this research here at Virginia Tech. Thank you for pushing me, encouraging me, teaching me, caring about me, and advising me though every decision I had to make over the past few years (it was a LOT, I know). I honestly couldn't have done it without you! Your continued support and optimism were what helped me get through even the hardest of times. To my other committee members, Dr. Cowieson, Dr. Dalloul, and Dr. Pierson, thank you so much for your advice, editing, interest in my work, and overall encouragement in every aspect of my work. It was very much appreciated!

Thanks to Diego, Jen, Hannah, Christa, Lindsay, Chasity, Sungwon, Carrie, Adriana, and Robert…the best lab mates and friends I could ever ask for. Thanks for helping with all of the ridiculously long sampling days and for talking through everything with me. Words can't really describe the gratitude I have for you all. A special thanks to Akash for helping with lab work and doing the important job of washing dishes! Dale and Curt, you guys did a great job at helping me take care of the birds. Barbara and Sarah, thanks for letting me take over your labs on many occasions.

Thanks to Ting for always listening and helping and to all of the other grad students in the department who helped me have fun and relax at Hokie Hops. Thank you to my friends at Northstar (especially GAP group!) for your prayers and all of the fun times (I'm coming back for GAPsgiving every year so be sure to plan around my schedule☺). Thanks to my Mechanicsburg, Penn State, and Auburn friends for listening to me talk about my research and pretending to be interested, even if you weren't☺. You are all a huge part of my life, and I thank you for that!

Mom, dad, and the rest of my family, your support for what I'm doing is very much appreciated. Thank you so much. To my future Harris family, thank you for your interest in what I do and your constant belief in me.

A final thanks to Kevin. I don't even really know where to begin, so thank you for being there for me since the day I met you. Thanks for helping me check birds, helping with lab work, driving me to campus, making me smile, making me dinner, and calming me down before my prelims, presentations, defense, etc. You are my daily source of encouragement and I thank you for believing in me so much that I believe in myself!

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Tables **Page**

CHAPTER I

INTRODUCTION

In poultry, twenty percent of the energy expenditure of the whole body is attributed to the gastrointestinal tract (Choct, 2009). Therefore, any nutritional or management strategies to improve the health of the intestinal tract and limit energy expenditure have great implications for improving efficiency of production. One major disease of the poultry industry that negatively affects intestinal health is coccidiosis. Previous approaches to management of the negative impacts of this disease included dietary anti-coccidial medications; however, development of resistance to some medications along with increasing consumer preference for antimicrobial-free diets has encouraged the use of anti-coccidial vaccines. Although protection from coccidiosis with the use of a vaccine is generally agreed upon, early detrimental effects to intestinal health and bird growth have limited complete commercial acceptance of the vaccine. Despite the hesitation to universally accept vaccinating for coccidiosis, in a society where demand drives the sale of products, it may be necessary to discover strategies to limit unfavorable consequences on bird performance.

One current nutritional strategy that is commonly employed to improve the performance of poultry is the use of exogenous dietary phytase. Originally it was added to diets to improve the availability of phosphorus in the intestinal tract in an attempt to reduce environmental pollution to waterways and limit the amount of expensive inorganic phosphorus added to the diet. However, extensive research on phytase and its substrate phytate has revealed the many anti-nutritional effects of phytate and the negative impact it has on intestinal health and overall bird efficiency. Concomitantly, the benefits of providing exogenous phytase beyond that of

improving phosphorus availability have been realized, and the extent to which phytase is utilized in poultry diets has now increased to over half of all poultry diets around the world.

With these current practices in mind, the research in this dissertation evaluated the consequences of a live coccidial oocyst vaccine on broiler performance and intestinal health parameters. The level of phytic acid in the diet was altered to determine if intestinal health would be exacerbated in the presence of two factors known to have a negative impact on the health and overall efficiency of the bird. The first study was done in the absence of exogenous phytase in order to get a baseline effect of the interaction between phytic acid and a live coccidial oocyst vaccine. The second experiment included phytase in an attempt to improve parameters of intestinal health and performance and to have a better understanding of how phytase alters intestinal pH and morphology and apparent digestibility of amino acids in birds that have received a live coccidial vaccine.

CHAPTER II

LITERATURE REVIEW

INTESTINAL HEALTH

The health of the intestinal tract is extremely important as nutrient digestion and assimilation for growth occur here. Subsequently, when disruption of this system occurs, major consequences in terms of bird health and growth follow. In order to understand the factors that may influence intestinal health, it is important to appreciate the normal structure and function of the intestine in order to be able to recognize when the equilibrium of the system has been disrupted.

Anatomy. The intestinal mucosa of the bird is covered in villi, which serve to increase the absorptive surface area of the gastrointestinal tract (GIT). Day old chicks tend to have generally smooth plate-like or broad, finger-shaped shaped villi that attain more folds as the bird matures (Bayer et al., 1975). The enterocytes, which are responsible for nutrient digestion and absorption, originate from the crypts of Lieberkuhn as stem cells (Miles et al., 2006). As these cells mature, they migrate along the surface of the villi. After about 48 hours in the jejunum or slightly longer in the duodenum and ileum, the cells reach the tip of the villi and are extruded into the lumen of the intestine to be replaced with newer cells (Imondi and Bird, 1966). Longer villi tend to be indicative of a greater surface area for digestion and more mature enterocytes for absorption of dietary nutrients. A large/deep crypt can indicate rapid tissue turnover to replace cells that have been extruded from the villi tip due to normal sloughing or as a result of inflammation (Miles et al., 2006; Choct, 2009). As tissue turnover increases, more energy is diverted to cell renewal and less energy remains for growth of other tissues.

Goblet cells produce and secrete mucins and are interspersed throughout the surface of the villi (Moran, 1985). Microvilli are present on the surface of the epithelial and goblet cells to further increase the surface area. It is believed that microvilli may cover goblet cell pores except during active secretion (Bayer et al., 1975). Superimposed on the microvilli is a network of glycoprotein fibers known as the glycocalyx layer (Moran, 1985). This structure, along with secreted mucin, may aid to immobilize water and limit the passage of certain molecules, except for simple polysaccharides, to the surface of the enterocytes.

Microflora. The development and stability of a healthy intestinal tract is dependent upon the establishment of diverse microflora. Some of the known benefits to the host include fermentation of nutrients and secretion of usable products, stimulation of immunity, and prevention of colonization by pathogens (Snel et al., 2002; Richards et al., 2005; Allen and Torres, 2008). In the GIT of broilers, the number of microbes is approximately 10^9 per gram of ileal digesta and 10^{11} per gram of cecal digesta (Apajalahti, 2004). A healthy GIT is known to contain more than 200 different species of bacteria that may aid in the ability of an animal to resist infections (Apajalahti, 2004; Choct, 2009). Commonly known as competitive exclusion, endogenous microflora are able to compete with pathogenic microbes for space, nutrients, and binding sites, thus limiting the potential for infection. Collins and Carter (1978) demonstrated in mice that a germ-free mouse can be killed with 10 cells of *Salmonella enteritidis* while a lethal dose in conventional mice is about 10^9, demonstrating the ability of beneficial commensal microflora to control undesirable growth of pathogenic bacteria.

Bacteria obtain nutrients from dietary compounds that are either unable to be broken down by digestive enzymes or that are absorbed too slowly by the GIT (Apajalahti, 2004). As a result, the density of bacteria in the intestine increases from the proximal to distal GIT (Richards

et al., 2005). In the duodenum, bacterial density is low because of a rapid passage rate, acidic pH, and dilution of contents with pancreatic and bile secretions. Towards the distal GIT, enzymatic activities are reduced, and the pH is not as low, producing an environment favorable for bacterial growth (Rehman et al., 2007). In the ceca, an anaerobic environment, a longer passage time, and availability of nutrient substrates that have escaped digestion by the bird are conducive to substantial fermentation (Rehman et al., 2007). In the process of utilizing these compounds, bacteria release amino acids (AA), vitamins B and K, and short-chain fatty acids (SCFAs) like lactate, acetate, propionate and butyrate that are beneficial to the bird and can reduce potentially pathogenic bacteria in the cecum (Snel et al., 2002).

While commensal bacteria are generally beneficial, their presence is not without an energetic cost to the host. It has been found that microflora compete with the host for nutrients, generate toxic compounds, stimulate rapid turnover of enterocytes, increase mucus secretion, alter intestinal morphology, and stimulate a constant immune response (Richards et al., 2005). Microflora in the intestine can increase cell turnover and require the host to secrete more protective mucus that the bacteria may digest (Gaskins, 2001; Forder et al., 2007). As a result, maintenance energy requirements may increase with increased bacterial load.

Endogenous Secretions. Endogenous secretions are those that include saliva, pancreatic secretions, sloughed epithelial cells, bile, serum albumin, and mucin (Nyachoti et al., 1997; Adedokun et al., 2011). They are present in all healthy animals and contribute to a variety of functions such as digestion, lubrication, growth, and protection from pathogens. They are present at a constant level (basal losses/secretion) but may also be influenced by mucin turnover rate, dietary protein or AA, dietary fiber, phytate, presence and type of indigestible index marker, gut health, age, species, or testing method (Adedokun et al., 2011).

One major endogenous secretion that is paramount to GIT health is mucus. Mucus in the intestinal tract is primarily composed of glycoproteins called mucins (Forstner and Forstner, 1994). Mucins contain a core peptide that is 1,500 to over 4,500 AA in length, and each core peptide contains a major domain and a minor domain. The major domain contains high amounts of serine, threonine, and proline and is resistant to proteases due to dense glycosylation. The minor domain is rich in cysteine and is susceptible to proteases due to little glycosylation (Neutra and Fortner, 1987).

The layer of mucus on the mucosal surface of the intestine can serve as a barrier to pathogens (Forstner and Forstner, 1994; Forder et al., 2007). It was suggested that the *N*-acetylglucosamine (GlcNAc) residues of goblet cell mucins may prevent attachment of pathogenic bacteria and protect the epithelial surface (Sharma et al., 1997). At the same time, mucin is composed of carbohydrates that specific bacteria are able to utilize for their nutrient requirement (Forder et al., 2007). Other functions of mucin include lubrication of epithelial surfaces, protection of the mucosal surface from proteases, and interaction with the immune surveillance system (Forstner and Forstner, 1994).

Digestibility. Ileal digestibility is used to estimate bioavailability of nutrients to determine what proportion of ingested dietary compounds are absorbed and utilized for metabolism and growth (Stein et al., 2007). Due to the fact that most nutrients are absorbed only from the small intestine and hindgut, fermentation may influence nutrient metabolism. Therefore, ileal digestibility is used more frequently and believed to be a more accurate estimation of digestibility compared to total tract digestibility. The apparent ileal digestibility (AID) of AA and other nutrients is considered the net disappearance of ingested dietary nutrients from the digestive tract proximal to the distal ileum (Stein et al., 2007). It should be noted that in addition

to non-digested/absorbed dietary nutrients, apparent digestibility includes endogenously-secreted nutrients that were not reabsorbed proximal to the distal ileum. It can be calculated using the following equation:

% AID = [(nutrient intake – ileal nutrient outflow)/(nutrient intake)] X 100

Some other important terminology that is imperative to understand is regarding phosphorus. Total P (tP) is that which is contained in all forms in the diet (organic, inorganic). Available P (aP) is phosphorus from the diet that can be absorbed by the bird. It is determined by subtracting the P in the distal ileum from the total amount of P in the diet. Retained P is that which remains in the body and is determined by subtracting excreta P from the P in the diet. Most of the P in a typical poultry diet is found in plant ingredients and bound to a molecule known as phytic acid, which will be discussed in detail later. Phosphorus bound to the phytic acid molecule is known as phytate-P (PP), and P not bound to the molecule is known as non-phytate P (nPP). Subtracting the PP content from the tP will yield the nPP content of the diet (Angel et al., 2002).

NUTRTIONAL INFLUENCES ON INTESTINAL HEALTH

The influence of nutrition on the intestinal health and dynamics of poultry is becoming an increasingly popular area of study. Due to the fact that the intestinal tract accounts for 20% of the energy expenditure of the whole body (Choct, 2009), any modulation of the diet to support intestinal health may aid in more efficient production. Nutrition has been shown to have effects on important areas of GIT health including structural integrity of the intestine, balance of microflora, and immune status.

Intestinal Morphology. Because the intestinal surface is such a dynamic structure, it is able to adapt to dietary and health conditions of the bird. The rapid turnover of the epithelial surface enables adjustment in the length of the villi and the depth of the crypts to support efficient digestion. An "ideal" length of villi is determined by considering the advantage of nutrient acquisition and the cost of maintenance of villi length (Moran, 1985). Due to the rapid intestinal development immediately post-hatch (Uni et al., 1998), fasting can reduce proliferation and migration of small intestinal enterocytes and hinder crypt and villus development in the duodenum and jejunum (Geyra et al., 2001; Uni et al., 2003). Villi lengthening is believed to occur when the animal has ad libitum access to feed but is faced with increased energetic demands. For example, if production or maintenance needs were increased while receiving the same dietary ration. Similarly if production needs were maintained, but the level of nutrition in the dietary ration was dropped, villi would likely lengthen to compensate (Moran, 1985).

Another factor that may be responsible for lengthening of villi is the presence of microflora. Several studies (Cook and Bird, 1973; Forder et al., 2007) have found that compared to germ-free birds, conventional birds had longer villi, deeper crypts, and faster cellular migration from the crypt to the villus tip. The use of an antibiotic can therefore cause shortening of villi and reduced depth of crypts, while simultaneously decreasing the overall length and weight of the intestinal tract (Miles et al., 2006). Therefore, it is no surprise that coccidiosis, an enteric disease that commonly affects poultry, also causes rapid epithelial cell turnover (Humphrey and Turk, 1974) and may transiently lengthen villi (Moran, 1985). Necrotic enteritis (NE), a common disease affecting poultry, may also initiate regeneration of epithelial cells and causes shortening of villi but deepening of the crypts (Long et al., 1974). It should be recognized though that structural changes tend to be localized and may be more noticeable in the upper

jejunum where a majority of nutrient digestion and absorption occurs (Moran, 1985). As approximately 12% of newly synthesized protein is utilized in the GIT, factors that increase cell turnover may ultimately increase the maintenance energy requirement of the bird (Choct, 2009).

Microflora. Intestinal microflora may be influenced by a variety of factors including hygiene, disease status, and stress. Although these factors are important, dietary alterations including composition (Apajalahti et al., 2004; Rehman et al., 2007), processing (Apajalahti et al., 2001), source of ingredients, and feed additives (Bedford and Apajalahti, 2001) all have a substantial effect on gut microflora. Dietary composition is important in determining microbial populations because each bacterial species has different nutritional requirements and different substrate preference (Apajalahti et al., 2004). Therefore, as dietary ingredients are altered, so are microflora populations. In the same way, as microflora are altered, the ability of the digestive system to acquire nutrients varies. Studies have shown shifts in bacterial communities dependent on the type of grain fed. Corn and sorghum tend to favor increases in *Enterococcus* and decreases in *Bifidobacterium*, while barley increases *Lactobacillus*, oat increases *Escherichia* and *Lactococcus*, and rye increases *Streptococcus* (Apajalahti, 2004).

Three well-known ways to alter the microbial communities are to feed relatively indigestible diets, diets high in water soluble non-starch polysaccharides (NSP), and to supplement enzymes into the diet. A diet that is indigestible to the bird will ultimately provide more substrate for the microbes that use those compounds for energy and subsequently increase the population numbers (Apajalahti, 2004). Diets that are high in soluble NSPs like β-glucans and arabinoxylans, such as those containing rye, barley, and wheat, increase the viscosity and slow the passage rate of digesta. Lower intestinal viscosity is found in birds given corn-based diets compared to wheat-based diets (Sharma et al., 1997). More viscous digesta enables the

microbes to have more time in contact with the nutrient substrates as well as time for colonization (Rehman et al., 2007). However, when a xylanase enzyme is used, NSPs are broken down enough to improve digestibility and reduce viscosity of diets (Sharma et al., 1997). Consequently, a 60% reduction in microbial numbers can be seen due to fewer nutrients being available to the microbes (Bedford and Apajalahti, 2001). A concomitant improvement in bird performance is frequently seen with the correct use of exogenous enzymes (Mathlouthi et al., 2003; Wu et al., 2004). The amount of protein in the diet is another factor that has been found to alter microbial concentration where a high protein diet can lead to a higher concentration of *C. perfringens* in the intestinal tract (Rehman et al., 2007).

A final way to alter microbial populations is with the use of antibiotics. Sub-therapeutic antibiotics, sometimes referred to as antibiotic growth promoters (AGP), have been used in animal production since the 1940s (Dibner and Richards, 2005). Antibiotics work to reduce the population of target species more than any other method; however, due to increased concern over antibiotic resistant bacteria, the use of subtherapeutic antibiotics in poultry diets is becoming more limited. It is believed that antibiotics primarily support growth of the birds by limiting the overall number of bacterial species in the gut, as indicated by the fact that antibiotics did not improve the growth of germ-free chicks (Coates et al., 1963). With limited growth of bacteria, energy previously used for tissue maintenance and renewal can be directed more towards bird growth or improved absorption of nutrients in the GIT (Miles et al., 2006). Additionally, a thinner mucosal surface due to reduced inflammation will also contribute to reduced energy costs (Miles et al., 2006). The use of AGPs can cause the most change in the ileal region where lactobacilli tend to dominate relative to all bacterial sequences. However, in drug-free diets,

Enterobacteriaceae typically predominates. However, after three weeks, this difference was not as evident (Wise and Siragusa, 2007).

Endogenous Secretions. Endogenous secretions are normal and occur in all healthy birds; however, there are certain feed ingredients and dietary strategies that can alter the frequency or amount of secretions. Though these secretions have many important functions in the GIT as mentioned earlier, a large increase can be energetically demanding. Just as delayed access to feed can alter the morphology of the intestine, it can also alter the secretions. Uni et al. (2003) found that delayed access to feed can cause an increase in the density of goblet cells that secrete mucin. As mentioned earlier, dietary protein, fiber, and phytate can also influence the rate of endogenous secretions (Adedokun et al., 2011). Some studies have also shown that as intestinal viscosity decreases, mucin secretion is enhanced (Sharma et al., 1997). While endogenous secretions have many important functions in the intestinal tract as mentioned previously, a large increase in the amount of secretions may increase the maintenance energy requirement of the bird and lead to less efficient production. Therefore, a healthy balance of secretions is desired that assures optimal protective function while still maintaining a low cost of maintenance.

PHYTIC ACID

Phytic Acid in Plants. Phytic acid (PA), or *myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (IP6), is the primary storage form of phosphorus naturally found in plants (Woyengo et al., 2009; Thavarajah et al., 2009). It contains 28.2% P (Selle et al., 2000), and in mature seeds, 50 to 80% of the total phosphorus can be contained in the PA molecule (Lott, 1984). Phytic acid is typically concentrated in seed or grain tissues (Graf, 1986) where it is found in

protein bodies or specialized protein storage vacuoles (Lott, 1980) bound with minerals to form phytate. In cereal grains, phytate is likely found in globoids within protein bodies in the embryo (Lin et al., 2005). A majority (88%) of the PP in corn is in the embryo, and the rest is in the aleurone layer. In rice and wheat, a majority of the PP is in the germ and aleurone layer (O'Dell et al., 1972). In soybean, phytate is found throughout the proteinaceous matrix of protein bodies as protein-phytate or K-phytate complexes (Lott, 1980). Though PA can bind monovalent cations, it strongly chelates with polyvalent cations (Graf, 1986) and occurs most frequently as a mixed salt of K^{+} and Mg^{++} (Lin et al., 2005; Prattley and Stanley, 1982; Lott, 1980).

Phytic Acid in Poultry Diets. Phytate has a beneficial role in plant development by serving as a storage site for important nutrients and by controlling the levels of inorganic phosphate in the developing seed and seedling (Lott et al., 2000). However, when plant ingredients are used in diets for monogastric animals, phytate becomes an anti-nutrient that limits the animal's effective digestive functions. Although poultry contain adequate endogenous phytase to hydrolyze phytate and release almost 70% of PP, the minerals contained in traditional poultry diets cause PP hydrolysis to drop down to about 25% (Tamim et al., 2004). Due to the lack of hydrolysis of the phytate molecule, phytate-bound phosphorus, minerals, and protein are essentially unavailable for digestion, resulting in reduced digestibility and utilization of these nutrients in broilers (Ravindran et al., 2006, 2000; Cowieson et al., 2006a). As a result, excretion of unabsorbed nutrients into the environment is substantially increased, and extra money is spent to supplement available phosphorus into the diet. These negative effects of phytate ultimately affect bird performance, especially when birds are given a diet marginal in specific nutrients (Cowieson et al., 2006a).

In order to understand how phytate exerts anti-nutritional effects, it is necessary to comprehend the chemical makeup of the molecule. Phytic acid contains six phosphate groups, each with two dissociable protons, resulting in potentially twelve negative charges on the molecule (Woyengo et al., 2009). With pKa values ranging from 1.5 to 10 (Costello et al., 1976), six of the protons can dissociate at an acidic pH, three at a neutral pH, and three at a basic pH, enabling PA to bind positively-charged molecules in a variety of pH conditions (Woyengo et al., 2009; Crea et al., 2008). A low pH environment increases solubility of phytate complexes; however, during the beginning of digestion, the pH in the crop is not low enough to cause dissociation of PA from cations. Since limited digestion occurs here, the effects of PA in the crop are minimal. As the pH drops to a more acidic level in the gastric phase, solubility increases and PA disassociates from cations. However, at a pH below the isoelectric point of proteins in the solution, amino groups on basic AA and proteins can become positively charged and bind with PA in the gastric phase. Once bound, the activity of many endogenous proteinaceous enzymes in the gastric phase is inhibited, initiating a feedback mechanism which increases production of digestive enzymes and HCl. As the nutrients exit the gastric phase, the buffering action of the small intestine increases the pH enabling mineral-phytate complexes to form (Angel et al., 2002; Cowieson et al., 2004). Phytic acid can bind to divalent and to a lesser extent, monovalent cations rendering them unavailable for absorption. Because digestion mainly occurs in the small intestine, a majority of the effects of PA are seen here.

Effect on Minerals. The ability of PA to bind mineral cations depends upon the relative concentrations of phytate to ions, the properties of the cation, the pH of the environment (Crea et al., 2008), and the degree of phosphorylation of the inositol ring (Cowieson et al., 2006a). In phytate excess, the formation of soluble complexes predominates, whereas in the presence of

excess metal ions, more insoluble complexes are formed (Crea et al., 2008,) which are unable to be effectively absorbed. Dependent on pH, PA can form stable complexes with Cu, Zn, Co, Mn, Fe, and Ca, in decreasing order (Crea et al., 2008). However, even though phytic acid has the lowest binding capacity to Ca, its ability to form insoluble complexes with phytic acid surpasses that of other minerals due to its abundance in the diet compared to other minerals, suggesting the relative importance of ion concentration on complex formation. Lower weight inositol phosphate esters that are less phosphorylated have a lower capacity to bind to cations and thus affect solubility to a lesser extent (Harland and Morris, 1995; Harland and Narula, 1999).

The obvious consequence that phytate complex formation has on bird health is a reduction in the digestibility of minerals (Woyengo et al., 2009) and AME (Ravindran et al., 2006). In a study done by Woyengo et al. (2009), increasing concentrations of dietary PA linearly reduced the apparent ileal digestibility (AID) of dry matter, Na, K, P, Ca, and Mg in pigs. Dietary PA at 20 g/kg can even result in a negative AID of Na and Mg, suggesting there is an additional effect beyond simple binding of nutrients (Woyengo et al., 2009). Due to the nature of complex formation and the abundance of certain minerals in the diet, the effect on different minerals varies and will be discussed later.

Due to the fact that the PA molecule is comprised of 28.2% P, it is well-known that PA reduces the availability and AID of P in poultry diets (Woyengo et al., 2009). In fact, in a typical poultry diet, only about 25% of the dietary PP is hydrolyzed, resulting in about 29% apparent absorption of P (Tamim et al., 2004). This knowledge is extremely useful for producers, as a large cost of poultry diets comes from the addition of an available source of P into the diet in the form of mono- or di-calcium phosphate.

Sodium is one mineral that tends to be highly affected by PA. High levels of dietary PA can cause a decrease in the AID of Na (Woyengo et al., 2009). Because pancreatic secretions tend to be higher in Na than other minerals (Zebrowska et al., 1983), it is believed that PA may cause an increase in pancreatic secretions and therefore, a reduction in the apparent digestibility of Na as the bird is unable to reabsorb the additional secretions. As the bird's source of Na is being used for pancreatic secretions, the activity of intestinal Na-K-ATPase is reduced with increased dietary PA (Liu et al., 2008). Consequently, a higher level of dietary Na may alleviate some negative effects of a high phytate diet.

Although the ability of PA to form stable complexes with calcium is lower than other minerals, due to its abundance in poultry diets, Ca is highly responsible for the formation of many insoluble Ca:phytate complexes. In the absence of Ca, phytate is able to be hydrolyzed and release 70% of PP. However, in a traditional poultry diet, the ability of a bird's endogenous phytase to hydrolyze PP drops down to 25% due to the formation of insoluble complexes with Ca (Tamim et al., 2004). Typically below pH 5, significant Ca binding does not occur to phytate (Crea et al., 2008). As pH increases, the strength of the Ca:phytate complexes increases and becomes more insoluble. However, it has been determined that even in a low pH environment, as the ratio of Ca:phytate increases, complexes may occur (Crea et al., 2008).

Effect on Protein. Another consequence of phytate is that it reduces ileal digestibility of protein (Cowieson et al., 2006a). Several theories have been proposed as to the mechanism of how phytate presumably binds to protein and reduces digestibility. It is believed that at an acidic pH, a binary protein:phytate complex may form where phytate can bind to the α-NH_2 groups and side chains of the basic AA arginine, histidine, and lysine (Selle et al., 2000). At a more neutral pH, a ternary protein:mineral:phytate complex may form via a cationic bridge (Selle et al., 2000).

Recent research has suggested another mechanism of protein interaction where phytate may alter the thermodynamics of the water matrix, resulting in protein:protein aggregation. It is believed that phytate may create an electrostatic hydration shell around itself that affects the solubility of other adjacent compounds due to the alteration of water potential. This is found to be especially true for hydrophobic proteins and may extend to other compounds as well (Cowieson and Cowieson, 2011).

It has been shown that the phytic acid: protein ratio may also be an important factor in the solubility of proteins (Mothes et al., 1990). At a phytic acid: protein ration of about 0.02, insoluble protein-phytic acid complexes begin to form. As the ratio increases to 0.045, the insolubility reaches its peak. It then begins to drop until the ratio reaches 0.07 to 0.1 where solubilization reoccurs. While this study examined the properties of rapeseed protein, it may be applicable to other proteins that are frequently contained in poultry diets and may have implications for poultry nutrition.

Despite the overall reduction in protein digestibility, it is clear that phytate may not alter the digestibility of all AA equally. It is possible that phytate may have a greater effect on the digestion and absorption of certain AA compared to others. Ravindran et al. (2006) found that dietary phytate has no effect on the digestibility of several AA, including serine, proline, isoleucine, cysteine, and tryptophan. In a study done by Cowieson et al. (2006), digestibility of alanine, serine, aspartic acid, and threonine were affected more by phytate than other AA. Because of the differences seen in AA digestibility, it is believed that phytate may somehow alter endogenous protein secretions, which are rich in methionine, cysteine, threonine, and serine (Cowieson et al., 2006a). This may explain why phytate frequently affects digestibility of those particular AA that comprise many endogenous secretions. In addition to different effects on

various AA, the level of dietary phytate may alter digestibility. Woyengo et al. (2009) saw a quadratic response in the AID of isoleucine, leucine, valine, glutamic acid, proline, and serine, where an increase in dietary PA from 0 to 10 g/kg resulted in an increase in AID, but an even greater increase in dietary PA from 10 to 20 g/kg resulted in a reduction of AID of these AA.

Endogenous Losses. Phytic acid is believed to be an anti-nutrient and interact with the gastrointestinal tract to increase endogenous secretions, including cell turnover and mucin secretion (Cowieson et al., 2004). Additionally, it is thought to hinder the functionality of endogenous enzymes by interacting with their polyvalent cation co-factors. As a result, the subsequent reduction in digestion may create a negative feedback loop and stimulate a hyper-secretion of endogenous enzymes and minerals to compensate (Cowiesion et al., 2006; Woyengo et al., 2009). Since only 70-80% of all endogenous protein secretions can be reabsorbed and utilized by the bird (Nyachoti et al., 1997), any extra production and secretion of protein is seen as a reduction in apparent digestibility and adds to the maintenance energy requirement of the bird, decreasing efficiency. Some evidence for the increased endogenous flow of minerals is that phytic acid has been found to result in a negative AID of Na and Mg, suggesting that there is an increased endogenous production of these specific minerals (Woyengo et al., 2009).

PHYTASE

Phytase, or *myo*-inositol hexaphosphate hydrolase, is an enzyme that is able break down the phytic acid molecule by catalyzing the hydrolysis of phosphate ester bonds (Angel et al., 2002). One unit of phytase (FTU) is considered to be the amount of enzyme that releases 1 mmol of inorganic phosphorus/minute from 0.00015 mol/l sodium phytate at pH 5.5 at 37˚C (Ravindran et al., 2000). The hydrolysis of phytic acid in a step-wise manner yields inorganic

phosphorus and lower inositol phosphate esters (*myo*-inositol pentaphosphate to *myo*-inositol monophosphate) (Selle et al., 2000; Türk et al., 2000). As such, it is able to improve mineral bioavailability as cations are released from the phytate complex. Phytase is naturally produced by plant-based ingredients (Viveros et al., 2000), endogenous intestinal microflora (Angel et al., 2002), and is found on the intestinal mucosal membrane of birds (Davies and Motzok, 1972; Maenz and Classen, 1998). However, due to the presence of calcium and other minerals present in typical poultry diets, the effectiveness of phytase from these sources is extremely reduced (McCuaig et al., 1972; Tamim et al., 2004; Selle et al., 2009). Since it is impractical to remove minerals from poultry diets, the alternative to improve P availability has been to include exogenous phytase in the diet.

Types of Phytases. The two types of phytases used in poultry diets are a 3-phytase and a 6-phytase, which are characterized by the point on the inositol ring where dephosphorylation is initiated (Selle et al., 2000; Türk et al., 2000; Angel et al., 2002). Typically 3-phytases originate from microbes, while 6-phytases are from plant origin; however, there are exceptions (Türk et al., 2000; Selle et al., 2000). A few commonly used commercial enzymes include a 3-phytase derived from *A. niger* and 6-phytases derived from *Peniophora lycii* and *Escherichia coli* (Selle and Ravindran, 2007). The source from which the phytase is obtained also has an influence on its functionality. Some of the microbes that produce phytase include bacteria, yeasts (Türk et al., 2000), and fungi (Pallauf and Rimbach, 1997). One major difference is the pH at which the phytase has optimal functionality. Microbial phytases generally have pH optima from 2 to 6 (Wodzinski and Ullah, 1996). Two types of microbial phytase are produced from the fungi *Aspergilli niger* and function at pH of 5.5 and 2.5 (phyA) and 2.0 (phyB) (Wodzinski and Ullah, 1996).

Influences on Phytase Functionality. At a pH from 4 to 7, similar to that found in the small intestine, the phytate-mineral complexes precipitate out of solution and the ability to be hydrolyzed by phytase is reduced (Angel et al., 2002). Another factor that has an impact on the efficacy of phytase is the mineral level in the diet. As Ca and P levels increase, phytate complexes also increase, and the activity of mucosal and exogenous phytase is reduced (McCuaig et al., 1972; Tamim et al., 2004).

Phytase in Poultry Diets. Phytases have been included in poultry diets since 1991 (Selle et al., 2009). Due to their ability to generate available P for broilers and the decreasing cost of the exogenous enzyme, phytase is now included in over half of the poultry diets around the world (Selle et al., 2009). Phytase was first introduced to poultry diets as a way to increase the availability of P from phytate. Due to environmental concerns regarding high levels of P being excreted and deposited into waterways, phytase was added to poultry diets in an attempt to increase the availability of P from phytate and reduce the amount of inorganic P that was added to poultry diets (Selle and Ravindran, 2007). Therefore, addition of phytase to poultry diets would be environmentally beneficial as well as economically advantageous as less high-cost P would need to be added to the diets.

The ability of phytase to improve P availability in poultry diets has been extensively researched and accepted (Namkung and Leeson, 1999; Selle et al., 2000; Ravindran et al., 2000; Adeola and Sands, 2003; Cowieson et al., 2006a; Cowieson et al., 2006b; Cowieson et al., 2009). However, through years of research on phytase, many "extra-phosphoric" benefits of phytase on bird performance have also been discovered. The addition of phytase to diets deficient in P, Ca, and/or energy has been shown to improve body weight, feed conversion, or feed intake (Namkung and Leeson, 1999; Cowieson et al., 2006b) compared to negative control diets with

no phytase. Through assumed hydrolysis of the phytate molecule and breakdown of phytate complexes, phytase supplementation has also been shown to improve retention of K, Na, Fe, Mg, S, Cu and metabolizability of N, dry matter, and/or AME_n (Namkung and Leeson, 1999; Ravindran et al., 2000; Cowieson et al., 2006b) .

Another extra-phosphoric advantage seen with phytase is improvement in protein and AA digestibility. Though some refute the ability of phytase supplementation to improve utilization of protein (Adeola and Sands, 2003), many studies have demonstrated improvement in AA digestibility (Ravindran et al., 1999; Namkung and Leeson, 1999; Ravindran et al., 2000; Cowieson et al., 2006a). It has been shown that improvements in apparent ileal AA digestibility with the use of phytase are more dependent on the inherent protein digestibility of the feedstuff rather than phytic acid concentration (Ravindran et al., 1999). Ingredients with inherently poor digestibility will respond better to addition of phytase than those with higher digestibility. Perhaps those studies without an improvement were using ingredients with inherently high digestibilities where the benefit of adding enzyme would be lost.

Another benefit of phytase has recently been studied, which is the ability of phytase to decrease the excretion of endogenous minerals and AA (Cowieson et al., 2006a; Cowieson et al., 2006b; Cowieson and Ravindran, 2007; Cowieson et al., 2008; Cowieson et al., 2009; Pirgozliev et al., 2011). This likely explains a portion of the improvement in AA digestibility and energy response seen with exogenous phytase use. Because approximately 50% of the nitrogen present in the small intestine is of endogenous origin, any improvements in absorption of secreted protein or a reduction in endogenous losses will improve energy balance in the bird and likely improve performance (Cowieson et al., 2009). Therefore, taking all effects of phytase into account, it is likely that the overall improved performance seen with phytase is a result of

improved nutrient availability as well as a reduction in endogenous loss (Cowieson et al., 2006b).

ENTERIC DISEASES

Under normal conditions, the GIT contains a variety of microbial species that help contribute to the overall health of the host. In a healthy state, a balance is maintained where microflora are confined to the lumen of the intestine and the mucosal immune system exists in a state of low reactivity (Allen and Torres, 2008). At times, however, disruptions occur to the balance of microflora, resulting in dysbiosis. When this occurs, populations of microbes normally present in the intestine proliferate beyond levels that are tolerated by the host, and disease results.

Coccidiosis

Coccidiosis is a disease that is caused by protozoan parasites of the genus *Eimeria* (McDougald and Reid, 1991). It is considered one of the most economically challenging diseases affecting the poultry industry (Williams, 2005). Seven pathogenic species of *Eimeria* that commonly infect chickens have been described by McDougald (2003) and include: *Eimeria acervulina, Eimeria tenella, Eimeria mitis, Eimeria praecox, Eimeria maxima, Eimeria necatrix,* and *Eimeria brunetti.* Each species infects a specific area in the intestine and is identified based on oocyst morphology, host specificity, immune specificity, appearance and location of host lesions, and length of prepatent period (McDougald and Reid, 1991). Cross-immunity between species is poor, so exposure to one species does not protect against outbreaks caused by another (McDougald and Reid, 1991). Coccidia are ubiquitous in commercial poultry houses, and outbreaks can be common due to the high reproductive potential of coccidia and the density of

birds in commercial poultry houses (McDougald and Reid, 1991). While birds of all ages are susceptible to coccidiosis, outbreaks normally occur in birds from three to six weeks of age.

Pathogenesis. The life cycle of *Eimeria* is complex. It is known that *Eimeria* causes disease in the intestinal tract of poultry as they multiply and damage tissue. Coccidial oocysts are excreted in the feces of infected birds and can remain in the environment until they are consumed by another bird. Oocysts located in the environment are enclosed in a thick shell and are not infective immediately after excretion from the bird. A process known as sporulation must first occur to enable oocysts to be infective. Sporulation, which lasts about 48 hours, is believed to require temperature, humidity, and access to oxygen (Kheysin, 1972; Yvore and Coudert, 1972). It is not believed to be favored by moist litter conditions, as 16% litter moisture content was found to be most advantageous to sporulation compared to 42 or 62% litter moisture conditions (Waldenstedt et al., 2001). During sporulation, four sporocysts, each containing two sporozoites, develop from a single cell in the oocyst. Sporulated oocysts are then ingested by the birds and sporocysts must be liberated from the oocysts before excystation of the sporozoites from the sporocysts can occur (Goodrich, 1944; Doran and Farr, 1962). Early work suggested that the sporocysts were liberated from the oocyst in the crop of the bird (Pratt, 1937). However, later work showed that sporocysts are released from oocysts primarily in the gizzard due to the physical grinding action, while bile salts and digestive enzymes from the pancreas play a role in the excystation of sporozoites from the sporocysts (Doran and Farr, 1962; McDougald and Reid, 1991). After the release of sporozoites, they invade the intestinal epithelial cells and begin the reproductive phase, which consists of at least two generations of asexual development, called schizogony or merogony, and a sexual phase where microgametes and macrogametes unite to

form a zygote that is eventually shed in the feces. The whole process takes anywhere from 4 to 6 days (McDougald and Reid, 1991).

Coccidiosis can cause tissue damage to the epithelium that negatively affects digestion, nutrient absorption, and result in dehydration and blood loss, reduction in weight gain, increased susceptibility to other enteric diseases, and even mortality (McDougald and Reid, 1991; Yegani and Korver, 2008). It is believed that maximum damage occurs during the reproductive phase when second-generation schizonts rupture to release merozoites (McDougald and Reid, 1991). The severity of an infection can vary depending on species of *Eimeria,* number of ingested oocysts, or immune status of the bird (McDougald and Reid, 1991).

Symptoms and Lesions. From the outward appearance, birds affected with coccidiosis can be identified based on poor weight gain, diarrhea, morbidity, emaciation, roughening of feathers, and mortality (McDougald and Reid, 1991; Williams, 2005). In addition, dehydration and blood loss is seen along with increased susceptibility to other diseases (Idris et al, 1997). Internally, *Eimeria* damage the intestinal mucosa and result in atrophied villi and reduced intestinal absorptive area (Idris et al., 1997; Williams, 2005; Assis et al., 2010). Other noted symptoms include increased intestinal passage time, decreased digesta viscosity, leakage of plasma proteins, and increased intestinal pH (Williams, 2005). Distinct lesions tend to be specific to the species of *Eimeria* causing the infection. This review will cover distinct symptoms and lesions of only those species contained in the commercial live coccidial oocyst vaccine, Coccivac®-B.

Eimeria maxima is known to affect the mid-gut region of the small intestine from the duodenal loop extending to Meckel's diverticulum. Gross observations of the bird can include reduced weight gain, nutrient malabsorption, watery diarrhea, and decreased carotenoid

pigmentation in the skin and yolk. In the intestine, gas build-up, petechial hemorrhages in the mucosa, yellow-orange mucus, and fluid in the mid-gut are observed (McDougald and Reid, 1991; Allen and Fetterer, 2002).

E. acervulina produces white striated plaques on the surface of the mucosa that are arranged transversely across the duodenum. Lesions may be seen from the serosal surface and typically localized to the duodenal loop, which may appear pale and contain watery fluid. Histological examination can reveal broken and/or fused villi and thickening of the mucosal surface (McDougald and Reid, 1991).

E. mivati is similar to *E. acervulina* in that it produces lesions that are comparable and able to be seen from the serosal surface, but that are more circular in shape. Early infection produces lesions in the duodenum, and later lesions appear in the mid-gut and lower small intestine (McDougald and Reid, 1991).

E. tenella inhabits the ceca and is considered the most pathogenic species found in chickens. Its symptoms include severe bleeding, high morbidity and mortality, loss of weight, and emaciation. Development of schizonts occurs deep in the mucosal tissue and causes extreme disruption as merozoites are released. The ceca can become enlarged and distended and eventually a cecal core of clotted blood and mucosa can form. In extreme cases, ceca can rupture, killing the host (McDougald and Reid, 1991).

Control Strategies. Coccidiosis in commercial poultry has typically been controlled with the use of anticoccidial medication in the feed. A variety of chemicals and ionophores have been used, each with varied modes of action. Some may be classified as coccidiocidal, meaning the parasite is killed, while others may be coccidiostatic, meaning development of the pathogen is arrested until removal of the medication (McDougald and Reid, 1991). With increasing

consumer concern regarding antibiotic resistance, acceptance of in-feed anticoccidial medication may be limited. Therefore, an alternate method for controlling coccidiosis has been developed.

Vaccinating for coccidiosis is becoming increasingly popular in the poultry industry as producers seek to find new methods of coccidiosis control that are accepted by consumers. The first vaccine for coccidiosis was introduced in 1952 (Edgar and King, 1952) and is typically used for broiler breeders and layers, and to a lesser extent, broilers. The theory behind live vaccines is that oocysts are administered to birds to induce a mild infection that is able to initiate an immune response and protect the bird from future infection (Dalloul and Lillehoj, 2006). Several methods of vaccine application have been attempted including administration in the drinking water, in the feed, in a colored edible gel containing oocysts, or as an eye spray (Dalloul and Lillehoj, 2006). However, the most practical and efficient method currently used is by way of a spray-cabinet (Chapman et al., 2002). With a spray-cabinet, the vaccine of live oocysts is sprayed on the bird as an aqueous suspension. As the birds preen themselves and each other, the oocysts are ingested, swallowed, and enter the intestinal tract where they are able to produce a mild active infection in the bird (Chapman et al., 2002) that is then able to induce immunity.

Because live vaccines cause a mild infection, performance of vaccinated broilers when compared to non-vaccinated birds is sometimes negatively impacted. However, vaccinated birds are able to compensate for the decline in performance so that by the end of most studies, differences between vaccinated and non-vaccinated birds are indistinguishable (Waldenstedt et al., 1999; Mathis, 1999; Parker, 2007; Lehman et al., 2009). Despite the transient decline in performance, the advantage in its use is that the initial mild infection is able to protect against a later more severe infection (Williams, 2005).

The physiological changes occurring in the small intestine of birds after administration of a live vaccine are similar to what occurs during the clinical disease and may be partly responsible for the reduction in performance. One response to a coccidiosis infection is an increase in the accumulation of mucus in the goblet cells as well as the number of goblet cells in the small intestine (Collier et al., 2008). The mucus layer of the intestine functions as a protective layer against pathogens (Horn et al., 2009), therefore the increase in mucus seen with infection is likely a protective mechanism against further infection. In addition, similar to the manner by which coccidiosis and the presence of microflora can cause sloughing of the villi (McDougald and Reid, 1991) and increase in crypt depth in the small intestine (Forder et al., 2007), vaccination induces similar changes to the structure of the intestine. It is likely that the increase in cell turnover to replace sloughed cells and an increased production of mucin may be responsible for some of the reduction in performance. Approximately 12% of newly synthesized protein is devoted to the digestive tract, therefore rapid turnover of these cells and production of extra mucin will likely increase the bird's energy requirement for maintenance (Choct, 2009).

Despite the advantages of using a live coccidial oocyst vaccine, one major disadvantage is their potential to predispose birds to further enteric diseases, such as necrotic enteritis (Chapman et al., 2002; Wages and Kenneth, 2003; Williams, 2005). Although the relationship between live vaccines and development of necrotic enteritis (NE) has not fully been explored, due to the fact that clinical coccidiosis may predispose birds to development of NE (Long, 1973), it is assumed that a mild coccidial infection, as that from a vaccine, may do the same. Additionally, as coccidiosis vaccines are frequently employed in an attempt to limit antimicrobials in feed, the use of an antibiotic would be avoided, as would any protection from NE. Perhaps the shift in microbial populations that occurs during a coccidiosis vaccination

(Oviedo-Rondón et al., 2006) in conjunction with an antibiotic-free diet could initiate the dysbiosis that leads to disease. As mentioned before, infection alters the intestinal mucin dynamics and causes leakage of plasma proteins as a result of intestinal damage (Van Immerseel et al., 2004). This in conjunction with an increase in endogenous secretions with vaccination (Miller et al., 1979) could provide the mucolytic *Clostridium perfringens* with an optimal source of nutrients as they are not able to produce all of the essential AA for survival (Cooper and Songer, 2009).

Necrotic Enteritis

Necrotic enteritis is an enteric disease of poultry caused by the gram negative bacteria *Clostridium perfringens,* type A or C. It has a huge economic impact on the commercial broiler industry, considering 1 to 40% of flocks in North America and the EU can be impacted (McDevitt et al., 2006). Typically controlled by antibiotics, the ban on antibiotic use in the EU and voluntary reduction of use in the US have contributed to increased incidence of the disease and prompted research of antibiotic-free methods of control (McDevitt et al., 2006).

Pathogenesis. Considered to be part of normal gut microflora, the presence of *C. perfringens* is not detrimental to birds as 75% to 95% of birds are colonized by this bacterium and few show symptoms of disease (McDevitt et al., 2006). Disease occurs when microbial populations are disrupted and overproliferation of these potentially pathogenic bacteria occurs. Perhaps it is necessary for multiplication of *C. perfringens* above a certain threshold to occur within a particular amount of time, to initiate disease (Long and Truscott, 1976). Typically, the disease initially occurs in broilers between two and four-and-a-half weeks of age and lasts about

a week (Long, 1973). It is believed that toxins secreted by the bacteria are responsible for the necrotic lesions that accompany this disease.

Necrotic enteritis is caused by the enterotoxins produced by *C. perfringens* (Immerseel et al., 2004). There are several strains of the bacterium that are characterized by their specific toxin-encoding genes, although all strains contain the α-toxin gene (plc) (McDevitt et al., 2006). While it was formerly believed that the alpha toxin was the main cause of necrotic enteritis in poultry, it has recently been determined that the alpha toxin is not necessary to induce necrotic enteritis in broiler chickens (Timbermont et al., 2009). However, the NetB toxin has been shown to be critical for induction of NE (Keyburn et al., 2010). It is believed that as inflammation occurs, toxins produced by bacteria may cause the lamina propria to become congested and edematous, upon which the epithelium lifts off the lamina propria. Gram positive bacteria then adhere to the outer edge of the lamina propria where proliferation and coagulation necrosis occurs on the villi. Beginning at the tip of the villi, necrosis continues until it reaches the crypts. In chronic cases, cystic structures are present in the crypts. Eventually, damaged cells on the villi are expelled into the lumen and replaced with newly formed cells (Long et al., 1974).

Symptoms and Lesions. There are two forms of NE commonly seen in the poultry industry, acute and subclinical. The acute form usually causes mild to severe mortality, while the subclinical form is less obvious and is diagnosed by focal necrotic lesions in the small intestinal mucosa, increased numbers of *C. perfringens*, and reduced growth rate and feed efficiency (Kaldhusdal and Hofshagen, 1992). With both forms, birds may huddle, have ruffled feathers, and diarrhea (Porter, 1998). In the acute form, birds can die quickly, as fast as 30 minutes after first signs of illness, and dead birds emit a strong odor (Long and Truscott, 1976). The intestine is typically very thin and friable and is distended with gas (Parish, 1961; Long et al., 1974).

When opened, a brown-colored fluid is released, and the mucosal surface is covered in an adherent diphtheritic membrane that is yellow-brown and frequently bile stained (Parish, 1961; Long et al., 1974). Most lesions occur in the jejunum, likely due to a favorable pH or lowered activity of digestive enzymes, but can also be seen in the duodenum, ileum, and ceca (Long et al., 1974). Upon microscopic examination, the villi are swollen and necrotic, especially on the apical part of the villi (Kaldhusdal and Hofshagen, 1992). The diphtheritic membrane is composed of degenerated epithelial cells, mononuclear cells, fibrin, and Gram-positive bacilli and is found adhered or adjacent to villi (Long et al., 1974). It has been found that infected birds have higher numbers of *C. perfringens* and lower numbers of coliform, lactobacilli, and streptococci bacteria compared to uninfected birds (Kaldhusdal and Hofshagen, 1992), demonstrating a shift in bacterial populations.

Predisposing Factors. Several factors may predispose birds to be more susceptible to developing NE. While no single factor is directly responsible for initiation of disease, some risk factors include diet composition, intestinal viscosity, and damage to the intestinal epithelium. One dietary factor that increases the incidence of NE is diets containing ingredients high in indigestible, water soluble NSPs like β-glucans and arabinoxylans (McDevitt et al., 2006). Some cereals that are high in NSPs are wheat, barley, and rye, which have all been shown to result in more frequent occurrence of NE in broilers compared to corn-based diets (Kaldhusdal and Hofshagen, 1992; Riddell and Kong, 1992; Annett et al., 2002). It is possible that the increased viscosity due to the water-soluble NSPs in these ingredients increases the intestinal transit time and enables bacteria more time to proliferate and colonize (Waldenstedt et al., 2000; Annett et al., 2002). The presence of NSPs may also suppress the growth of other non-pathogenic bacteria, making it easier for *C. perfringens* to proliferate (Annett et al., 2002). Increased viscosity may

also be responsible for a reduction in digestibility, although evidence to support this may be sparse (McDevitt et al., 2006).

Another favorable condition for development of NE may be an increase in the level of protein or a particular AA that the bacteria in the lower intestinal tract may use as a nutrient source (Long, 1973; Drew et al., 2004; Yegani and Korver, 2008). *C. perfringens* is not able to produce 13 essential AA, so provision of these AA is conducive to bacterial growth (McDevitt et al., 2006). High protein diets or diets with an imbalanced energy:protein ratio may cause birds to consume beyond their protein requirement. In such a case, the high amount of nitrogen will not be completely digested in the upper GIT and will pass to the lower GIT where microbes can use it for nutrient substrates since minimal digestion by the bird occurs here (McDevitt et al., 2006).

As mentioned earlier, coccidiosis or a live coccidial oocyst vaccine may be a factor in predisposing birds to developing NE (Chapman et al., 2002; Wages and Kenneth, 2003; Williams, 2005). The pathogenesis is quite complicated and several factors may be responsible for development of disease. Damage to enterocytes caused by *Eimeria* can induce leakage of plasma proteins and increase mucus production, both of which can serve as a nutrient source for *C. perfringens* and promote proliferation of the pathogenic bacteria. Bacteriocins produced by the virulent strains of *C. perfringens* may inhibit growth of other non-pathogenic strains. Subsequently, collagenases and enzymes secreted by the host and/or the bacteria induce necrotic enteritis at the basal and lateral domains of the enterocytes before extending into the lamina propria. The NetB toxin produced from the virulent *C. perfringens* forms pores in the enterocytes, initiating cell death. Then as the extracellular matrix molecules begin to leak into the lumen as a result of cell damage, *C. perfringens* is able to bind and begin further colonization (Timbermount et al., 2011).

SUMMARY

From the background information and studies presented in this review, it is clear that intestinal health is paramount to overall heath and performance of poultry. Therefore, any nutritional, management, or other strategies to maintain optimal gastrointestinal tract conditions would aid in efficient production. Here, the focus is on nutritional modulation of intestinal health, as nutrition plays a large economic role in animal agriculture. With changing consumer preferences, one constant challenge of poultry production is to cater to the desires of the public while maintaining economically feasible production practices.

In the following experiments, the consequences of a live coccidial oocyst vaccine on broiler performance and intestinal health are studied as coccidiosis vaccination is becoming increasingly popular to satisfy public desire for antimicrobial free diets. Along with the effect of the vaccine on intestinal health, dietary phytic acid is also examined to determine their combined effect on the GIT and bird performance and establish areas in which nutritional modulation may be advantageous. In the second experiment, exogenous phytase was added to the diets in an attempt to decrease hindrances to performance caused by vaccination and/or phytic acid or to understand in greater depth some of the extra-phosphoric benefits of phytase. In addition to live performance (body weight, feed intake, feed conversion), tibia ash, small intestinal pH and morphology, and apparent ileal AA digestibility were evaluated as early changes in intestinal health may translate to ultimate differences in performance.

REFERENCES

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737-1748.

Adeola, O., and J. S. Sands. 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci. 81:E78-E85

Allen, C. A., and A. G. Torres. 2008. Host-microbe communication within the GI tract. Pages 93-101 *In* GI microbiota and regulation of the immune system. Gary B. Huffnagle and Mairi C. Noverr, eds. Landes Bioscience and Springer Science.

Angel, R., N. M. Tamim, T. J. Applegate, A. S. Dhandu, and L. E. Ellestad. 2002. Phytic acid chemistry: Influence on phytin-phosphorus availability and phytase efficacy. J. Appl. Poult. Res. 11:471-480.

Annett, C. B., J. R. Viste, M. Chirino-Trejo, H. L. Classen, D. M. Middleton, and E. Simko. 2002. Necrotic enteritis: effect of barley, wheat, and corn diets on proliferation of *Clostridium perfringens* type A. Avian Pathol. 31:598-601.

Apajalahti, J., A. Kettunen, M. Bedford, and W. Holben. 2001. Percent G + C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. Appl. Environ. Microbiol. 67:5656-5667.

Apajalahti, J. 2004. Structure and dietary modulation of a intestinal microbial communities. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.

Assis, R. C. L., F. D. Luns, M. E. Beletti, R. L. Assis, N. M. Nasser, E. S. M. Maria, and M. C. Cury. 2010. Histomorphometry and macroscopic intestinal lesions in broilers infected with *Eimeria acervulina.* Vet. Parasitol. 168:185-189.

Bayer, R. C., C. B. Chawen, F. H Bird, and S. D. Musgrave. 1975. Characteristics of the absorptive surface of the small intestine of the chicken from 1 day to 14 weeks of age. Poult. Sci. 54:155-169.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition – their current value and future benefits. Anim. Feed Sci. Technol. 86:1–13.

Bedford, M. R., and J. Apajalahti. 2001. Microbial interactions in the response to exogenous enzyme utilization. Pages 229-314. *In* : Enzymes in Farm Animal Nutrition. M.R. Bedford, and G.G Partridge, eds., CABI Publishing, Wallingford.

Chapman, H. D., T. E. Cherry, H. D. Danforth, G. Richards, M. W. Shirley, and R. B. Williams. 2002. Sustainable coccidiosis control in poultry production: the role of live vaccines. Int. J. Parasitol. 32:617-629.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9-15.

Coates, M. E., R. Fuller, G. F. Harrison, M. Lev, and S. F. Suffolk. 1963. Comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17:141-151.

Collins, F. M., and P. B. Carter. 1978 Growth of *Salmonellae* in orally infected germfree mice. Infect. Immun. 21:41-47.

Cook, R. H., and F. H. Bird. 1973. Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. Poult. Sci. 52:2276-2280.

Cooper, K. K., and J. G. Songer. 2009. Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A. Anaerobe 15:55-60.

Crea, F., C. De Stefano, D. Milea, and S. Sammartano. 2008. Formation and stability of phytate complexes in solution. Coord. Chem. Rev. 252:1108-1120.

Coweison, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45:101-108.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006a. Phytic acid and phytase: Implications for protein utilisation by poultry. Poult. Sci. 85:878-885.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006b. Supplementation of corn-soy based diets with an *Escherichia coli*-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and the metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Cowieson, A. J. and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98:745-752.

Cowieson, A. J., V. Ravindran, and P. H. Selle. 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult. Sci. 87:2287-2299.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401-418.

Cowieson, A.J., and N. P. Cowieson. 2011. Phytate and the thermodynamics of water. Proc. Aust. Poult. Sci. Symp. 22:22-25.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. World Poult. Sci. J. 67:225-236.

Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancement in control measures and vaccine development. Expert Rev. Vaccines. 5:143-163.

Davies, M. I., and I. Motzok. 1972. Properties of chick intestinal phytase. Poult. Sci. 51:494-501.

Dibner, J. J. and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84:634-643.

Doran, D. J., and M. M. Farr. 1962. Excystation of the poultry coccidium, *Eimeria acervulina.* J. Protozool. 9:154-161.

Drew, M. D., N. A. Syed, B. G. Goldade, B. Laarveld, and A. G. Van Kessel. 2004. Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. Poult. Sci. 83:414-420.

Edgar, S. A., and D. A. King. 1952. Breeding and immunizing chickens for resistance to coccidiosis. Ann. Rep. Alabama Polytech. Inst. Agr. Exp. Stn. 62/63:36-37.

Erdman, Jr., J. W. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56:736-741.

Forder, R. E. A., G. S. Howarth, D. R. Tivey, and R. J. Hughes. 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult. Sci. 86:2396-2403.

Forstner, J. F., and G. G. Forstner. 1994. Gastrointestinal mucus. *In* Physiology of the gastrointestinal tract, 3rd ed. Leonard R. Johnson, ed. New York: Raven Press. Pgs. 1255-1283.

Gaskins, H. R. 2001. Intestinal bacteria and their influence on swine growth. Pages 585-608 *in* Swine nutrition. A. J. Lewis and L. L. Southern, eds. 2nd ed. CRC Press, Boca Raton, FL.

Geyra, A., Z. Uni, and D. Sklan. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr. 86:53-61.

Goodrich, H. P. 1944. Coccidian oocysts. Parasitol. 36:72-79.

Graf, E. 1986. Chemistry and applications of phytic acid: an overview. *In* Phytic acid: Chemistry and applications. *Edited by* E. Graf. Pilatus Press, Minneapolis, Minn. Pp. 1-21.

Harland, B. F., and E. R. Morris. 1995. Phytate: a good or a bad food component? Nutr. Res. 15:733-754.

Harland, B. F., and Narula, G. 1999. Food phytate and its hydrolysis products. Nutr. Res. 19:947-961.

Humphrey, C. D., and D. E. Turk. 1974. The ultrastructure of chick intestinal absorptive cells during *Eimeria acervulina* infection. Poult. Sci. 53:1001-1008.

Idris, A., D. Bounous, M. Goodwin, J. Brown, and E. Krushinskie. 1997. Quantitative pathology of small intestinal coccidiosis caused by *Eimeria maxima* in young broilers. Avian Pathol. 26(4): 731-741.

Immerseel, F. V., J. D. Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol. 33:537-549.

Imondi, A. R., and F. H. Bird. 1966. The turnover of intestinal epithelium in the chick. Poult. Sci. 45:142-147.

Kaldhusdal, M., and M. Hofshagen. 1992. Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. Poult. Sci. 71:1145-1153.

Keyburn, A. L., X. X. Yan, T. L. Bannam, F. Van Immerseel, J. I. Rood, and R. J. Moore. 2010. Association between avian necrotic enteritis and *Clostridium perfringens* strains expressing NetB toxin. Vet. Res. 41(2):21.

Kheysin, Y. M. 1972. Sporulation of oocysts and their survival in the external environment. Pages 149-177 *in* Life cycles of coccidia of domestic animals. K. S. Todd Jr., ed. University Park Press, London, UK.

Lehman, R., E. T. Moran, and J. B. Hess. 2009. Response of coccidiostat- versus vaccination-protected broilers to gelatin inclusion in high and low crude protein diets. Poult. Sci. 88:984-993.

Li, Neng., O. Wahlberg, I. Puigdomenech, and L.O. Öhman. 1989. Equilibrium studies of phytate ions. 1. Equilibria between phytate ions and protons in 3M (Na)ClO_4 medium. Acta. Chem. Scand. 43:331-339.

Lin, L., I. Ockenden, and J. N. A. Lott. 2005. The concentrations and distributions of phytic acid phosphorus and other mineral nutrients in wild-type and *low phytic acid* 1-1 (*lpa1-1)* corn (*Zea mays* L.) grain parts. Can. J. Bot. 83:131-141.

Liu, N. Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432-3439.

Long, J. R. 1973. Necrotic enteritis in broiler chickens I. A review of the literature and the prevalence of the disease in Ontario. Can. J. Comp. Med. 37:302-308.

Long, J. R., J. R. Pettit, and D. A. Barnum. 1974. Necrotic enteritis in broiler chickens II. Pathology and proposed pathogenesis. J. Comp. Med. 38:467-474.

Long, J. R., and R. B. Truscott. 1976. Necrotic enteritis in broiler chicksn. III. Reproduction of the disease. Can. J. Comp. Med. 40:53-59.

Lott, J. N. A. 1980. Protein bodies. *In* The biochemistry of plants. Vol. 1. Edited by N. E. Tolbert. Academic Press, Inc., New York. Pp. 589-623.

Lott, J. N. A. 1984. Accumulation of see reserves of phosphorus and other minerals. *In* Seed physiology. Vol. 1. *Edited by* D. R. Murray. Academic Press, Sydney, Australia. Pp. 139-166.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci. Res. 10:11-33.

Maenz, D. D., an H. L. Classen. 1998. Phytase activity in the small intestinal brush border membrane of the chicken. Poult. Sci. 77:557-563.

Mathis, G. F. 1999. The influence of the coccidiosis vaccine, Coccivac-B®, on compensatory weight gain of broiler chickens in comparison with the anticoccidial, salinomycin. Poult. Sci. 78 (Suppl. 1):46-49.

Mathlouthi, N., H. Juin, and M. Larbier. 2003. Effect of xylanase and beta-glucanase supplementation of wheat- or wheat- and barley-based diets on the performance of male turkeys. Br. Poult. Sci. 44:291-298.

McCuaig, L. W., M. I. Davies, and I. Motzok. 1972. Intestinal alkaline phosphatase and phytase of chicks: Effect of dietary magnesium, calcium, phosphorus, and thyroactive casein. Poult. Sci. 51:526-530.

McDevitt, R. M., J. D. Brooker, T. Acamovic, and N. H. C. Sparks. 2006. Necrotic enteritis: A continuing challenge for the poultry industry. World's Poult. Sci. J. 62:221-247.

McDougald, L. R. 1998. Intestinal protozoa important to poultry. Poult. Sci. 77:1156-1158.

McDougald, L. R. 2003. Protozoal infections. Pages 974–990 in Diseases of Poultry. J. R. G. Y. M. Saif, A. M. Fadly, L. R. McDougald, and D. E. Swayne, ed. Iowa State Press, Ames.

McDougald, L. R., and W. M. Reid, 1991. Coccidiosis. In Diseases of Poultry, B. W. Calnek, ed., Iowa State University Press, Ames, 780-797.

Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476-485.

Miller, H. R. P., Y. Narva, and C. R. Parishm. 1979. Intestinal goblet cell differentiation in *Nippostrongylus*-infected rats after transfer of fractionated thoracic duct lymphocytes. Int. Arch. Allergy Appl. Immunol. 59(3):281-285.

Mothes, R., K. D. Schwenke, D. Zirwer, and K. Gast. 1990. Rapeseed protein – polyanionic interactions. Soluble complexes between the 2 S protein fraction (napin) and phytic acid. Die Nahrung 34:375-385.

Moran, E. T. 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. J. Nutr. 115:665-674.

Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78:1317-1319.

Nyachoti, C. M., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Can. J. Anim. Sci. 77:149-163.

O'Dell, B.L., A. R. de Boland, and S. R. Koirtyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20:718-721.

Oviedo-Rondón, E. O., M. E. Hume, C. Hernández, and S. Clemente-Hernández. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poult. Sci. 85:854-860.

Pallauf, J., and G. Rimbach. 1997. Effect of supplemental phytase on mineral and trace element bioavailability and heavy metal accumulation in pigs with different type diets. *In* Phytase in Animal Nutrition and Waste Management. M. B. Coehlbo and E. T. Kornegay, ed. BASF Publication DC9601. BASF Corporation, Mount Olive, NJ.

Parish, W. E. 1961. Necrotic enteritis in the fowl (Gallus Gallus Domesticus) I. Histopathology of the disease and isolation of a strain of *Clostridium welchii*. J. Comp. Pathol. 71:377-393.

Parker, J., E. O. Oviedo-Rondón, B. A. Clack, S. Clemente-Hernández, J. Osborne, J. C. Remus, H. Kettunen, H. Mäkivuokko, and E. M. Pierson. 2007. Enzymes as feed additive to aid responses against *Eimeria* species in coccidia-vaccinated broilers fed corn-soybean meal diets with different protein levels. Poult. Sci. 86: 643-653.

Pirgozliev, V., M. R. Bedford, T. Acamovic, and M. Allimehr. 2011. The effects of supplementary bacterial phytase on dietary true metabolizable energy, nutrient digestibility and endogenous losses in precision fed turkeys. Br. Poult. Sci. 52:214-220.

Porter, R. E. 1998. Bacterial enterides of poultry. Poult. Sci. 77:1159-1165.

Pratt, I. 1937. Excystation of the coccidia, *Eimeria tenella*. J. Parasitol. 23:426-427.

Prattley, C. A. and D. W. Stanely. 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6:243-253.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78:699-706.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility, and nutrient retention. Br. Poult. Sci. 41:193–200.

Ravindran, V., P. C. H. Morel, G. G. Partridge, et al… 2006. Influence of an *Escherichia coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82–89.

Rehman, H. U., W. Vahjen, W. A. Awad, and J. Zentek. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch. Anim. Nutr. 61:319-335.

Richards, J. D., J. Gong, and C. F. M. de Lange. 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: Current understanding, possible modulations, and new technologies for ecological studies. Can. J. Anim. Sci. 85:421-435.

Riddell, C., and X. M. Kong. 1992. The influence of diet on necrotic enteritis in broiler chickens. Avian Dis. 36:499-503.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13:255-278.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126-141.

Snel, J., H. J. M Harmssen, P. W. J. J van der Wielen, and B. A. Williams. 2002. Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. Pages 37-69 *in* Nutrition and health of the gastrointestinal tract. M. C. Blok, H. A. Vahl, L. de Lange, A. E. van de Braak, G. Hemke, and M. Hessing, eds. Wageningen Academic Publishers, the Netherlands.

Stein, H. H., B. Sève, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180.

Tamim, N. M., R. Angel, and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83:1358-1367.

Thavarajah, P., D. Thavarajah, and A. Vandenberg. 2009. Low phytic acid lentils (*Lens culinaris* L.): A potential solution for increased micronutrient bioavailability. J. Agric. Food Chem. 57:9044-9049.

Timbermont, L., A. Lanckriet, A. R. Gholamiandehkordi, F. Pasmans, A. Martel, F. Haesebrouck, R. Ducatelle, F. Van Immerseel. 2009. Origin of *Clostridium perfringens* isolates determines the ability to induce necrotic enteritis in broilers. Comp. Immunol. Microbiol. Inf. Dis. 32:503-512.

Timbermont, L., F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2011. Necrotic enteritis in broilers: an updated review on the pathogenesis. Avian Pathol. 40:341-347.

Türk, M., A. S. Sandberg, N. G. Carlsson, and T. Andlid. 2000. Inositol hexaphosphate hydrolysis by baker's yeast. Capacity, kinetics, and degradation products. J. Agric. Food Chem. 48:100-104.

Uni, Z., S. Ganot, and D. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci. 75:1104-1108.

Uni, Z., S. Smirnov, and D. Sklan. 2003. Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. Poult. Sci. 82:320-327.

Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol. 33:537-549.

Viveros, A., C. Centeno, A. Brenes, R. Canales, and A. Lozano. Phytase and acid phosphatase activities in plant feedstuffs. J. Agric. Food Chem. 48:4009-4013.

Wages, D.P. and O. Kenneth. 2003. Necrotic enteritis in Diseases of Poultry. 11th ed. Y.M. Saif, ed. Blackwell Publishing Co., Ames, IA. Pgs. 781-785.

Waldenstedt, L., A. Lundén, K. Elwinger, P. Thebo, and A. Uggla. 1999. Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore, on performance of broilers raised with or without a growth promoter, in an initially *Eimeria* free environment. Acta Vet. Scand. 40:11-21.

Waldenstedt, L., K. Elwinger, A. Lundén, P. Thebo, M. R. Bedford, and A. Uggla. 2000. Intestinal digesta viscosity decreases during coccidial infection in broilers. Br. Poult. Sci. 41:459-464.

Waldenstedt, L., K. Elwinger, A. Lundén, P. Thebo, and A. Uggla. 2001. Sporulation of *Eimeria maxima* oocysts in litter with different moisture contents. Poult. Sci. 80:1412-1415.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159-180.

Wise, M. G., and G. R. Siragusa. 2007. Quantitative analysis of the intestinal bacterial community in one- to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. J. Appl. Microbiol. 102:1138-1149.

Woyengo, T. A., A. J. Cowieson, O. Adeola, and C. M. Nyachoti. 2009. Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. Br. Poult. Sci. 102:428–433.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measures and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br. Poult. Sci. 45:76-84.

Yegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052-2063.

Yvore, P., and P. Coudert. 1972. Etude de la respiration endogene et de la segmentation de l'oocyste d'*Eimeria tenella* Durant la sporgonie. Ann. Recherches Veterinaires. 3:131-143.

Zebrowska, T., A. G. Low, and H. Zebrowska. 1983. Studies on gastric digestion of protein and carbohydrate, gastric secretion, and exocrine pancreatic secretions in the growing pig. Br. J. Nutr. 49:401-410.

CHAPTER III

Dietary phytic acid effects on broiler performance, small intestinal morphology, and apparent digestibility during a coccidial vaccination

R. N. Lehman[1], A. J. Cowieson[2], C. L. Walk[2], and A. P. McElroy[1]

[1] Virginia Tech, Department of Animal and Poultry Sciences, Blacksburg, VA
[2] AB Vista, Wiltshire, Marlborough, UK

ABSTRACT The effects of dietary phytic acid (**PA**) and a live coccidial oocyst vaccine on broiler performance, intestinal morphology, and nutrient digestibility were evaluated in a 30 d floor pen experiment. Purified PA in an aqueous solution was included in the diet at 0 g/kg, 6 g/kg, or 12 g/kg to alter the level of phytic acid. The three diets were given to birds that were either spray-vaccinated on d 1 with a live coccidial oocyst vaccine or birds that received no coccidiosis prevention, resulting in 6 treatments. A starter diet was fed from d 0 to 18, and a grower diet was fed from d 18 to 30. Vaccinated birds given diets with added PA had lower feed intake ($P \leq 0.01$) and body weight gain ($P \leq 0.05$) compared to the non-vaccinated birds given the same diets. Feed conversion was increased ($P = 0.0120$) from d 18 to 30 by vaccination, but birds given the medium PA diet generally had improved feed conversion ($P \leq 0.05$) compared to birds given the low or high PA diets. A significant increase was seen in mortality ($P \leq 0.001$) in which the vaccinated birds given the medium PA diet had higher mortality than all other treatments. Phytic acid alone generally resulted in longer intestinal villi while vaccination alone tended to increased crypt depth. Vaccination and PA both had main effects on apparent ileal amino acid digestibility (**IAAD**) where vaccination decreased ($P < 0.01$) apparent IAAD of most amino acids on d 21 and addition of PA improved ($P = 0.01$) total IAAD. These results suggest that a PA solution added to the diets of broilers could improve FI and BWG through increased P digestibility. Medium concentrations of PA combined with a live *Eimeria* oocyst vaccine induce

severe necrotic enteritis. Dietary calcium may also have an important impact on bird performance in relation to phytate solubility and nutrient digestibility.

Keywords: phytate, broiler, coccidia, vaccination, necrotic enteritis

INTRODUCTION

A major component of commercial poultry diets is plant-based ingredients containing variable levels of the anti-nutrient phytate. Phytate, or the salt of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), derives its anti-nutrient properties from its ability to carry a negative charge and bind biologically important nutrient cations at virtually all pH levels found in the avian gastrointestinal tract (Maenz et al., 1999). While poultry possess endogenous phytase, the enzyme necessary for the hydrolysis of phytate, in the presence of Ca in the diets, its efficacy is drastically reduced such that apparent ileal phytate-P hydrolysis drops from 69.2% to 25.4% (Tamim et al., 2004). As a result, the availability of P and other minerals is lower.

In addition to reducing the availability of nutrients through direct interactions, phytate decreases apparent ileal digestibility of protein (Ravindran et al., 2006; Cowieson et al., 2006), presumably through lower solubility as a result of protein:protein aggregation (Cowieson and Cowieson, 2011). It has recently been suggested that phytate can electrostatically create a hydration shell around itself and alter the thermodynamics of the water matrix likely negatively affecting the solubility of other compounds and transport of nutrients (Cowieson and Cowieson, 2011). Consequently, the efficiency of nutrient utilization may be reduced through the overproduction of endogenous secretions (Cowieson and Ravindran, 2007; Cowieson et al., 2009; Woyengo et al., 2009), which likely increase the maintenance energy requirement of the bird. Poorer protein solubility in the gastric phase of digestion may stimulate an increase in

pepsin and HCl secretion to compensate for the reduction in solubility (Cowieson et al., 2008). In the small intestine, the secretion of amino acids is exaggerated, specifically those contained in mucin, pancreatic enzymes, and bile secretions (Cowieson et al., 2004), while the ability to reabsorb these endogenous proteins and other minerals is compromised (Cowieson et al., 2008). Consequently, a negative net N balance may result, and dietary requirements for specific amino acids or minerals that comprise these endogenous secretions may be altered (Cowieson et al., 2008).

Administration of live coccidial oocyst vaccines to commercial broilers has become increasingly common. Many studies have shown no difference in the final performance of coccidiosis-vaccinated birds compared to non-vaccinated birds (Bedrnik et al., 1989; Mathis, 1999; Williams et al., 1999). However, others have demonstrated the mild infection induced by a live coccidial vaccination limits optimal bird performance, especially within the first three weeks following vaccination (Newman, 1999; Waldenstedt et al., 1999; Mathis, 1999; Parker et al., 2007; Lehman et al., 2009). The infection and its ensuing damage to the intestinal surface can reduce the absorptive surface area of the villi (Idris et al., 1997), likely contributing to the decrease in body weight gain and poorer feed conversion and overall performance. In addition, intestinal damage may release plasma proteins into the lumen (Van Immerseel et al., 2004) and inflammation can enhance mucogenesis. While mucins are typically necessary for the protection of mucosal surfaces (Forstner and Forstner, 1994), overproduction of mucin and the concomitant release of plasma proteins can serve as nutrient substrates for mucolytic bacteria like *C. perfringens* and detrimentally promote their proliferation (Van Immerseel et al., 2004; Collier et al., 2008). Therefore, as an indirect result, vaccination against coccidiosis could predispose birds to necrotic enteritis (**NE**) (Van Immerseel et al., 2004; Williams, 2005) and cause an exaggerated

dietary requirement for certain amino acids such as those needed for mucin production and intestinal repair.

Bird response to vaccination depends on a variety of factors, including vaccine dose, heath status of the bird, and management; but it is also critical to understand the role that diet composition has in determining bird response to coccidiosis vaccination. It is possible that reduced nutrient availability from phytate combined with compromised absorption from vaccination will synergistically diminish bird performance, as the antinutritive effects of PA may be more severe when intestinal absorptive capacity is limited (Cowieson et al., 2008). Therefore, the purpose of this study was to determine bird performance, intestinal morphology, and apparent ileal amino acid digestibility when given various levels of dietary phytic acid in conjunction with a live coccidial oocyst vaccine.

MATERIALS AND METHODS

Bird Husbandry and Diets

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. A total of 2,520 day-old Cobb 700 male broilers were obtained from a commercial hatchery and transported to the poultry research farm at Virginia Tech. Upon arrival, half of the chicks were spray-vaccinated with a vaccine[1] that contained live oocysts of *Eimeria acervulina*, *Eimeria mivati*, *Eimeria maxima*, and *Eimeria tenella* following manufacturer recommendations. Birds that were not vaccinated did not receive any anti-coccidial treatment. Chicks were randomly selected, weighed, and placed 35 chicks per pen in one of 72 floor pens with clean pine shavings and a final stocking density of 9.32 kg/m^2. Birds were

[1] Coccivac® - B, Intervet, Schering-Plough Animal Health, Omaha, NE

provided ad libitum access to feed and water and reared with a commercial lighting program in a closed-sided house with temperature control.

All diets were formulated on a corn-soybean meal basis to meet Cobb broiler nutrient recommendations (Table 3.1) and fed as a crumble (starter) or as whole pellets (grower and finisher) with 0.1% titanium oxide[2] as an indigestible marker. Percent available phosphorus was held constant while allowing the amount of total phosphorus to fluctuate, depending on the amount of phytic acid that was added to the diet. Vaccinated and non-vaccinated birds were given one of three experimental diets containing different levels of purified phytic acid (**PA**)[3] in liquid form, for a total of 6 treatments with 12 replicate pens/treatment. The low PA diet contained no added phytic acid while PA was added in at 0.6% of the diet for the medium PA diet and at 1.2% of the diet for the high PA diet. Water was added to the low and medium PA diets to ensure all diets had an added volume of liquid equivalent to that of the high phytic acid diet.

Sample Collection

Body weight (**BW**) and feed intake (**FI**) of birds were measured by pen on d 0, d 18, and d 30 to calculate body weight gain (**BWG**) and feed conversion (**FC**) for each feeding period (starter - d 0 to d 18; grower - d 18 to d 30) and cumulatively (d 0 to 30). Mortality was recorded daily, and birds removed for mortality or sampling were weighed to adjust the FI and FC respective of the number of bird days. Birds were euthanized by cervical dislocation for collection of digesta and tissue samples.

On d 7 and 21, intestinal tissues were obtained from one bird/pen (n=12 birds/treatment) for histological evaluation of morphology, including villus height (**VH**), crypt depth (**CD**), villus

[2] Sigma-Aldrich, St. Louis, MO
[3] Sigma-Aldrich, St. Louis, MO

height to crypt depth ratio (**VCR**), and goblet cell number. Tissue samples, approximately 1 inch in length, were taken from the midpoint of the duodenum, jejunum, and ileum and gently flushed with PBS to remove luminal contents before placement in 10% neutral-buffered formalin until further processing. Each segment was cut into five 5 mm sections, embedded in paraffin, cut to 5 μm, and mounted onto slides. Slides were stained using Periodic Acid-Schiff's reagent and Alcian Blue and examined with light microscopy[4]. Measurements were obtained using Sigma Scan Pro 5[5] and digitized using Image Pro Plus[6]. Both villus height and crypt depth (n=12) were measured from 3 of the 5 tissue sections/slide, following procedures according to Sun et al. (2005). Briefly, VH was measured from the tip of the villus to the base where it intersects with the opening of the crypt; CD was measured from the crypt opening to the base of the crypt; and VCR was calculated and normalized using the natural log. The goblet cell number/villus area was calculated by determining the total number of goblet cells/respective villus area and normalized using the natural log (though not discussed in this manuscript, results from goblet cell counts are available in the Appendix in tables A.1 and A.2).

On d 7 and 21, digesta from five birds/pen (n=60 birds/treatment) was collected from the ileum, as defined as the area immediately posterior to Meckel's diverticulum to the ileo-cecal junction, and pooled before being snap-frozen on dry ice and stored at -80˚C until further analysis. Digesta samples were later lyophilized and diet and digesta samples ground to pass through a 1mm screen. Titanium oxide concentration in the diet and digesta samples was determined according to methods described by Short et al. (1996). Amino acid concentrations of the diet and digesta samples were determined using HPLC following acid hydrolysis according to modified methods of Albin et al. (2000). Briefly, 100mg of sample was purged for 30 seconds

[4] Olympus Polaroid DMC-IE camera, Polaroid Corporation, MA
[5] SPSS, Chicago, IL
[6] Media Cybernetics, Silver Springs, MD

with N_2 and hydrolyzed for 24 hours at 100˚C in approximately 6ml of 6M HCl. Hydrolyzed samples were filtered using 0.45µm luer lock syringe filters. Filtered, hydrolyzed samples were added to 100µl of the internal standard Norleucine, centrifuged, dried twice, and analyzed using a Pico-Tag®[7] column and HPLC. Amino acids were identified and integrated using Pierce hydrolyzed standard, (Fisher P120088) and grams amino acid/ 100 g of sample was calculated. Amino acid values were then used to calculate apparent ileal amino acid digestibility (**IAAD**) using the following equation:

Digestibility Coefficient = $[(\text{amino acid/TiO})_d - (\text{amino acid/TiO})_i) / (\text{amino acid/TiO})_d * 100$, where $_d$ = amino acid and titanium oxide concentration of the diet, and $_i$ = amino acid and TiO concentration of the ileal samples. Phosphorus concentration of the digesta samples was determined using a Genesys 5[8] spectrophotometer following nitric/perchloric acid wet-ash digestion.

Statistical Analysis

Performance data were subjected to ANOVA as a 2 X 3 factorial design consisting of vaccination and phytic acid as main effects and analyzed using the MIXED procedure of SAS. Pen served as the experimental unit for BW, BWG, FI, FC, percent mortality, and apparent IAAD. Percent mortality data were arc sine transformed prior to analysis. Bird served as the experimental unit for morphological measurements. Mean differences were adjusted using the Tukey method, and statistical significance was accepted at $P \leq 0.05$. Day 0 BW was used as a covariate to analyze d18 BW due to initial differences unrelated to treatment. Also, d18 BW was used as a covariate to analyze differences in d30 BW.

[7] Waters Corporation, Milford, MA
[8] Thermo Electron Corportation, Madison, WI

RESULTS

Growth Performance

Diet analysis revealed percentages of Ca and crude protein were generally higher than formulated values (Table 3.1), especially for the starter diet. Lysine and threonine values were also higher than formulated; however, the Thr:Lys ratio was still maintained between suggested values (NRC, 1994; Baker et al., 2002).

Phytic acid and vaccination also resulted in a significant interaction for BW and BWG ($P \leq 0.05$; Tables 3.2 and 3.3). The interaction appeared to be a result of the non-vaccinated birds having a heavier BW and greater BWG than the vaccinated birds when given the medium and high PA diets as compared to the low PA diet.

There was a greater intake of feed for the non-vaccinated birds given the medium and high PA diets compared to the vaccinated birds given those diets, resulting in an interaction ($P < 0.01$) between PA and vaccination during each feeding period and overall (Table 3.4). For each period and cumulatively, in the non-vaccinated birds both medium and high PA resulted in increased FI ($P < 0.01$) as compared to low PA. However, in the vaccinated birds, low PA and medium PA resulted in similar FI, and birds on high PA diets had higher FI ($P < 0.01$). There were no significant interactions between PA and vaccination that affected FC. Vaccination resulted in less efficient ($P = 0.012$) feed conversion from d 18 to 30 (Table 3.5). Birds fed the medium PA diet had improved FC compared to birds fed the low or high PA diets ($P < 0.05$), except during d 0 to 18 where birds fed the medium PA diet only had improved FC compared to birds given the low PA diet.

On d 16, there was an increase in mortality due to necrotic enteritis that lasted until about d 21. The significant increase in mortality resulted in a PA by vaccination interaction ($P \leq 0.001$)

during each period and cumulatively (Table 3.6) in which vaccinated birds given the medium PA diet had a larger percent mortality than all other treatments.

Small Intestinal Morphology

In each segment of the small intestine, villi height (VH) and crypt depth (CD) were measured as indicators of the effect of treatment on intestinal morphology (Tables 3.7 and 3.8). On day 7 (Table 3.7), vaccination increased ($P < 0.0001$) the depth of crypts in the duodenum. High PA resulted in longer villi in the duodenum ($P = 0.0003$) and jejunum ($P=0.0338$) compared to the low PA diet, with the medium PA diet being intermediate between the two. The only significant ($P=0.0353$) interaction between PA and vaccination on intestinal morphology was seen in ileal CD where CD did not change in non-vaccinated birds with addition of PA; however, in the vaccinated birds, the medium and high PA diets resulted in shallower crypts compared to those birds given the low PA diet.

On day 21 (Table 3.8), there was a significant PA by vaccination interaction on VH in the duodenum ($P = 0.0009$) and jejunum ($P = 0.0037$) and on the crypt depth in the duodenum ($P = 0.0389$). In the duodenum, the level of PA in the diet had no effect on VH in vaccinated birds but in the non-vaccinated birds, the medium and high PA diets increased the height of the villi compared to that in birds given the low PA diet. In the jejunum, the effect was rather opposite where the level of PA in the diet of non-vaccinated birds had no effect on VH, while in the vaccinated birds, the medium and high PA diets resulted in shorter villi compared to the low PA diet. Vaccination lead to deeper crypts in the duodenum, regardless of the level of PA in the diet, but the interaction is due to a more significant change in the depths of the crypts of birds given the medium PA diet compared to the other two diets. Vaccinated resulted in deeper crypts ($P < 0.0001$) in the jejunum and shorter villi ($P < 0.006$), and deeper crypts ($P < 0.0155$) in the ileum.

Apparent Ileal Amino Acid and Phosphorus Digestibility

Apparent IAAD was measured on d 7 and d 21. On d 7 (Table 3.9a and 3.9b), vaccination had no effect on apparent IAAD, but PA had a significant effect on the digestibility of all amino acids and total digestibility ($P < 0.05$). The diets containing medium or high PA did not differ from each other but had greater digestibility than the low PA diet. The only significant interaction between PA and vaccination ($P = 0.0225$) was seen in aspartic acid digestibility where vaccination caused a decrease in digestibility in birds given the low PA diet as compared to non-vaccinated birds on this diet. This difference between vaccinated and non-vaccinated birds was not seen with the medium or high PA diets.

On day 21 (Tables 3.10a and 3.10b), interactions between PA and vaccination were only seen for arginine ($P = 0.0354$) and proline ($P = 0.0427$). Phytic acid level did not affect the apparent IAAD of arginine in non-vaccinated birds, whereas in vaccinated birds, the high PA diet resulted in a higher IAAD compared to the other two diets. For proline, the interaction was due to vaccination decreasing the apparent IAAD in birds given the medium PA diet but having no effect on birds given the low or high PA diet. Vaccination caused a decrease ($P < 0.01$) in total apparent ileal digestibility and digestibility of all individual amino acids except for arginine and aspartic acid. All individual amino acids except threonine and leucine were affected ($P < 0.05$) by dietary PA. For all amino acids where dietary PA impacted digestibility, with the exception of histidine, aspartic acid, and glutamic acid, high PA resulted in increased digestibility compared to either low or medium PA, which had similar digestibilities. For total amino acid digestibility, birds given the high PA diet had higher total digestibility ($P<0.01$) than the other two diets, which were not different from each other.

Digestibility coefficient for P, digestible P content, and digestible P intake on d 7 (Table 3.11) was higher ($P < 0.0001$) in the medium and high PA diets compared to the low PA diet. On d 21 (Table 3.12), there was an interaction in the digestibility coefficient and digestible P content ($P < 0.05$) where a lower value was seen in the vaccinated birds given the low and medium PA diets but similar in the vaccinated and non-vaccinated birds given the high PA diet. For digestible P intake, there was an increase ($P < 0.0001$) in digestible P intake as PA was added to the diet.

DISCUSSION

Some of the initial negative effects on broiler performance associated with a live coccidial oocyst vaccination, such as reduced FI and BWG and increased FC, have been previously reported (Newman, 1999; Parker et al., 2007; Lehman et al., 2009) and are consistent with current results. Conversely, the improvement in FI and BWG seen with increased concentration of dietary PA is contrary to previous research that found that PA decreased FI and BW and increased FCR (Liu et al., 2008; Onyango and Adeola, 2009). Interestingly, other researchers found that dietary phytate caused no change in performance, especially when available P was similar among diets (Jang et al., 2003; Linares et al., 2007; Salarmoini et al., 2008). It should be taken into account that the studies in which PA resulted in no change in performance compared low phytate and conventional grain, while the other studies generally looked at alternative dietary ingredients such as sodium phytate, rice bran, and corn germ meal to alter phytic acid concentration. It should be considered whether it was the actual phytate content in these diets or the addition of an alternative ingredient that substantially affected performance. Generally, ingredients that are higher in phytate also tend to be higher in fiber, which is

associated with reduced protein utilization and increased secretion of endogenous AA, possibly contributing to negative effects on performance (Angkanaporn et al., 1994). Additionally, the source of phytate may play a role the bird response. Phytic acid in a free form has less of an effect on mucin loss from the intestinal tract of broilers compared to magnesium-potassium phytate (Onyango et al., 2009). This may suggest PA in free form as used in this experiment may be less detrimental to intestinal health than the magnesium-potassium phytate form more commonly found in nature.

In addition to dietary phytic acid, the presence of other ingredients needs to be considered. The role of dietary mineral levels, especially calcium, in the hydrolysis of phytate and availability of nutrients has been previously documented (Maenz et al., 1999; Tamim et al., 2004). Although formulated to be at similar levels across diets, the actual value of Ca in the low PA diet in this study was much higher than that of the other diets. Excess Ca in the diet can reduce nutrient utilization potentially through the formation of Ca-phytate complexes that bind important minerals (Wise, 1983) or by depressing the activity of alkaline phosphatase and phytase (McCuaig et al., 1972). It is possible that the reduction in nutrient digestibility, as a result of Ca-phytate complex formation, may have contributed to the unexpected decrease in FI and BW in birds given the low PA. Previous findings have reported that an increase in Ca:tP beyond 1.1:1 lowered BW, FI, P and Ca retention, and activity and efficacy of supplemented phytase (Qian et al., 1997). The Ca:tP in this study was much higher in the low PA diet (1.83 and 1.56 for the starter and grower, respectively) than in the medium or high PA diets. Additionally, the increase in digestibility of P that occurred with the addition of PA may also help to explain the improvement in FI and BWG. It is possible that the PA used in this study had a greater digestibility than what was originally formulated, and the additional digestible P contributed to a

higher FI and BWG. Perhaps the level of calcium or digestible P in the diet is of greater importance than the level of phytate itself as hydrolysis of phytate is influenced greater by calcium and phytate source rather than concentration of phytate or level of dietary fiber (Ballam et al., 1984).

The spike in mortality starting on d 16 in the vaccinated birds given the medium PA diet was determined to be NE upon post-mortem inspection, and quite fittingly, coincides with the last stage in the vaccine-initiated coccidial life cycle before peak oocyst shedding (Schering-Plough, 2007). In addition to causing higher mortality, the combination of vaccination and medium PA reduced FI. Anorexia during infection can be a mechanism of host defense that reduces nutrients available for pathogenic organisms (Murray and Murray, 1979). It is possible that the reduced FI for the vaccinated birds given the medium PA diet was a physiological attempt to suppress the NE infection. Furthermore, any stimulation of the immune system, even in the absence of tissue damage or major pathological alterations, can reduce FI and feed efficiency (Klasing et al., 1987). The similar reduction in BWG of the vaccinated birds given the medium PA diet is likely a direct consequence of the reduced FI, or conceivably nutrients may have been redirected to support an immune response rather than being utilized for growth (Barnes et al., 2002). Despite the high mortality, there was a general improvement in FC in vaccinated birds given the medium PA diet. Though unexpected, this outcome is likely related to mortality as an improved FC frequently follows high mortality. Presumably the remaining birds that did not die from NE were generally more efficient than those that became infected.

The use of live coccidial oocyst vaccines could predispose birds to other enteric infections such as NE (Williams, 2005). Though not completely understood, perhaps mild lesions caused by vaccination in some birds are enough to create an environment hospitable to

other microorganisms that may cause disease. Additionally, it is possible that the small shift in intestinal microbial communities that accompanies coccidiosis vaccination (Oviedo-Rondón et al., 2006) was exaggerated by the fact that antibiotic growth promotants and anticoccidial ionophores were not used in this trial. Consequently, the shift in microflora from vaccination may intensify into a more dramatic and potentially pathogenic population. In the present study, severe NE infection and mortality did not occur in all of the vaccinated birds, suggesting that vaccination alone is not enough to predispose birds to NE, but other factors may be necessary. In addition to disruption of intestinal integrity, the provision of an acceptable food source is necessary for the growth of *C. perfringens*, which cannot produce all essential amino acids and needs to obtain them from other sources (Cooper and Songer, 2009). Therefore, the disruption of intestinal mucin dynamics or the leakage of plasma protein into the lumen from intestinal damage (Van Immerseel et al., 2004) may provide an optimal nutrient source for the growth of these bacteria. Any change in mucin secretion can affect the carbohydrate profile of mucins and ultimately the adhesion of bacteria and competitive exclusion of pathogens (Horn et al., 2009). Both vaccination (Miller et al., 1979) and phytate (Cowieson et al., 2004) increase endogenous secretions and create an optimal environment for mucolytic bacteria, which may help to explain why the vaccinated birds given PA became infected with NE.

It is interesting to note, however, that the vaccinated birds given the high PA diet did not experience severe mortality. One consideration for the unique pattern of mortality may relate to the phytate: protein ratio of the diets. In 1990, Mothes et al. described how different phytate: protein ratios could be more or less conducive to protein solubility. They determined a phytate: protein ratio turbidity curve in which the ratio determined the turbidity, and hence, solubility of the protein. A ratio between 0.04 and 0.05 had the greatest turbidity (least solubility), and ratios

above or below that range had improved solubility. It may prove significant that the phytate: protein ratio of the medium PA diet is located directly within the low solubility range while the low and the high PA diets are below and above the range, respectively. If protein solubility in the medium PA diet was reduced, it is likely that more protein would have bypassed digestion and entered the distal GI tract, providing nutrients for proteolytic bacteria like *C. perfringens.* In addition, the insoluble protein-phytate complexes may have stimulated secretion of more digestive enzymes, aggravating the need for amino acids and potentially diverting the supply away from proper immune system function. It should be taken into consideration, though, that rapeseed protein was used in the Mothes et al. experiment and a more traditional poultry diet with an alternate protein source may have varied results.

Intestinal morphology is an important aspect of overall bird health as it relates considerably to nutrient acquisition (Kuzmuk et al., 2005). Absorptive epithelial cells that line the villi are generated in the crypts and migrate up the villi to replace old desquamated cells (Schat and Myers, 1991). These cells are essential for nutrient absorption, therefore maximal nutrient uptake and bird performance is attained with an optimal balance of villi height and crypt depth. Longer villi increase the surface area of the intestine, which is necessary to increase absorptive capacity (Caspary, 1992). Disruption to the migration of epithelial cells by infections, toxins, etc. may interfere with the intestinal barrier, likely having detrimental consequences on bird health (Schat and Myers, 1991). In this experiment, vaccination generally resulted in increased depth of the crypts. Deeper crypts are commonly found in coccidiosis infections (Morris et al., 2004) as a result of faster tissue turnover in an attempt to compensate for epithelial destruction (Rose et al., 1992). Vaccination with live oocysts induces a mild, transient infection necessary to induce immunity (Shirley, 1992; Williams, 2002b); therefore, the deeper crypts in

vaccinated birds are likely in response to inflammation and sloughing of cells on the villi (Yason et al., 1987). On d 7, longer villi in the duodenum and jejunum resulted with increasing PA concentration. Though this was not expected, birds given this treatment had heavier BW and hence may have had proportionally larger villi. The lack of difference seen in the ileum may be due to the fact that there is less digestive function in the ileum (Yamauchi et al., 1996), which may have resulted in a muted response to dietary ingredients. Interestingly, ileal CD was not affected with addition of PA in the non-vaccinated birds, but inclusion of medium or high levels of PA in diets of vaccinated bird resulted in shallower crypts. Little to no change was expected to occur in the ileum of vaccinated birds due to the fact that the vaccine contains oocysts of *E. acervulina*, *E. maxima*, *E. mivati*, and *E. tenella* that invade the duodenum, jejunum, duodenum/jejunum, and ceca of broilers, respectively. In terms of intestinal morphology, it should also be taken into consideration that alterations observed in one segment of the intestinal tract may be in response to treatment-related changes that occurred in previous intestinal sections (Oviedo-Rondón et al., 2006).

On d 21, vaccination increased the depths of the crypts in the jejunum and ileum. Most likely there was no significant difference in the duodenum due to the fact that CD was significantly increased in that section on d 7 and probably had less of a dramatic change from d 7 until d 21. Vaccination also resulted in shorter villi in the ileum. Previously, it has been shown that a coccidiosis infection can result in shorter villi (Klasing et al., 2002; Assis et al., 2010), and because d 21 is during the peak of oocyst shedding with this vaccine (Schering-Plough, 2007), it is possible the oocysts caused a mild infection that increased villi sloughing. The crypts in the duodenum of the vaccinated birds were generally deeper than those in the non-vaccinated birds, regardless of PA level. As previously discussed, this is expected in order to increase cell turnover

to repair vaccination-damaged villi (Yason et al., 1987). Interestingly, birds given the medium PA diet had significantly deeper crypts than the other treatments. As demonstrated by the high mortality, this treatment had the most severe NE. Bacterial infections have been shown to damage enterocytes and increase CD (Miles et al., 2006; Jiang et al., 2009), so the severe infection in these birds may have lead to the deeper crypts.

Apparent digestibility values encompass the inherent digestibility characteristics of an ingredient as well as an animal's physiological response to that ingredient. A diet that has a lower apparent digestibility may be reflective of amino acids from the diet that were not absorbed in addition to any endogenous secretions that were secreted as a result of the diet. Because of the ability of PA to bind nutrients and increase endogenous protein secretions (Cowieson et al., 2004), the increase in apparent IAAD in birds receiving the diets with added PA was unexpected. Perhaps Ca levels in the diets, as mentioned previously, play a larger role in terms of phytate solubility and subsequent digestibility of other nutrients such as AA. Because the high PA diet had lower Ca levels than that of the low PA diet, it is possible that the Ca in the low PA diet formed complexes with the phytic acid and lead to a reduction in digestibility. Additionally, PA has been shown to have variable effects on amino acid digestibility in pigs and does not always result in reduced IAAD (Woyengo et al., 2009). In terms of endogenous secretions, increasing PA concentration does not affect all amino acids similarly, but rather the composition of endogenous secretions changes to reflect the amino acid profile of the secretions (Cowieson et al., 2008). This potentially explains differences with the high PA diet where PA had no effect on the IAAD of threonine or leucine but increased that of arginine in vaccinated birds. The reduction in apparent IAAD from vaccination on d 21 is likely a result of the infection and damage to the villi, which reduces nutrient absorption (Preston-Mafham and Sykes, 1970;

Ruff and Wilkins, 1980). Perhaps the apparent reduction in IAAD is a result of increased secretion of various proteins that accompanies coccidiosis vaccination. The lack of many significant interactions may be due to the fact that apparent IAAD only gives a measurement for a specific point in time and may not be representative of the subtle changes that occur throughout the infection cycle and different feeding periods.

The use of the phytic acid solution may be subject to criticism as it is not representative of commercial feeding applications. However the use of a purified form eliminated any confounding factors that an altered dietary composition may have had on bird performance. The introduction of an ingredient such as rice bran or canola meal to alter the phytate concentration may introduce a number of variables like amino acid balance, fiber, and other anti-nutrients. Additionally, the use of low phytate grains may not be practical in a commercial situation as the cost of phytase is inexpensive and relatively easy to obtain compared to the altered grain.

Addition of PA generally improved FI and BWG, likely a result of improved digestible P content. Medium concentrations of dietary PA given to coccidiosis-vaccinated broilers allowed overproliferation of *C. perfringens* and subsequent NE. It is possible that a specific phytate: protein ratio combined with the vaccination is responsible for greater insolubility of the diet, increased production of endogenous proteins, and potentially less nutrient allocation to the immune system. Furthermore, the effect of Ca or Ca:tP levels in the diet should not be neglected in terms of a possible explanation of varied responses attributed to phytate.

REFERENCES

Albin, D. M., J. E. Wubben, and V. M. Gabert. 2000. Effect of hydrolysis time on the determination of amino acids in samples of soybean products with ion-exchange chromatography or precolumn derivatization with phenyl isothiocyanate. J. Agric. Food Chem. 48:1684-1691.

Angkanaporn, K., M. Choct, W. L. Bryden, and E. F. Annison. 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. J. Sci. Food Agric. 66:399-404.

Assis, R. C. L., F. D. Luns, M. E. Beletti, R. L. Assis, N. M. Nasser, E. S. M. Faria, and M. C. Cury. 2010. Histomorphometry and macroscopic intestinal lesions in broilers infected with *Eimeria acervulina.* Vet. Parasitol. 168:185-189.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ileal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485-494.

Ballam, G. C., T. S. Nelson, and L. K. Kirby. 1984. Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. Poult. Sci. 63:333-338.

Barnes, D. M., Z. Song, K. C. Klasing, and W. Bottje. 2002. Protein metabolism during an acute phase response in chickens. Amino Acids. 22:15-26.

Bedrnik, P., J. Kucera, A. Firmanova, and P. Jurkovic. 1989. Field vaccination of broilers against coccidiosis. Avian Pathol. 18:255-264.

Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299s-308s.

Collier, C. T., C. L. Hofacre, A. M. Payne, D. B. Anderson, P. Daiser, R. I. Mackie, and H. R. Gaskins. 2008. Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting *Clostridium perfringens* growth. Vet. Immunol. Immunopathol. 122:104-115.

Cooper, K. K., and J. G. Songer. 2009. Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A. Anaerobe. 15:55-60.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals form broiler chickens. Br. Poult. Sci. 45:101-108.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006. Phytic acid and phytase: Implication for protein utilization by poultry. Poult. Sci. 85:878-885.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401-418.

Cowieson, A. J., and N. P. Cowieson. 2011. Phytate and the thermodynamics of water. Proc. Aust. Poult. Sci. Symp. 22:22-25.

Cowieson, A. J. and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow of amino acid composition of endogenous protein at the terminal ileum of grown broiler chickens. Br. J. Nutr. 98:745-752.

Cowieson, A. J., V. Ravindran, and P. H. Selle. 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult. Sci. 87:2287-2299.

Forstner, J. F. and G. G. Forstner. 1994. Gastrointestinal mucus. Pages 1255-1283 in Physiology of the Gastrointestinal Tract, 3rd ed. L. R. Johnson, ed. Raven Press, New York.

Horn, N. L., S. S. Donkin, T. J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. Poult. Sci. 88:1906-1914.

Idris, A., D. Bounous, M. Goodwin, J. Brown, and E. Krushinskie. 1997. Quantitative pathology of small intestinal coccidiosis caused by *Eimeria maxima* in young broilers. Avian Pathol. 26:731-741.

Jang, D. A., J. G. Fadel, K. C. Klasing, A. J. Mireles, Jr., R. A. Ernst, K. A. Young, A. Cook, and V. Raboy. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. Poult. Sci. 82:1914-1924.

Jiang, Z. Y., L. H. Sun, Y. C. Lin, X. Y. Ma, C. T. Zheng, G. L. Zhou, F. Chen, and S. T. Zou. 2009. Effects of dietary glycyl-glutamine on growth performance, small intestinal integrity, and immune responses of weaning piglets challenged with lipopolysaccharide. J. Anim. Sci. 87:4050-4056.

Klasing, K. C., D. E. Laurin, R. K. Peng, and M. Fry. 1987. Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone, and interleukin-1. J. Nutr. 117:1629-1637.

Klasing, K. C., K. L. Adler, J. C. Remus, and C. C. Calvert. 2002. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidia-infected chicks and increases functional properties of phagocytes. J. Nutr. 132:2274-2282.

Kuzmuk, K. N., K. S. Swanson, K. A. Tappenden, L. B. Schook, and G. C. Fahey, Jr. 2005. Diet and age affect intestinal morphology and large bowel fermentative end-product concentrations in senior and young adult dogs. J. Nutr. 135:1940-1945.

Lehman, R., E. T. Moran, and J. B. Hess. 2009. Response of coccidiostat- versus vaccination-protected broilers to gelatin inclusion in high and low crude protein diets. Poult. Sci. 88:984-993.

Linares, L. B., J. N. Broomhead, E. A. Guaiume, D. R. Ledoux, T. L. Veum, and V. Raboy. 2007. Effect of low phytate barley on zinc utilization in young broiler chicks. Poult. Sci. 86:299-308.

Liu, N., Y. J. Ru, A. J. Cowieson, F. D. Li, and X. CH. Cheng. 2008. Effects of phytate and phytase on the performance and immune function of broilers fed nutritionally marginal diets. Poult. Sci. 87:1105-1111.

Maenz, D. D., C. M. Engle-Schaan, R. W. Newkirk, and H. L. Classen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

Mathis, G. F. 1999. The influence of the coccidiosis vaccine, Coccivac-B on compensatory weight gain of broiler chickens in comparison with the anticoccidial, salinomycin. Poult. Sci. 78(Suppl. 1): 117.

McCuaig, L. W., M. I. Davies, and I. Motzok. 1972. Intestinal alkaline phosphatase and phytase of chicks: Effect of dietary magnesium, calcium, phosphorus and thyroactive casein. Poult. Sci. 51:526–530.

Miles, R. D., G. D. Butcher, P. R. Henry, and R. C. Littell. 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 85:476-485.

Miller, H. R. P., Y. Narva, and C. R. Parishm. 1979. Intestinal goblet cell differentiation in *Nippostrongylus*-infected rats after transfer of fractionated thoracic duct lymphocytes. Int. Arch. Allergy Appl. Immunol. 59:281-285.

Morris, B. C., H. D. Danforth, D. J. Caldwell, F. W. Pierson, and A. P. McElroy. 2004. Intestinal mucosal mast cell immune response and pathogenesis of two *Eimeria acervulina* isolates in broiler chickens. Poult. Sci. 83:1667-1674.

Mothes, R., K. D. Schwenke, and S. Grossmann. 1990. Modified method of phytic acid analysis in rapeseed. Nahrung. 34:99-101.

Murray, M. J., and A. B. Murray. 1979. Anorexia of infection as a mechanism of host defense. Am. J. Clin. Nutr. 32:593-596.

Newman, L. J. 1999. Coccidiosis control with vaccine: Been there, done that. Or have we? Proc. Arkansas Poult. Symp. Springdale, Arkansas. pp 52-55.

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. National Academy Press, Washington, DC.

Onyango, E. M., and O. Adeola. 2009. Dietary phytate (inositol hexaphosphate) regulates the activity of intestinal mucosa phytase. J. Anim. Physiol. Anim. Nutr. 93:639-646.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836-842.

Oviedo-Rondón, E. O., M. E. Hume, C. Hernández, and S. Clemente-Hernández. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poult. Sci. 85:854-860.

Parker, J., E. O. Oviedo-Rondón, B. A. Clack, S. Clemente-Hernández, J. Osborne, J. C. Remus, H. Kettunen, H. Mäkivuokko, and E. M. Pierson. 2007. Enzymes as feed additive to aid in responses against *Eimeria* species in coccidia-vaccinated broilers fed corn-soybean meal diets with different protein levels. Poult. Sci. 86:643-653.

Preston-Mafham, R. A., and A. H. Sykes. 1970. Changes in body weight and intestinal absorption during infections with *Eimeria acervulina* in the chicken. Parasitol. 61:417-424.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37–46.

Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an *Escherichia coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82-89.

Rose, M. E., B. J. Millard, and P. Hesketh. 1992. Intestinal changes associated with expression of immunity to challenge with *Eimeria vermiformis.* Infect. Immun. 60:5282-5290.

Ruff, M. D., and G. C. Wilkins. 1980. Total intestinal absorption of glucose and L-methionine in broilers infected with *Eimeria acervulina, E. mivati, E. maxima,* or *E. brunetti.* Parasitol. 80:555-569.

Salarmoini, M., G. L. Campbell, B. G. Rossnagel, and V. Raboy. 2008. Nutrient retention and growth performance of chicks given low-phytate conventional or hull-less barleys. Br. Poult. Sci. 49:321-328.

Schat, K. A., and T. J. Myers. 1991. Avian intestinal immunity. Crit. Rev. Poult. Biol. 3:19-34.

Schering-Plough Animal Health. 2007. Coccivac®-B Normal Reaction: Oocyst Shedding Pattern Technical Service Bulletin.

Shirley, M. W. 1992. Research on avian coccidia: An update. Br. Vet. J. 148:479-499.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215-221.

Sun, X., A. McElroy, K. E. Webb, Jr., A. E. Sefton, and C. Novak. 2005. Broiler performance and intestinal alterations when fed drug-free diets. Poult. Sci. 84:1294-1302.

Tamim, N. M., R. Angel, and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83:1358-1367.

Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol. 33:537-549.

Waldenstedt, L., A. Lundén, K. Elwinger, P. Thebo, and A. Uggla. 1999. Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore on performance of broilers raised with or without a growth promoter in an initially *Eimeria* free environment. Acta. Vet. Scand. 40:11-21.

Williams, R. B., W. W. H. Carlyle, D. R. Bond, and I. A. G. Brown. 1999. The efficacy and economic benefits of Paracox®, alive attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. Int. J. Parasitol. 29:341-355.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159-180.

Wise, A. 1983. Dietary factors determining the biological activities of phytate. Nutr. Abstr. Rev. 53:791–806.

Woyengo, T. A., A. J. Cowieson, O. Adeola, and C. M. Nyachoti. 2009. Ileal digestibility and endogenous flow of minerals and amino acids: Responses to dietary phytic acid in piglets. Br. J. Nutr. 102:428-433.

Yamauchi, K., H. Kamisoyama, and Y. Isshiki. 1996. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. Br. Poult. Sci. 37:909-921.

Yason, C. V., B. A. Summers, and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. Am. J. Vet. Res. 6:927-938.

Table 3.1 Composition and nutrient content of diets

	Starter (2998 kcal/kg)			Grower (3142 kcal/kg)		
Ingredients	Low PA	Med PA	High PA	Low PA	Med PA	High PA
Corn	55.98	54.76	53.54	59.35	58.13	56.91
Soybean meal	37.67	37.89	38.12	32.96	33.17	33.39
Poultry Fat	2.29	2.72	3.16	4.09	4.52	4.95
Limestone	0.81	0.85	0.89	0.75	0.79	0.82
Dicalcium phosphate	1.75	1.68	1.62	1.58	1.52	1.45
Salt	0.45	0.45	0.45	0.40	0.40	0.41
L-Lysine	0.30	0.30	0.30	0.20	0.20	0.194
DL-Methionine	0.36	0.36	0.36	0.30	0.30	0.30
L-Threonine	0.062	0.061	0.061	0.055	0.054	0.054
Selenium premix[1]	0.016	0.016	0.016	0.017	0.017	0.017
Phytic acid[2]	0.0	0.6	1.2	0.0	0.6	1.2
Constant[3]						
Calculated Nutrient Comp.						
Crude Protein	22	22	22	20	20	20
Lysine	1.40	1.40	1.40	1.20	1.20	1.20
Threonine	0.88	0.88	0.88	0.80	0.80	0.80
Ca	0.90	0.90	0.90	0.82	0.82	0.82
Total P	0.64	0.71	0.78	0.59	0.66	0.73
Available P	0.45	0.45	0.45	0.41	0.41	0.41
Analyzed Composition						
Crude Protein	23.49	23.81	21.31	20.33	20.73	20.76
Lysine	2.023	1.833	1.735	1.628	1.413	1.499
Threonine	1.227	1.161	1.126	1.001	0.918	0.980
Ca	1.26	0.77	1.09	1.06	0.82	0.84
Total P	0.69	0.83	0.90	0.68	0.74	0.82
Phytic Acid	0.68	0.91	1.08	0.80	0.83	1.15

[1] Supplied per kilogram mix: 600 ppm

[2] Sigma-Alrich, 50% (w/w) in H_2O

[3]Constant included 0.10% each titanium oxide, trace mineral and vitamin premix. Mineral and vitamin premixes provided the following per kg of mix: Vitamin – vitamin A, 8,818,400 IU; vitamin D_3 2,645,520 ICU; vitamin E, 22,046 IU; vitamin B_{12}, 26 mg; riboflavin, 8,818 mg; niacin, 88,184 mg; d-pantothenic acid, 22,046 mg; vitamin K, 2,646 mg; folic acid, 2,205 mg; vitamin B_6, 4,339 mg; thiamine, 3,732 mg; d-biotin, 220 mg; Mineral – iron (ferrous sulfate), 40 mg; manganese (manganese sulfate and manganous oxide), 120 mg; zinc (zinc oxide), 210 mg; cobalt (cobalt carbonate), 2.2 mg; iodine (calcium iodate), 132mg.

Table 3.2 Effect of a live coccidial vaccination and dietary phytic acid concentration on average body weight of broilers (g)

Treatment		Day 0	Day 18	Day 30
Low PA		38.4	554.9[c]	1559.7[b]
Med PA		37.9	644.9[b]	1620.1[a]
High PA		38.2	682.6[a]	1642.0[a]
Pooled SEM		0.21	5.5	11.87
Non-Vaccinated		38.6[a]	639.6[a]	1632.3[a]
Vaccination		37.7[b]	615.3[b]	1582.2[b]
Pooled SEM		0.17	4.71	7.23
Low PA	-[1]	38.8	554.0[d]	1566.2[c]
	+[2]	38.1	555.8[d]	1553.3[c]
Med PA	-	38.5	664.5[b]	1662.1[ab]
	+	37.3	625.3[c]	1578.0[c]
High PA	-	38.6	700.4[a]	1668.8[a]
	+	37.7	664.9[b]	1615.3[bc]
Pooled SEM		0.30	6.6	14.81
P-Value				
PA		0.2001	0.0008	0.0043
Vaccination		0.0004	<0.0001	<0.0001
PA*Vaccination		0.7691	0.0170	0.0173

[a-d] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

Table 3.3 Effect of a live coccidial vaccination and dietary phytic acid concentration on average body weight gain of broilers (g)

Treatment		d 0-18	d 18-30	d 0-30
Low PA		519[c]	879[c]	1,398[c]
Med PA		604[b]	1,004[b]	1,608[b]
High PA		644[a]	1,054[a]	1,701[a]
Pooled SEM		5.7	9.0	12.9
Non-Vaccinated		605[a]	1,017[a]	1,623[a]
Vaccination		573[b]	941[b]	1,515[b]
Pooled SEM		4.7	7.3	10.6
Low PA	-[1]	521[d]	887[d]	1,409[d]
	+[2]	517[d]	871[d]	1,388[d]
Med PA	-	629[b]	1,065[a]	1,694[ab]
	+	580[c]	943[c]	1,522[c]
High PA	-	666[a]	1,100[a]	1,766[a]
	+	623[b]	1,009[b]	1,636[b]
Pooled SEM		8.1	12.7	18.3
P-Value				
PA		<0.0001	<0.0001	<0.0001
Vaccination		<0.0001	<0.0001	<0.0001
PA*Vaccination		0.0145	0.0003	0.0004

[a-d] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

Table 3.4 Effect of a live coccidial vaccination and dietary phytic acid concentration on feed intake of broilers (g)

Treatment		d 0-18	d 18-30	d 0-30
Low PA		621[c]	1332[c]	1953[c]
Med PA		700[b]	1455[b]	2155[b]
High PA		755[a]	1564[a]	2319[a]
Pooled SEM		8.5	14.7	20.2
Non-Vaccinated		713[a]	1488[a]	2201[a]
Vaccination		671[b]	1413[b]	2083[b]
Pooled SEM		7.0	12.0	16.5
Low PA	-[1]	621[c]	1323[c]	1943[c]
	+[2]	621[c]	1342[c]	1962[c]
Med PA	-	737[ab]	1528[ab]	2266[b]
	+	663[c]	1381[c]	2044[c]
High PA	-	782[a]	1613[a]	2395[a]
	+	729[b]	1515[b]	2244[b]
Pooled SEM		12.1	20.8	28.6
P-Value				
PA		<0.0001	<0.0001	<0.0001
Vaccination		<0.0001	<0.0001	<0.0001
PA*Vaccination		0.0089	0.0005	0.0003

[a-c] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

Table 3.5 Effect of a live coccidial vaccination and dietary phytic acid concentration on feed conversion of broilers (g/g)

Treatment		d 0-18	d 18-30	d 0-30
Low PA		1.196[a]	1.518[a]	1.398[a]
Med PA		1.158[b]	1.450[b]	1.340[c]
High PA		1.172[ab]	1.484[a]	1.364[b]
Pooled SEM		0.0091	0.0120	0.0081
Non-Vaccinated		1.179	1.465[b]	1.358
Vaccination		1.172	1.503[a]	1.376
Pooled SEM		0.0074	0.0098	0.0066
Low PA	-[1]	1.191	1.492	1.380
	+[2]	1.201	1.543	1.415
Med PA	-	1.172	1.436	1.338
	+	1.144	1.465	1.342
High PA	-	1.174	1.467	1.357
	+	1.169	1.501	1.372
Pooled SEM		0.0129	0.0170	0.0115
P-Value				
PA		0.0158	0.0008	0.0001
Vaccination		0.4797	0.0075	0.0595
PA*Vaccination		0.3321	0.7903	0.3971

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

Table 3.6 Effect of a live coccidial vaccination and dietary phytic acid concentration on broiler mortality[1] (%)

Treatment		d 0-18	d 18-30	d 0-30
Low PA		1.58[b]	1.64[b]	3.01[b]
Med PA		6.78[a]	7.08[a]	10.8[a]
High PA		2.86[b]	3.93[b]	5.48[ab]
Pooled SEM				
Non-Vaccinated		1.77[b]	1.01[b]	2.49[b]
Vaccination		5.70[a]	7.42[a]	10.38[a]
Pooled SEM				
Low PA	-[2]	1.73[b]	0.96[bc]	2.44[b]
	+[3]	1.43[b]	2.32[bc]	3.58[b]
Med PA	-	1.45[b]	0.36[c]	1.69[b]
	+	12.11[a]	13.79[a]	19.94[a]
High PA	-	2.14[b]	1.71[bc]	3.33[b]
	+	3.57[b]	6.16[b]	7.62[b]
Pooled SEM				
P-Value				
PA		0.0009	0.0043	0.0068
Vaccination		0.0004	<0.0001	<0.0001
PA*Vaccination		0.0009	0.0001	0.0004

[a-c] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

[1] The arcsine transformation (arcsine √%) was used for mortality percentages prior to analysis.

[2]Vaccinated birds, Coccivac-B, Schering-Plough

[3]Non-vacciated birds

Table 3.7 Effect of dietary phytate levels and live coccidiosis vaccination on intestinal histology of 7-day-old broilers[1]

Treatment		Duo VH[2]	Duo CD[3]	Jej VH	Jej CD	Ile VH	Ile CD
Low PA		1.3710[b]	0.2010	0.7264[b]	0.1855	0.5203	0.1474[a]
Med PA		1.4386[ab]	0.2083	0.7608[b]	0.1850	0.5305	0.1359[b]
High PA		1.5161[a]	0.2036	0.8155[a]	0.1819	0.5466	0.1390[ab]
Pooled SEM		0.02373	0.003231	0.02381	0.0039	0.0107	0.00295
Non-Vaccinated		1.4263	0.1954[b]	0.7537	0.1801	0.5241	0.1398
Vaccination		1.4576	0.2133[a]	0.7815	0.1882	0.5408	0.1418
Pooled SEM		0.01937	0.002638	0.01944	0.0032	0.0087	0.00241
Low PA	-[2]	1.3590	0.1873	0.7410	0.1770	0.5108	0.1401[ab]
	+[3]	1.3830	0.2148	0.7117	0.1940	0.5298	0.1548[a]
Med PA	-	1.4075	0.2039	0.7104	0.1830	0.5089	0.1383[ab]
	+	1.4697	0.2127	0.8113	0.1870	0.5520	0.1335[b]
High PA	-	1.5123	0.1949	0.8096	0.1802	0.5525	0.1410[ab]
	+	1.5200	0.2123	0.8215	0.1836	0.5406	0.1371[b]
Pooled SEM		0.03355	0.004570	0.03367	0.0056	0.0151	0.00418
P-Value							
PA		0.0003	0.2805	0.0338	0.7841	0.2292	0.0203
Vaccination		0.2573	0.0001	0.3153	0.0822	0.1819	0.5588
PA*Vaccination		0.7073	0.1339	0.1503	0.4010	0.2037	0.0353

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Each vaccination and phytic acid combination represents the means of 12 replications.
[2] VH = villus height (mm).
[3] CD = crypt depth (mm).
[4] Non-vaccinated birds.
[5] Vaccinated birds.

Table 3.8 Effect of dietary phytate levels and live coccidiosis vaccination on intestinal histology of 21-day-old broilers[1]

Treatment		Duo VH[2]	Duo CD[3]	Jej VH	Jej CD	Ile VH	Ile CD
Low PA		1.9642	0.2783^{b}	1.4936	0.2380	0.8414	0.1939
Med PA		2.0507	0.3386^{a}	1.3534	0.2418	0.8325	0.2067
High PA		2.0687	0.3029^{ab}	1.4213	0.2488	0.8762	0.2021
Pooled SEM		0.04398	0.01164	0.04213	0.00923	0.02475	0.00669
Non-Vacc		2.1240^{a}	0.2038^{b}	1.4239	0.1839^{b}	0.8907^{a}	0.1913^{b}
Vaccination		1.9318^{b}	0.4094^{a}	1.4216	0.3018^{a}	0.8094^{b}	0.2105^{a}
Pooled SEM		0.03591	0.00951	0.03441	0.00754	0.02021	0.00547
Low PA	-[4]	1.9189^{b}	0.1903^{c}	1.3753^{ab}	0.1728	0.9029	0.1860
	+[5]	2.0095^{ab}	0.3663^{b}	1.6119^{a}	0.3032	0.7799	0.2018
Med PA	-	2.2245^{a}	0.2111^{c}	1.4153^{ab}	0.1875	0.8742	0.1899
	+	1.8769^{b}	0.4661^{a}	1.2915^{b}	0.2960	0.7907	0.2235
High PA	-	2.2285^{a}	0.2099^{c}	1.4812^{ab}	0.1915	0.8950	0.1979
	+	1.9090^{b}	0.3959^{b}	1.3614^{b}	0.3060	0.8575	0.2063
Pooled SEM		0.06220	0.01647	0.05956	0.01305	0.03500	0.00947
P-Value							
Diet		0.2068	0.0021	0.0730	0.6988	0.4321	0.3845
Vaccination		0.0003	0.0001	0.9630	0.0001	0.0060	0.0155
Diet*Vaccination		0.0009	0.0389	0.0037	0.6872	0.4833	0.3998

[a-c] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Each vaccination and phytic acid combination represents the means of 12 replications.
[2] VH = villus height (mm).
[3] CD = crypt depth (mm).
[4] Non-vaccinated birds.
[5] Vaccinated birds.

Table 3.9a Effect of dietary phytate levels and Coccivac®-B on apparent ileal essential amino acid digestibility coefficients of 7-day-old broilers[1]

Treatment		HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS
Low PA		0.7987[b]	0.8094[b]	0.7743[b]	0.7992[b]	0.8105[b]	0.8121[b]	0.8144[b]	0.8550[b]
Medium PA		0.8797[a]	0.8476[a]	0.8186[a]	0.8426[a]	0.8498[a]	0.8520[a]	0.8571[a]	0.9067[a]
High PA		0.8718[a]	0.8378[ab]	0.8122[a]	0.8358[a]	0.8421[a]	0.8462[a]	0.8530[a]	0.9023[a]
Pooled SEM		0.00816	0.01050	0.00791	0.00744	0.00725	0.00722	0.00707	0.00545
Low PA	-[2]	0.8125	0.8125	0.7775	0.8085	0.8214	0.8243	0.8273	0.8663
	+[3]	0.7848	0.8063	0.7711	0.7898	0.7995	0.7999	0.8015	0.8437
Med PA	-	0.8788	0.8454	0.8154	0.8423	0.8504	0.8534	0.8588	0.9074
	+	0.8807	0.8498	0.8217	0.8429	0.8493	0.8507	0.8554	0.9059
High PA	-	0.8673	0.8162	0.7951	0.8215	0.8292	0.8342	0.8431	0.8969
	+	0.8762	0.8593	0.8293	0.8501	0.8550	0.8583	0.8629	0.9077
Pooled SEM		0.01154	0.01484	0.01118	0.01052	0.01025	0.01022	0.00999	0.00770
P-Value									
Diet		0.0001	0.0337	0.0003	0.0002	0.0006	0.0004	0.0001	0.0001
Vaccination		0.5536	0.2588	0.2167	0.6849	0.9131	0.9049	0.7067	0.4824
PA*Vaccination		0.2501	0.2239	0.1852	0.0856	0.0728	0.0657	0.0818	0.0970

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

[1] Each vaccination and phytic acid combination represents the means of 12 replications.

[2] Non-vaccinated birds.

[3] Vaccinated birds.

Table 3.9b Effect of dietary phytate levels and Coccivac®-B on apparent ileal essential amino acid digestibility coefficients of 7-day-old broilers[1]

Treatment		ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low PA		0.7291[b]	0.8510[b]	0.7782[b]	0.7982[b]	0.8134[b]	0.8042[b]	0.8228[b]	0.8136[b]
Medium PA		0.9244[a]	0.9168[a]	0.8220[a]	0.8339[a]	0.8600[a]	0.8412[a]	0.8615[a]	0.8673[a]
High PA		0.9110[a]	0.9105[a]	0.8090[a]	0.8228[ab]	0.8543[a]	0.8335[a]	0.8533[a]	0.8597[a]
Pooled SEM		0.01427	0.00675	0.00755	0.00753	0.00717	0.00733	0.00677	0.00693
Low PA	-[2]	0.7791[b]	0.8685	0.7873	0.8023	0.8233	0.8087	0.8317	0.8253
	+[3]	0.6792[c]	0.8334	0.7691	0.7941	0.8034	0.7998	0.8139	0.8020
Med PA	-	0.9209[a]	0.9176	0.8218	0.8318	0.8595	0.8394	0.8631	0.8670
	+	0.9278[a]	0.9159	0.8223	0.8359	0.8605	0.8430	0.8599	0.8675
High PA	-	0.9125[a]	0.9082	0.7975	0.8058	0.8420	0.8158	0.8406	0.8487
	+	0.9095[a]	0.9128	0.8204	0.8399	0.8665	0.8513	0.8660	0.8707
Pooled SEM		0.02082	0.00955	0.01068	0.01064	0.01014	0.01037	0.00958	0.00979
P-Value									
Diet		0.0001	0.0001	0.0004	0.0044	0.0001	0.0016	0.0003	0.0001
Vaccination		0.0641	0.1748	0.8416	0.2527	0.8228	0.2391	0.8484	0.9724
PA*Vaccination		0.0225	0.0898	0.1647	0.1313	0.0984	0.0949	0.0796	0.0774

[a-c] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Each vaccination and phytic acid combination represents the means of 12 replications.
[2] Non-vaccinated birds.
[3] Vaccinated birds.

Table 3.10a Effect of dietary phytate levels and Coccivac®-B on apparent ileal essential amino acid digestibility coefficients of 21-day-old broilers[1]

Treatment		HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS
Low PA		0.7350[b]	0.8590[ab]	0.8498	0.8541[ab]	0.8617[ab]	0.8770	0.8679[b]	0.9070[b]
Med PA		0.7865[a]	0.8512[b]	0.8493	0.8507[b]	0.8581[b]	0.8750	0.8714[ab]	0.9141[ab]
High PA		0.8088[a]	0.8704[a]	0.8654	0.8711[a]	0.8773[a]	0.8888	0.8858[a]	0.9211[a]
Pooled SEM		0.007353	0.004465	0.005288	0.005177	0.004948	0.004489	0.004766	0.003509
Non-Vacc		0.7948[a]	0.8615	0.8652[a]	0.8706[a]	0.8766[a]	0.8911[a]	0.8848[a]	0.9225[a]
Vaccinated		0.7587[b]	0.8588	0.8445[b]	0.8467[b]	0.8548[b]	0.8694[b]	0.8654[b]	0.9056[b]
Pooled SEM		0.006003	0.003646	0.004318	0.004227	0.004040	0.003665	0.003891	0.002865
Low PA	-[2]	0.7525	0.8682[ab]	0.8636	0.8646	0.8713	0.8860	0.8761	0.9140
	+[3]	0.7174	0.8498[b]	0.8361	0.8436	0.8522	0.8680	0.8598	0.8999
Med PA	-	0.8149	0.8535[ab]	0.8656	0.8721	0.8783	0.8942	0.8898	0.9285
	+	0.7580	0.8489[b]	0.8329	0.8293	0.8379	0.8558	0.8531	0.8997
High PA	-	0.8170	0.8629[ab]	0.8663	0.8750	0.8803	0.8931	0.8884	0.9251
	+	0.8006	0.8778[a]	0.8645	0.8672	0.8743	0.8845	0.8832	0.9171
Pooled SEM		0.01040	0.00631	0.00748	0.00732	0.00700	0.00635	0.00674	0.00496
P-Value									
Diet		0.0001	0.0128	0.0562	0.0150	0.0189	0.0708	0.0235	0.0219
Vaccination		0.0001	0.6030	0.0012	0.0002	0.0003	0.0001	0.0008	0.0001
Diet*Vaccination		0.1576	0.0354	0.0952	0.0605	0.0524	0.0639	0.0676	0.1052

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

[1] Each vaccination and phytic acid combination represents the means of 12 replications.

[2] Non-vaccinated birds.

[3] Vaccinated birds.

Table 3.10b Effect of dietary phytate levels and Coccivac®-B on apparent ileal non-essential amino acid digestibility coefficients of 21-day-old broilers[1]

Treatment		ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low PA		0.7918^{b}	0.8938^{b}	0.8454^{ab}	0.8524^{ab}	0.8716^{b}	0.8588	0.8717^{ab}	0.8600^{b}
Med PA		0.9001^{a}	0.9111^{a}	0.8301^{b}	0.8434^{b}	0.8750^{ab}	0.8578	0.8705^{b}	0.8696^{b}
High PA		0.9145^{a}	0.9243^{a}	0.8572^{a}	0.8662^{a}	0.8906^{a}	0.8731	0.8868^{a}	0.8857^{a}
Pooled SEM		0.01142	0.00515	0.00567	0.00480	0.00484	0.00469	0.00465	0.00471
Non-Vacc		0.8803	0.9184^{a}	0.8574^{a}	0.8661^{a}	0.8896^{a}	0.8754^{a}	0.8874^{a}	0.8821^{a}
Vaccinated		0.8572	0.9011^{b}	0.8311^{b}	0.8419^{b}	0.8685^{b}	0.8511^{b}	0.8653^{b}	0.8614^{b}
Pooled SEM		0.00933	0.00420	0.00463	0.00392	0.00395	0.00383	0.00380	0.00385
Low PA	-[2]	0.7971	0.8962	0.8511	0.8623	0.8820	0.8715^{ab}	0.8829	0.8693
	+[3]	0.7864	0.8915	0.8397	0.8426	0.8613	0.8462^{bc}	0.8605	0.8508
Med PA	-	0.9157	0.9268	0.8516	0.8641	0.8930	0.8782^{a}	0.8894	0.8869
	+	0.8845	0.8954	0.8086	0.8228	0.8570	0.8374^{c}	0.8515	0.8523
High PA	-	0.9283	0.9324	0.8694	0.8721	0.8939	0.8764^{a}	0.8898	0.8903
	+	0.9007	0.9162	0.8450	0.8602	0.8872	0.8697^{ab}	0.8838	0.8812
Pooled SEM		0.01615	0.00728	0.00801	0.00679	0.00685	0.00663	0.00658	0.00666
P-Value									
Diet		0.0001	0.0004	0.0049	0.0053	0.0166	0.0424	0.0271	0.0011
Vaccination		0.0844	0.0047	0.0002	0.0001	0.0003	0.0001	0.0001	0.0003
Diet*Vaccination		0.7960	0.1924	0.1472	0.0874	0.1088	0.0427	0.0607	0.1612

[a-c] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

[1] Each vaccination and phytic acid combination represents the means of 12 replications.

[2] Non-vaccinated birds.

[3] Vaccinated birds

Table 3.11 Effect of a live coccidial vaccination and dietary phytic acid concentration on P digestibility coefficients, content, and digestible intake of broilers on d 7

Treatment		Digestibility coefficient	Digestible P content	Digestible P Intake
Low PA		0.4543[b]	0.3134[b]	86.69[b]
Med PA		0.5889[a]	0.4888[a]	129.14[a]
High PA		0.5644[a]	0.5079[a]	135.55[a]
Pooled SEM		0.012	0.0098	3.717
Non-Vaccinated		0.5344	0.4345	114.34
Vaccination		0.5373	0.4389	119.91
Pooled SEM		0.0098	0.0080	3.035
Low PA	-[1]	0.4691	0.3236	84.05
	+[2]	0.4394	0.3032	89.33
Med PA	-	0.5818	0.4828	124.69
	+	0.5960	0.4947	133.59
High PA	-	0.5522	0.4970	134.28
	+	0.5766	0.5189	136.82
Pooled SEM		0.017	0.01386	5.256
P-Value				
PA		< 0.0001	< 0.0001	< 0.0001
Vaccination		0.8307	0.6967	0.1991
PA*Vaccination		0.2468	0.2878	0.8315

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

Table 3.12 Effect of a live coccidial vaccination and dietary phytic acid concentration on P digestibility coefficients, content, and digestible intake of broilers on d 21

Treatment		Digestibility coefficient	Digestible P content	Digestible P Intake
Low PA		0.4676[c]	0.3180[c]	343.77[c]
Med PA		0.5439[b]	0.4025[b]	414.62[b]
High PA		0.5965[a]	0.4891[a]	513.20[a]
Pooled SEM		0.009	0.0067	10.839
Non-Vaccinated		0.5571[a]	0.4181[a]	433.14
Vaccination		0.5149[b]	0.3883[b]	414.58
Pooled SEM		0.0075	0.0055	8.850
Low PA	-[1]	0.5015[b]	0.3410[d]	350.60
	+[2]	0.4337[c]	0.2949[e]	336.93
Med PA	-	0.5733[a]	0.4242[b]	427.35
	+	0.5146[b]	0.3808[c]	401.88
High PA	-	0.5965[a]	0.4891[a]	521.49
	+	0.5965[a]	0.4891[a]	504.91
Pooled SEM		0.013	0.0095	15.329
P-Value				
PA		< 0.0001	< 0.0001	< 0.0001
Vaccination		0.0002	0.0003	0.1427
PA*Vaccination		0.0231	0.0295	0.9228

[a-e] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1]Vaccinated birds, Coccivac-B, Schering-Plough
[2]Non-vacciated birds

CHAPTER IV

The effect of dietary phytic acid concentration and addition of exogenous phytase on vaccinated and non-vaccinated broiler performance, tibia ash, and small intestinal health

R. N. Lehman[1], A. J. Cowieson[2], C. L. Walk[3], and A. P. McElroy[1]

[1] Virginia Tech, Department of Animal and Poultry Sciences, Blacksburg, VA
[2] Poultry Research Foundation, The University of Sydney, Camden, Australia
[3] AB Vista, Wiltshire, Marlborough, UK

ABSTRACT Broiler performance, tibia ash, apparent ileal amino acid digestibility (**IAAD**), and small intestinal pH and morphology were evaluated as a response to dietary phytic acid levels, phytase inclusion, and a live coccidial oocyst vaccine. Vaccinated and non-vaccinated birds were raised in floor pens for 28 d and given one of four diets: low phytic acid (**PA**) without phytase; low PA with phytase; high PA without phytase; and high PA with phytase, resulting in a 2 X 2 X 2 factorial arrangement of treatments. Low and high PA diets were formulated to contain 0.21% and 0.29% phytate-P, respectively while maintaining similar available P levels. Phytase was supplemented on top of the diet at 1000 FTU/kg. Vaccinated birds were spray vaccinated on day-of-hatch with a live coccidial oocyst vaccine while non-vaccinated birds received no coccidiosis prevention treatment. The high PA diet and vaccination each caused a reduction in feed intake ($P \leq 0.01$), body weight ($P \leq 0.01$), and body weight gain ($P \leq 0.001$); however, effects from PA were no longer observed by d 28 while vaccination effects were seen throughout the duration of the trial. Mortality was also increased ($P \leq 0.01$) by vaccination. The only effect phytase had on live performance was a three-way interaction with phytic acid and vaccination where phytase addition tended to decrease mortality of young birds, with the exception of non-vaccinated birds given the high PA diet ($P = 0.0145$). Tibia ash was not affected ($P > 0.05$) by any treatment. Small intestinal pH was more significantly altered in

younger birds where vaccination reduced ($P \leq 0.05$) the pH of most intestinal sections. In the proximal gizzard, phytic acid addition decreased ($P \leq 0.01$) the pH on d 7 and 28 while phytase increased ($P = 0.0302$) the pH on d 7. In general, d 7 IAAD was reduced ($P \leq 0.05$) by both vaccination and a high PA diet. On d 28, IAAD was increased ($P \leq 0.05$) by addition of phytase and by the high PA diet. Vaccination alone generally increased ($P \leq 0.05$) the crypt depth (**CD**) and decreased ($P \leq 0.01$) the villus height to crypt depth ratio (**VCR**). Phytase had the opposite effect by decreasing the CD ($P \leq 0.05$) and increasing ($P \leq 0.01$) the VCR. The addition of phytic acid only decreased ($P \leq 0.05$) the villi height and CD in the duodenum and decreased ($P = 0.0011$) the CD and increased ($P = 0.0101$) the VCR in the ileum of birds on d 7. From these results, it can be inferred that addition of phytase to the diet can improve the intestinal morphology and IAAD, especially when other nutrient-limiting factors like phytic acid and a live coccidiosis vaccination are present. However, due to the diets being nutritionally adequate, any further improvements in performance or intestinal health seen with addition of phytase were not observed in the present study.

Keywords: phytic acid, coccidiosis vaccination, phytase, broiler, intestinal health

INTRODUCTION

The deleterious effects of phytate on bird performance have been extensively researched. Dietary phytate has been implicated in binding important nutrient cations (Maenz et al., 1999), reducing apparent ileal digestibility of protein (Cowieson et al., 2006b; Ravindran et al., 2006), stimulating overproduction of endogenous secretions (Cowieson and Ravindran, 2007; Cowieson et al., 2008, 2009; Onyango et al., 2009; Woyengo et al., 2009), reducing activity of digestive enzymes (Liu et al., 2008), and interfering with intestinal transport and absorption mechanisms

(Mansoori, 2010), all of which have negative impacts on bird performance. Fortunately, phytase has enabled producers to avoid many of these consequences associated with phytate-containing diets.

The inclusion of phytase in poultry diets is becoming increasingly common as economic feasibility and product reliability is enhanced. Since the first commercial introduction of the feed enzyme in 1991 (Selle and Ravindran, 2007), much more is understood concerning the beneficial effects of phytase, even beyond that of improved phosphorus availability. Exogenous phytase can also benefit protein and amino acid digestibility (Ravindran et al., 1999; Cowieson et al., 2006a) and improve overall bird performance, possibly through a reduction in nutrient loss and an increase in nutrient availability (Cowieson et al., 2006b).

Another extra-phosphoric benefit of phytase is the improvement in feed intake (Pirgozliev et al., 2011). It is possible that the use of phytase could increase digestible nutrient intake and aid in ameliorating consequences of limited nutrient absorption in a variety of situations, such as when birds are given a live coccidiosis vaccine. While live performance of birds given an anticoccidial vaccine have been inconsistent (Bedrnik et al., 1989; Mathis, 1999; Williams et al., 1999; Newman, 1999; Waldenstedt et al., 1999; Parker et al., 2007; Lehman et al., 2009), it is accepted that a live vaccine produces immunity through induction of a mild form of the disease. In its clinical form, coccidiosis is a disease that is known to damage the intestinal surface through reproduction of protozoan parasites and result in reduced absorptive surface area of the villi (Idris et al., 1997). A reduction in the viable intestinal surface area ultimately results in the reduced capacity to digest and absorb nutrients, which is implicated in the poorer performance frequently seen with vaccinated birds.

Several studies have looked at the combined effects of phytase and coccidiosis vaccination or infection. Walk et al. (2011) found that phytase did not alleviate reduced growth performance in coccidial vaccinated birds. Similarly, others observed that phytase supplementation was unable to remedy P utilization or performance deficiencies seen with coccidiosis vaccination and infection (Watson et al., 2005; Shaw et al., 2011). While no obvious improvements in performance were previously observed, it is known that phytase efficacy is dependent on concentration of its substrate (phytate), and a diet with intrinsically high digestibility may not have a strong response to enzyme addition (Cowieson and Bedford, 2009). Therefore, in the presence of a high-phytate diet, it is possible that phytase would induce noticeable improvements in performance when birds are subjected to a mild disruption of nutrient absorption from administration of a live coccidiosis vaccine.

Previous research in our laboratory has shown that when combined with a live coccidial vaccine, a diet higher in phytate (0.87%) induce severe necrotic enteritis (**NE**), believed to be associated with altered nutrient digestibility. Therefore, the objective of this experiment was to determine if the use of phytase could alleviate potential intestinal damage, which may predispose the birds to overgrowth of *C. perfringens*, and related performance deficiencies as a result of the combination of a coccidiosis vaccine and elevated dietary phytate levels.

MATERIALS AND METHODS

Bird Husbandry and Diets

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. A total of 2,232 day-old Cobb 500 male broilers were obtained from a commercial hatchery and transported to the poultry research farm at Virginia Tech. Upon

arrival, half of the chicks were spray-vaccinated with a vaccine[1] that contained live oocysts of *Eimeria acervulina*, *Eimeria mivati*, *Eimeria maxima*, and *Eimeria tenella* following manufacturer recommendations. Birds that were not vaccinated did not receive any anti-coccidial medication. Chicks were randomly selected (with respect to non-vaccinated and vaccinated groups), weighed, and placed 31 chicks per pen in one of 72 floor pens with clean pine shavings at a final stocking density of 14.15 kg/m^2. Birds were provided ad libitum access to feed and water and reared with a commercial lighting program in a negative pressure ventilated house with temperature control.

All diets were formulated on a corn-soybean meal basis to meet Cobb broiler nutrient recommendations (Tables 4.1 and 4.2). Diets were fed as a crumble (starter) or as whole pellets (grower) with 0.1% titanium oxide as an indigestible marker. Percent available phosphorus was held constant while allowing the amount of total phosphorus to fluctuate, depending on the amount of phytic acid (**PA**)[2] that was added to the diet. Vaccinated and non-vaccinated birds were given one of four experimental diets containing supplemental phytic acid in liquid form at either 0.1% (low PA) or 0.7% (high PA) of the diet with or without the addition of exogenous phytase over the top, for a total of 8 treatments with 9 replicate pens/treatment. Water was added to the low PA diet to ensure diets had an added volume of liquid equivalent to that of the high phytic acid diet. The phytase used was an evolved *E. coli* 6-phytase (AB Vista[3]) expressed in *Trichoderma reesei* and formulated to provide 1,000 FTU/ kg diet. Corn was added in place of phytase in the diets not containing phytase. Phytase activity in the diets was analyzed by Enzyme Services and Consultancy[4].

[1] Coccivac®- B, Intervet, Schering-Plough Animal Health, Omaha, NE
[2] Sigma-Aldrich, St. Louis, MO
[3] AB Vista Feed Ingredients, Marlborough, UK
[4] Pengam, Blackwood NP12 3SP, Wales UK

Sample Collection

Body weight (**BW**) and feed intake (**FI**) of birds were measured by pen on d 0, d 7, and d 28 and used to determine body weight gain (**BWG**) and feed conversion (**FC**) for each feeding period (starter: d 0 to 7; grower: d 7 to 28) and cumulatively (d 0 to 28). Birds removed for mortality or sampling were recorded daily to adjust the FI and FC respective of the number of bird days. Birds sampled were euthanized by cervical dislocation before collection of digesta and tissue samples.

On d 7 and 28, intestinal tissue samples were obtained from one bird/pen (n=9 birds/treatment) for histological evaluation of morphology, including villus height (**VH**), crypt depth (**CD**), and villus height to crypt depth ratio (**VCR**). Tissue samples, approximately 1 inch in length, were taken from the midpoint of the duodenum, jejunum, and ileum and gently flushed with phosphate buffered saline to remove luminal contents before placement in 10% neutral-buffered formalin until further processing. Each segment was cut into five 5 mm sections, embedded in paraffin wax, cut to 5 μm, and mounted onto slides. Slides were stained with hematoxylin and eosin and examined with light microscopy[5]. Measurements of VH and CD were obtained using Sigma Scan Pro 5[6] and digitized using NIS Elements imaging software[7]. Both VH and CD (n=9) were measured from 3 tissues of the 5 tissue sections/slide, following procedures according to Sun et al. (2005). Briefly, VH was measured from the tip of the villus to the base where it intersects with the opening of the crypt; CD was measured from the crypt opening to the base of the crypt; and VCR was calculated and normalized using the natural log.

Digesta from eight birds/pen (n=72 birds/treatment) on d 7 and five birds/pen (n=45 birds/treatment) on d 28 was collected from the ileum. Digesta was taken from Meckel's

[5] Olympus Polaroid DMC-IE camera, Polaroid Corporation, MA
[6] SPSS, Chicago, IL
[7] Nikon Instruments, Linthicum, MD

diverticulum to an inch proximal to the ileal-cecal junction and pooled by pen before being snap-frozen on dry ice and stored at -80˚C until further analysis. Digesta samples were later lyophilized, and diet and digesta samples were ground to pass through a 1mm screen. Titanium oxide concentration in the diet and digesta samples was determined according to methods described by Short et al. (1996). Amino acid concentrations of the diet and digesta samples were determined by the Experiment Station Chemical Laboratories[8]. Amino acid values were then used to calculate apparent ileal amino acid digestibility (IAAD) using the following equation: Digestibility Coefficient = $[(\text{amino acid/TiO})_d - (\text{amino acid/TiO})_i) / (\text{amino acid/TiO})_d * 100$, where $_d$ = amino acid and titanium oxide concentration of the diet, and $_i$ = amino acid and TiO concentration of the ileal samples.

Left tibias were obtained from three birds/ pen (n=27 birds/treatment) on d 7 and 28 and pooled for determination of bone ash. Tibias were stripped of tissue, wrapped in cheese cloth, and dried overnight at 100°C. Fat was extracted from tibias using a Soxhlet apparatus and 100% ethyl ether according to modified methods adapted from Watson et al. (2006). Fat-extracted tibias were then dried for 24 hours at 100°C and ashed in a muffle furnace for 24 hours at 600°C to determine bone ash.

Immediately after cervical dislocation, small intestinal pH was measured in two birds/pen using an Accumet Basic pH meter[9] with a spearhead tip. Measurements were taken a quarter inch into the proximal and distal ends of the gizzard and the ileum, after the small intestine was cut at Meckel's diverticulum, and at the ileo-cecal junction.

[8] ESCL, University of Missouri, Colombia, MO.
[9] Denver Instrument Company, 2542 Fig Street Arvada, CO

Statistical Analysis

Performance data were subjected to ANOVA as a 2 X 2 X 2 factorial design consisting of vaccination, phytic acid, and phytase and analyzed using the MIXED procedure of SAS. All main effects, two-way interactions and three-way interactions were analyzed. Pen served as the experimental unit for BW, BWG, FI, FC, percent mortality, apparent IAAD, and bone ash. Percent mortality data were arc sine transformed prior to analysis. Bird served as the experimental unit for morphological measurements and intestinal pH. Mean differences were adjusted using the Tukey method, and statistical significance was accepted at $P \leq 0.05$.

RESULTS

Growth Performance

Diet analysis showed similar levels of nutrients to those which were formulated; however, crude protein was 0.45 to 2.4% higher than what was formulated (Table 1). Though enzyme recovery was slightly lower than 1000 FTU, levels across all diets were similar and within an acceptable range (Table 4.2). Diets without added phytase had low levels of naturally occurring phytase, which was expected. Interactions between dietary treatments and vaccination were not significant for FI, BW, BWG, or FC. In addition, phytase did not have a significant main effect on these parameters, so reported values represent main effects of PA or vaccination (Tables 4.3 to 4.6). During the d 0 to 7 feeding period, high PA reduced FI ($P \leq 0.01$), while vaccination caused an increase ($P \leq 0.0001$) in FI (Table 4.3). From d 7 to 28 and cumulatively (d 0 to 28), vaccination decreased FI ($P \leq 0.01$). On d 7 bird fed the low PA diet were heavier than birds fed the high PA diet ($P = 0.0002$; Table 4.4). On d 0 and d 7, vaccinated birds weighed more than non-vaccinated birds ($P < 0.01$), but by d 28, non-vaccinated birds had a greater BW

compared to vaccinated birds (P = 0.0004). During the d 0-7 period, birds given the low PA diet gained more weight (P ≤ 0.001), but by the end of the experiment, the BWG of birds given the low or the high PA diet was statistically the same (Table 4.5). Vaccinated birds gained more weight than non-vaccinated birds during d 0 to 7, but from d 7 to 28 and cumulatively (d 0 to 28), the non-vaccinated birds had a higher weight gain (P ≤ 0.001). The only significant effect on FC was caused by vaccination, where an improved FC was seen in the vaccinated birds from d 0 to 7 (P ≤ 0.05; Table 4.6). There was a significant interaction between PA, vaccination, and phytase (P ≤ 0.05; Figure 4.1) from d 0 to 7 that indicated an increase in mortality was seen in the non-vaccinated birds given the high PA diets when phytase was added to the diet, while addition of phytase decreased mortality of all other treatments, although at different rates. Around day 14, birds developed NE. Therefore, from d 7 to 28 and cumulatively, vaccinated birds had a significantly higher mortality compared to non-vaccinated birds (P ≤ 0.01; Table 4.7).

Tibia Ash & Weight

Tibia ash and weight was determined as an indicator of bone mineralization (Table 4.8). There were no significant interactions between treatments and no main effect of treatments on tibia ash. Low PA diets resulted in heavier tibias (P ≤ 0.05) on d 7 compared to the high PA diet. By d 28, there was no difference in weight of tibias of birds given the low or high PA diets; however, non-vaccinated birds had heavier tibias (P ≤ 0.01) than vaccinated birds.

Small Intestinal pH

The pH was measured on d 7 and d 28 in four areas of the small intestine: proximal and distal gizzard and proximal and distal ileum. On d 7, no significant interactions were found, however low PA caused a higher pH in the proximal gizzard (P ≤ 0.01) and distal ileum (P ≤

0.05) compared to birds given a high PA diet (Table 4.9). Vaccination caused a lower pH in all sections measured except for the proximal gizzard ($P \leq 0.05$). Addition of phytase to the diet increased the pH in the proximal gizzard compared to diets with no added phytase ($P \leq 0.05$).

On d 28 (Table 4.10), the low PA diets continued to result in higher pH only in the proximal gizzard ($P \leq 0.01$) compared to that in birds fed the high PA diets. Vaccination caused a lower pH in the proximal gizzard ($P \leq 0.05$), whereas no other areas of the small intestine exhibited a pH difference. There was a significant PA*phytase interaction ($P \leq 0.05$) as non-vaccinated birds given the low PA diet had the highest pH in the proximal ileum and vaccinated birds given the low PA diet had the lowest pH in this section, with birds given the high PA diet being intermediate between these two treatments.

Apparent Ileal Amino Acid Digestibility

Ileal amino acid digestibility was measured on days 7 and 28. On day 7 (Tables 4.11 and 4.12), birds given the low PA diet had a higher ($P \leq 0.05$) apparent IAAD than birds given the high PA diet for all AA measured, with the exception of methionine. Vaccination decreased ($P \leq 0.05$) AA digestibility for all AA ($P \leq 0.05$). Phytase had no effect on ileal AA digestibility on d 7. On d 28 (Tables 4.13 and 4.14), PA had the opposite effect than observed on d 7 as high PA diets caused an increase in ileal AA digestibility for all AA ($P \leq 0.05$). Vaccination increased the apparent ileal digestibility of serine ($P \leq 0.05$), but had no effect on other AA. At d 28, dietary phytase significantly improved ($P \leq 0.05$) apparent digestibility of all AA except for threonine, methionine, and alanine.

Small Intestinal Morphology

The VH and CD were measured in each segment of the small intestine. On d 7 (Table 4.15), the only interaction occurred between phytase and vaccination on duodenal VH.

Vaccinated birds without phytase had longer villi compared to non-vaccinated birds without phytase and non-vaccinated birds with phytase ($P \leq 0.01$). Vaccinated birds that received phytase had duodenal VH intermediate to the other treatments. The high concentration of PA decreased ($P \leq 0.05$) VH compared to the low PA diet. High dietary PA also decreased CD in the duodenum and ileum ($P \leq 0.05$). In the ileum, though VH was not affected by PA, the VCR was significantly increased with the high PA diet ($P \leq 0.05$). Vaccination decreased VH in the jejunum ($P \leq 0.05$) and increased CD ($P \leq 0.001$) and subsequently reduced VCR ($P \leq 0.01$) in all intestinal sections. Phytase had no effect on VH, but reduced CD ($P \leq 0.05$) in all intestinal sections and increased ($P \leq 0.01$) the VCR in the duodenum and ileum.

On d 28, there was a 3-way interaction (Table 4.17) between PA, phytase, and vaccination on the jejunal and ileal VCR and ileal CD ($P \leq 0.05$). In the jejunum, VCR of the vaccinated birds was similar when given either the low or high PA diets. The response of the non-vaccinated birds was quite different where high PA in the diet caused a decrease in the VCR in the birds without phytase; however, when phytase was in the diet, high PA caused an increase in VCR. In the ileum, the high PA diet decreased VCR in the non-vaccinated birds without phytase but caused an increase in all other treatments. Also in the ileum, crypts were deeper in the vaccinated birds given the low PA diet without phytase compared to the non-vaccinated birds given the high PA diet with phytase. Vaccination increased the depth of the crypts in the duodenum and in the jejunum ($P \leq 0.01$), but did not affect the CD in the ileum. Addition of phytase to the diet decreased duodenal CD ($P \leq 0.01$) and decreased ileal VH ($P \leq 0.01$).

Though not significant, all treatment means for each measured parameter is included in the Appendix in tables A.3 to A.14.

DISCUSSION

Live coccidial oocyst vaccination reduces FI and BWG and increases FC (Newman, 1999; Parker et al., 2007; Lehman et al., 2009). In the current study, there was an expected reduction in FI and BWG in the vaccinated birds during the d 7-28 period and cumulatively. Conversely, FI, BWG, and FC were improved during the d 0 to 7 period, which is not typical of results seen with a live coccidial vaccine. On day 0, vaccinated birds randomly had a higher body weight than non-vaccinated birds, and this initial advantage in body weight over the non-vaccinated birds may have been enough of a benefit to enable them to perform better than the non-vaccinated birds, despite the vaccination. Addition of PA lead to a reduction in FI and BWG during the d 0 to7 period, consistent with previous research (Liu et al., 2008; Onyango and Adeola, 2009; Liu et al., 2010).

The addition of phytase did not affect performance. While diets were formulated to contain 1000 FTU, diet analysis revealed enzyme activity between 720 and 860 FTU. Super levels of phytase (>1,000 FTU/kg) in poultry and swine diets have a considerable impact on performance beyond that of conventional levels and may offer an explanation as to why a value below 1000 FTU may not have resulted in improved performance (Shirley and Edwards, 2003; Cowieson et al., 2011). However, lower levels of phytase (400 and 800 FTU/kg) have also been shown to improve performance of birds (Cabahug et al., 1999). It should be noted though, that the level of non-phytate P was reduced in the previously mentioned study, whereas, in the current study phytase was added to the top of diets that were adequate in P and all other nutrients.

The ability of exogenous phytase to be effective is a product of dietary nutrient levels, phytate concentration, and inclusion level of phytase. Any hindrance to optimal performance due

to phytate may be partly mitigated by improved nutrition, and as a consequence, further improvements from phytase may be minimal (Selle and Ravindran, 2007). If a feed is already adequate in required nutrients, the bird will experience little to no advantage in performance with the addition of enzymes, as the bird is already performing to its optimal potential. This is likely what occurred in this experiment as diets were adequate in nutrients and addition of phytase did not substantially improve nutrient availability or alleviate phytate-induced nutrient losses. Similarly, Selle et al. (1999) demonstrated that when phytase is supplemented to P-adequate broiler diets, growth performance is not altered. However, when it was added to diets with a reduced P, Ca, protein/AA, and energy density, a significant weight gain and feed efficiency improvement was seen. Others have also supported this fact as birds given diets adequate in total P did not have a pronounced response to addition of dietary phytase (Cabahug et al., 1999; Ravindran et al., 2000). Additionally, an enzyme is only effective if there is substantial substrate on which it is able to work. Therefore, the level of phytate may have a substantial impact on the perceived improvement with phytase, especially when nutrient specifications are not reduced. The low and high PA diets contained a calculated 0.21% and 0.29% phytate-P, respectively. Perhaps a more distinguishable improvement would have been seen with addition of phytase to these diets if the level of phytate-P was beyond the threshold level of 0.3% (Selle and Ravindran, 2007).

The high mortality was determined to be a result of NE. It began on d 14 in the vaccinated birds and lasted for about five days. Mortality then declined and was maintained somewhat steady for the duration of the trial. Coccidiosis infection is believed to be a major predisposing factor to the development of NE (Williams, 2005), so it is fitting that the birds vaccinated against coccidiosis experienced a severe infection compared to the non-vaccinated

birds. Although coccidiosis is believed to play a role in development of NE, many other factors such as diet, environment, and intestinal conditions are all believed to contribute to a possible outbreak (Wu et al., 2010). Factors that disrupt the intestinal integrity, alter mucin dynamics, or cause leakage of plasma protein (Van Immerseel et al., 2004) play an important role in providing an optimal nutrient source for the growth of *C. perfringens* since the bacteria cannot produce all essential nutrients (Cooper and Songer, 2009). The vaccination, combined with the fact that no antibiotics were used in any of the diets, created an environment that was conducive to the development of NE, as antibiotics offer some protection from *Clostridium* (Elwinger et al., 1998). Additionally, the presence of antibiotics may help to stabilize the shift in intestinal microbial populations that may occur during vaccination (Oviedo-Rondón et al., 2006) and thus prevent dysbacteriosis and development of intestinal diseases. As previously observed (Powell et al., 2011), the addition of phytase in this experiment was able to lower mortality during the first week of the trial.

Tibia ash was measured as an indicator of bone mineralization. On d 7, birds given low PA diets had heavier tibias compared to those of birds on the high PA diet. During the first 7 days, birds given the low PA diet ate more and had a higher BWG, which could explain why those birds had a greater tibia weight. Percent tibia ash was not affected on d 7 or d 28. Perhaps because the diets were sufficient in all nutrients, any amount of minerals bound by the PA was not enough to negatively affect bone mineralization. By d 28, only vaccination had an effect on tibia weight, which was expected due to the fact that non-vaccinated birds ate more and had a higher BWG than the vaccinated birds at this time.

Intestinal pH can be altered by a variety of factors including manipulation of dietary ingredients (March et al., 1958), feed form (Gabriel et al., 2003), or disease (Kouwenhoven and

van der Horst, 1972; Ruff and Reid, 1975). Increase in acidity is known to improve the solubility of salts like calcium phosphate (Bergeim, 1926). This has lead to the discovery that increases in acidity due to lactose have improved the intestinal absorption of calcium (Bergeim, 1926). However, others disagree with these findings and suggest that large changes to intestinal pH are unlikely, regardless of pH of the diet (Mussehl et al., 1933). The decrease in pH seen in the proximal gizzard from the high PA diet was expected due to the high acidity of the PA itself. As the bolus moved through the gizzard and the rest of the intestinal tract, PA had less of an effect on pH likely due to the fact that it was broken down. There was a slight effect of PA on the pH of the distal ileum, which may have been caused by secondary factors rather than through direct action of the phytic acid itself.

The decrease in pH seen with vaccination was expected due to the fact that *Eimeria* infection decreases intestinal pH, typically related to the area of intestine that is affected by the oocysts (Kouwenhoven and van der Horst, 1972; Ruff and Reid, 1975). A greater effect of vaccination was seen on d 7 compared to d 28 possibly due to the fact that development of immunity is believed to be achieved by d 28 as oocyst shedding dramatically decreases by this time and infection is not likely to still be present (Schering-Plough, 2007).

The apparent IAAD was measured as an indicator of the nutrients that are assumed to be digested, based on what nutrients remain in the lumen. Previously, coccidiosis has been found to reduce the absorption of AA (Preston-Mafham and Sykes, 1970), and in this study, vaccination resulted in a decrease in apparent digestibility of AA on d 7 but not d 28. This is likely due to the fact that cycling of oocysts and infection was no longer present at d 28. Part of the coccidia life cycle involves invasion of the host epithelial cells (Shirley, 1992), which is needed to cause infection and induce immunity. During a coccidiosis challenge or vaccination-induced infection,

villus atrophy can occur (Pout, 1967) that decreases the surface area of the intestine. The resulting damage to the villi may be responsible for a reduction in nutrient absorption and thus apparent IAAD (Preston-Mafham and Sykes, 1970; Ruff and Wilkins, 1980)

The addition of phytase resulted in no improvements in AA digestibility in the younger birds but improved AA digestibility in the older birds. There are conflicting reports on the ability of phytase to improve apparent IAAD in poultry where some have found an improvement with the use of phytase (Yi et al., 1996; Ravindran et al., 1999, 2006; Cowieson et al., 2006b; Liu et al., 2010), while others have seen no noticeable improvement in digestibility (Zhang et al., 1999; Onyango et al., 2005). In addition, the type of ingredients used in the diet is important. Phytase has been demonstrated to improve the ileal digestibility of AA in wheat-based diets to a much greater extent than when a corn-based diet is used (Ravindran et al., 1999; Rutherfurd et al., 2002), which is likely attributed to the protein storage differences between the two grains (Selle and Ravindran, 2007). One reason an improvement was not seen in this study initially is the fact that the birds were still relatively young during which time many changes to the structure and functionality of the intestine are still occurring. In the previous studies where improvements were seen, birds were no younger than 16 days. In the older birds, improvements in digestibility were seen in most AA; however, phytase had little effect on methionine in comparison to the other AA. Considering methionine is found in low levels in endogenous secretions, the fact that phytase was able to improve apparent digestibility of the other AA but not methionine suggests that one mechanism by which phytase worked was by limiting the secretion of endogenous proteins (Cowieson and Ravindran, 2007).

It is interesting to note that PA affected digestibility of AA on both d 7 and d 28; however, in the younger birds, high PA decreased digestibility whereas in the older birds, it

increased digestibility. The reduction in digestibility caused by PA is in agreement with previous studies (Cowieson et al., 2006a; Ravindran et al., 2006). The reduction in apparent digestibility of protein is due to a number of interacting factors. One explanation is that nutrients are bound in a complex and unable to be hydrolyzed and subsequently digested. This can occur by protein forming a complex with phytate either where phytate binds to the α-NH_2 groups and side chains of basic AA or where a protein:mineral:phytate complex is formed (Selle et al., 2000). It is also hypothesized that PA is able to alter the water matrix of the intestinal tract so that solubility of compounds and transport of nutrients are affected (Cowieson and Cowieson, 2011). Another mechanism by which PA is believed to reduce apparent digestibility of AA is through an increase in endogenous secretions. These secretions that are rich in AA are then unable to be completely reabsorbed (Cowieson and Ravindran, 2007; Cowieson et al., 2009; Woyengo et al., 2009) and decrease the apparent digestibility of AA due to the fact that there are a greater number of AA now in the lumen. The increase in endogenous secretions is economically relevant to producers because the heightened secretion of endogenous proteins increases the maintenance energy requirements of the bird and may lower efficiency. During the first 7 d, phytase had no effect on apparent IAAD, therefore it is possible that PA would negatively impact digestibility. However, when the birds were older, phytase improved the IAAD, and birds given high PA had improved digestibility. The exact cause for the improved digestibility seen in birds given high PA is unknown but perhaps by d 28, the birds given the high PA were able to adapt to the diet and increase their production of endogenous phytase to mitigate the consequences of more PA in the diet. The ability to adapt to chronic challenge has been attributed to the dynamic nature of the intestine (Yunus et al., 2011).

The morphology of the small intestine was measured as an indicator of intestinal health and potential for nutrient absorption. Longer villi are an indication of greater surface area and absorptive capacity (Caspary, 1992), whereas shorter villi tend to have poor nutrient absorption and reduce bird performance (Xu et al., 2003). The size and number of crypts influence the amount of cell proliferation and villi growth (Geyra et al., 2001). Deeper crypts commonly signify more rapid tissue turnover, possibly in response to epithelial cell destruction (Rose et al., 1992), inflammation and sloughing (Yason et al., 1987), or disease (Morris et al., 2004; Golder et al., 2011). In general, all treatments altered the intestinal morphology of birds more on d 7 compared to d 28. The small intestinal structure of birds is still developing a few days after hatch (Geyra et al., 2001), so nutritional influences are likely to have a greater initial impact on the developing structure of the intestine. However, as the intestinal tract becomes more mature, the initial influence diet had on morphology will no longer be seen. This is evident in the fact that PA did not affect morphology in the older birds but caused noticeable changes in the younger birds.

Vaccination, as expected, increased the depth of the crypts due to the mild infection caused by the live oocysts (Shirley, 1992; Williams, 2002). While the height of the villi was only slightly affected by vaccination, the VCR was consistently lower in the vaccinated birds due to the effect that vaccination had on crypt depth. A lower VCR is due to shorter villi and/or deeper crypts and tends to indicate sloughing of cells from the villi and an attempt by the crypts to replace lost cells. On the contrary, the addition of phytase resulted in improved intestinal morphology by decreasing the CD and increasing the VCR. Relatively fewer changes occurred in the ileum compared to the jejunum and the duodenum. This is likely a result of the vaccine containing oocysts of *E. acervulina*, *E. maxima*, *and E. tenella* that primarily invade the

duodenum, jejunum, and ceca, respectively, the slower migration rate of enterocytes in the ileum, or the fact that ileal enterocytes may be relatively mature at hatch (Geyra et al., 2001).

The use of high phytate diets negatively affected performance and apparent IAAD of broilers. A live coccidial vaccine decreased performance and IAAD and disrupted small intestinal morphology of young birds, while little difference was seen in older birds. Although few interactions were seen with phytase and vaccination or PA, the addition of dietary phytase did improve mortality, IAAD, and intestinal morphology. It is concluded that if feeding diets adequate in nutrients, few noticeable improvements in performance will be seen with the addition of phytase. However, the ability of exogenous enzymes like phytase to improve intestinal health was seen, despite ample nutrients being present in the diet. The importance of using matrix values assigned to exogenous enzymes such as phytase is imperative in order to realize the economic advantage as well obtain noticeable improvements in performance.

REFERENCES

Bedrnik, P., J. Kucera, A. Firmanova, and P. Jurkovic. 1989. Field vaccination of broilers against coccidiosis. Avian Pathol. 18:255-264.

Bergeim, O. 1926. Intestinal chemistry. V. Carbohydrates and calcium and phosphorus absorption. J. Biol. Chem. 70:29-33.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase as influenced by dietary phytic acid and non-phytate phosphorus levels. I. Effects on bird performance and toe ash content. Br. Poult. Sci. 40:660-666.

Caspary, W. F. 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299s-308s.

Cooper, K. K., and J. G. Songer. 2009. Necrotic enteritis in chickens: A paradigm of enteric infection by *Clostridium perfringens* type A. Anaerobe. 15:55-60.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006a. Phytic acid and phytase: Implication for protein utilization by poultry. Poult. Sci. 85:878-885.

Cowieson, A. J., T. Acamovic, and M. R. Bedford. 2006b. Supplementation of corn-soy based diets with an *Escherichia coli*-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and the metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Cowieson, A. J., and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow of amino acid composition of endogenous protein at the terminal ileum of grown broiler chickens. Br. J. Nutr. 98:745-752.

Cowieson, A. J., V. Ravindran, and P. H. Selle. 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult. Sci. 87:2287-2299.

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: Complimentary mode of action? Worlds Poult. Sci. J. 65:609-624.

Cowieson, A. J., M. R. Bedford, P. H. Selle, and V. Ravindran. 2009. Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. Worlds Poult. Sci. J. 65:401-418.

Cowieson, A. J., and N. P. Cowieson. 2011. Phytate and the thermodynamics of water. Proc. Aust. Poult. Sci. Symp. 22: 22-25.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds Poult. Sci. J. 67:225-236.

Elwinger, K., B. Engström, O. Fossum, and L. Waldenstedt. 1998. Effect of antibiotic growth promoters and anticoccidials on growth of *Clostridium perfringens* in the caeca and on performance of broiler chickens. Acta. Vet. Scand. 39:433-441.

Gabriel, I., S. Mallet, and M. Leconte. 2003. Differences in the digestive tract characteristics of broiler chickens fed on complete pelleted diet or on whole wheat added to pelleted protein concentrate. Br. Poult. Sci. 44:283-290.

Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776-782.

Golder, H. M., M. S. Geier, R. E. A. Forder, P. I. Hynd, and R. J. Hughes. 2011. Effects of necrotic enteritis challenge on intestinal micro-architecture and mucin profile. Br. Poult. Sci. 52:500-506.

Idris, A., D. Bounous, M. Goodwin, J. Brown, and E. Krushinskie. 1997. Quantitative pathology of small intestinal coccidiosis caused by *Eimeria maxima* in young broilers. Avian Pathol. 26:731-741.

Kouwenhoven, B., and C. J. G. van der Horst. 1972. Disturbed intestinal absorption of vitamin A and carotenes and the effect of a low pH during *Eimeria acervulina* infection in the domestic fowl (Gallus domesticus). Z. Parasitenk. 38:152-161.

Lehman, R., E. T. Moran, and J. B. Hess. 2009. Response of coccidiostat- versus vaccination-protected broilers to gelatin inclusion in high and low crude protein diets. Poult. Sci. 88:984-993.

Liu, N., Y. J. Ru, F. D. Li, and A. J. Cowieson. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. J. Anim. Sci. 86:3432-3439.

Liu, N., Y. Ru, J. Wang, and T. Xu. 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. Br. J. Nutr. 103:862-868.

Maenz, D. D., C. M. Engle-Schaan, R. W. Newkirk, and H. L. Classen. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed Sci. Technol. 81:177-192.

Mansoori, B. 2010. D-xylose absorption capacity of broiler intestine in response to phytic acid. Br. Poult. Sci. 51:158-161.

March, B., R. Tuckey, and J. Biely. 1958. The effect of diet on pH in the intestinal tract of chicks. Poult. Sci. 37:405-410.

Mathis, G. F. 1999. The influence of the coccidiosis vaccine, Coccivac-B on compensatory weight gain of broiler chickens in comparison with the anticoccidial, salinomycin. Poult. Sci. 78(Suppl. 1):117.

Morris, B. C., H. D. Danforth, D. J. Caldwell, F. W. Pierson, and A. P. McElroy. 2004. Intestinal mucosal mast cell immune response and pathogenesis of two *Eimeria acervulina* isolates in broiler chickens. Poult. Sci. 83:1667-1674.

Mussehl, F. E., M. J. Blish, and C. W. Ackerson. 1933. Effect of dietary and environmental factors on the pH of the intestinal tract. Poult. Sci. 12:120-123.

Newman, L. J. 1999. Coccidiosis control with vaccine: Been there, done that. Or have we? Proc. Arkansas Poult. Symp. Springdale, Arkansas. pp 52-55.

Onyango, E. M., and O. Adeola. 2009. Dietary phytate (inositol hexaphosphate) regulates the activity of intestinal mucosa phytase. J. Anim. Physiol. Anim. Nutr. 93:639-646.

Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous amino acid loss from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836-842.

Onyango, E. M., M. R. Bedford, and O. Adeola. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. Poult. Sci. 84:248-255.

Oviedo-Rondón, E. O., M. E. Hume, C. Hernández, and S. Clemente-Hernández. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poult. Sci. 85:854-860.

Parker, J., E. O. Oviedo-Rondón, B. A. Clack, S. Clemente-Hernández, J. Osborne, J.C. Remus, H. Kettunen, H. Mäkivuokko, and E. M. Pierson. 2007. Enzymes as feed additive to aid in responses against *Eimeria* species in coccidia-vaccinated broilers fed corn-soybean meal diets with different protein levels. Poult. Sci. 86:643-653.

Pirgozliev, V., M. R. Bedford, T. Acamovic, P. Mares, and M. Allymehr. 2011. The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. Br. Poult. Sci. 52:245-254.

Pout, D. D. 1967. Villous atrophy and coccidiosis. Nature, London. 213:306-307.

Powell, S., T. D. Bidner, and L. L. Southern. 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poult. Sci. 90:604-608.

Preston-Mafham, R. A., and A. H. Sykes. 1970. Changes in body weight and intestinal absorption during infections with *Eimeria acervulina* in the chicken. Parasitology. 61:417-424.

Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78:699-706.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193-200.

Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an *Escherichia coli*-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82-89.

Rose, M. E., B. J. Millard, and P. Hesketh. 1992. Intestinal changes associated with expression of immunity to challenge with *Eimeria vermiformis.* Infect. Immun. 60:5282-5290.

Ruff, M. D., and W. M. Reid. 1975. Coccidiosis and intestinal pH in chickens. Avian Dis. 19:52-58.

Ruff, M. D., and G. C. Wilkins. 1980. Total intestinal absorption of glucose and L-methionine in broilers infected with *Eimeria acervulina, E. mivati, E. maxima,* or *E. brunetti.* Parasitology. 80:555-569.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 44:598-606.

Schering-Plough Animal Health. 2007. Coccivac®-B Normal Reaction: Oocyst Shedding Pattern Technical Service Bulletin.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13:255-278.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Tech. 135:1-41.

Selle, P. H., V. Ravindran, P. H. Pittolo, and W. L. Bryden. 1999. An evaluation of microbial phytase in sorghum-based broiler diets. Proc. Aust. Poult. Sci. Symp. 11:97-100.

Shaw, A. L., F. W. van Ginkel, K. S. Macklin, and J. P. Blake. 2011. Effects of phytase supplementation in broiler diets on a natural *Eimeria* challenge in native and vaccinated birds. Poult. Sci. 90:781-790

Shirley, M. W. 1992. Research on avian coccidia: An update. Br. Vet. J. 148:479-499.

Shirley, R. B., and H. M. Edwards. 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82:671-680.

Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: An emerging threat for animal and public health. Avian Pathol. 33:537-549.

Waldenstedt, L., A. Lundén, K. Elwinger, P. Thebo, and A. Uggla. 1999. Comparison between a live, attenuated anticoccidial vaccine and an anticoccidial ionophore on performance of broilers raised with or without a growth promoter in an initially *Eimeria* free environment. Acta. Vet. Scand. 40:11-21.

Walk, C. L., A. J. Cowieson, J. C. Remus, C. L. Novak, and A. P. McElroy. 2011. Effects of dietary enzymes on performance and intestinal goblet cell number of broilers exposed to a live coccidian oocyst vaccine. Poult. Sci. 90:91-98.

Watson, B. C., J. O. Matthews, L. L. Southern, and J. L. Shelton. 2005. The interactive effects of *Eimeria acervulina* infection and phytase for broiler chicks. Poult. Sci. 84:910-913.

Watson, B. C., J. O. Matthews, L. L. Southern, and J. L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult. Sci. 85:493-497.

Williams, R. B., W. W. H. Carlyle, D. R. Bond, and I. A. G. Brown. 1999. The efficacy and economic benefits of Paracox®, alive attenuated anticoccidial vaccine, in commercial trials with standard broiler chickens in the United Kingdom. Int. J. Parasitol. 29:341-355.

Williams, R. B. 2002. Anticoccidial vaccines for broiler chickens: Pathways to success. Avian Pathol. 31:317-353.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159-180.

Woyengo, T. A., A. J. Cowieson, O. Adeola, and C. M. Nyachoti. 2009. Ileal digestibility and endogenous flow of minerals and amino acids: Responses to dietary phytic acid in piglets. Br. J. Nutr. 102:428-433.

Wu, S. B, N. Rodgers, and M. Choct. 2010. Optimized necrotic enteritis model producing clinical and subclinical infection of *Clostridium perfringens* in broiler chickens. Avian Dis. 54:1058-1065.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82:1030-1036.

Yason, C. V., B. A. Summers, and K. A. Schat. 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. Am. J. Vet. Res. 6:927-938.

Yi, Z., E. T. Kornegay, and D. M. Denbow. 1996. Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. Poult. Sci. 75:979-990.

Yunus, A. W., K. Ghareeb, A. A. M. Abd-El-Fattah, M. Twaruzek, and J. Böhm. 2011. Gross intestinal adaptations in relation to broiler performance during chronic aflatoxin exposure. Poult. Sci. 90:1683-1689.

Zhang, X., D. A. Roland, G. R. McDaniel, and S. K. Rao. 1999. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. Poult. Sci. 78:1567-1572.

Table 4.1 Composition and nutrient content of diets

Ingredients	Starter		Grower	
	Low PA	High PA	Low PA	High PA
Corn	67.365	66.188	70.899	69.722
Soybean Meal	26.723	26.915	22.81	23.003
Poultry Fat	1.403	1.823	2.206	2.625
Limestone	0.87	0.909	0.853	0.892
Di-calcium Phosphate	1.85	1.783	1.714	1.646
Salt	0.379	0.375	0.355	0.351
L-Lysine	0.631	0.628	0.447	0.443
DL-Methionine	0.361	0.362	0.299	0.30
Selenium Premix[1]	0.017	0.017	0.018	0.018
Vitamin Premix[2]	0.10	0.10	0.10	0.10
Trace Mineral Premix[3]	0.10	0.10	0.10	0.10
Titanium oxide	0.10	0.10	0.10	0.10
Phytic Acid[4]	0.10	0.70	0.10	0.70
Nutrient Composition				
Energy (ME; kcal/lb)	1360	1360	1400	1400
Calculated Composition				
Crude Protein	21	21	19	19
Lysine	1.45	1.45	1.20	1.20
TSAA	0.958	0.958	0.857	0.857
Threonine	0.735	0.735	0.675	0.674
Ca	0.9	0.9	0.85	0.85
Total P	0.733	0.806	0.691	0.764
Available P	0.45	0.45	0.42	0.42
Analyzed Composition				
Crude Protein	23.4	21.55	20.5	19.45
Lysine	1.59	1.48	1.19	1.22
Threonine	0.73	0.69	0.63	0.63
Ca	1.155	0.91	1.035	0.95
Total P	0.67	0.77	0.70	0.76
Crude Fat	3.815	4.96	3.35	4.51

[1] Supplied per kilogram mix: 600 ppm.
[2] Supplied per kilogram mix: vitamin A, 8,818,400 IU; vitamin D_3 2,645,520 ICU; vitamin E, 22,046 IU; vitamin B_{12}, 26 mg; riboflavin, 8,818 mg; niacin, 88,184 mg; d-pantothenic acid, 22,046 mg; vitamin K, 2,646 mg; folic acid, 2,205 mg; vitamin B_6, 4,339 mg; thiamine, 3,732 mg; d-biotin, 220 mg.
[3] Supplied per kilogram mix: iron (Ferrous Sulfate), 40 g; manganese (Manganese Sulfate and Manganous Oxide), 120 g; zinc (Zinc Oxide), 210 g; cobalt (Cobalt Carbonate), 2.2 g; iodine (Calcium Iodate), 132 g.
[4] Sigma-Alrich, 50% (w/w) in H_2O

Table 4.2 Recovery of enzyme activity in experimental diets

Diet	Determined Phytase Activity[1]	
	Starter	Grower
Low PA	~ 66	~ 64
Low PA + 1000 FTU	728	856
High PA	~ 68	~ 38
High PA + 1000 FTU	795	855

[1]One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1 µmole of inorganic P per minute from sodium phytate at pH 5.5 and 37°C.

Table 4.3 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on feed intake of broilers (g)

Treatment	d 0-7	d 7-28	d 0-28
Low PA	134.32[a]	1882.56	2016.88
High PA	131.00[b]	1924.11	2055.12
Non-vaccinated	130.30[b]	1967.11[a]	2097.41[a]
Vaccinated	135.02[a]	1839.56[b]	1974.58[b]
- Phytase	132.95	1887.69	2020.64
+ Phytase	132.37	1918.98	2051.36
Pooled SEM	0.7762	30.8328	31.0358
P-Value			
PA	0.0036	0.3442	0.3870
Vaccination	< 0.0001	0.0048	0.0068
Phytase	0.6004	0.4756	0.4866

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.4 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on body weight of broilers (g)

Treatment	d 0	d 7	d 28
Low PA	38.8494	142.48[a]	1422.57
High PA	38.8323	138.67[b]	1426.98
Non-vaccinated	38.6517[b]	137.44[b]	1463.36[a]
Vaccinated	39.0301[a]	143.71[a]	1386.20[b]
- Phytase	38.8403	140.27	1404.14
+ Phytase	38.8414	140.88	1445.42
Pooled SEM	0.08978	0.66995	14.7276
P-Value			
PA	0.8930	0.0002	0.8330
Vaccination	0.0041	< 0.0001	0.0004
Phytase	0.9935	0.5170	0.0519

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.5 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on body weight gain of broilers (g/g)

Treatment	d 0-7	d 7-28	d 0-28
Low PA	103.63[a]	1280.82	1384.45
High PA	99.8422[b]	1288.32	1388.16
Non-vaccinated	98.7824[b]	1326.77[a]	1425.55[a]
Vaccinated	104.69[a]	1242.37[b]	1347.06[b]
- Phytase	101.42	1264.61	1366.04
+ Phytase	102.05	1304.53	1406.58
Pooled SEM	0.6663	14.59145	14.7091
P-Value			
PA	0.0002	0.7176	0.8588
Vaccination	< 0.0001	0.0001	0.0004
Phytase	0.5115	0.0576	0.0558

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.6 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on feed conversion of broilers (g/g)

Treatment	d 0-7	d 7-28	d 0-28
Low PA	1.2977	1.4687	1.4558
High PA	1.3135	1.4930	1.4799
Non-vaccinated	1.3206[a]	1.4847	1.4731
Vaccinated	1.2906[b]	1.4770	1.4793
- Phytase	1.3123	1.4930	1.4793
+ Phytase	1.2989	1.4687	1.4564
Pooled SEM	0.00793	0.1663	0.01525
P-Value			
PA	0.1644	0.3059	0.2679
Vaccination	0.0097	0.7469	0.6258
Phytase	0.2389	0.3048	0.2927

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.7 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on mortality[2] of broilers (%)

Treatment	d 0-7	d 7-28	d 0-28
Low PA	1.7131	13.6239	15.3369
High PA	1.4357	8.4159	9.8513
Non-vaccinated	1.2772	3.8189[b]	5.0961[b]
Vaccinated	1.8715	18.2209[a]	20.0921[a]
- Phytase	2.1595[a]	10.7523	12.9116
+ Phytase	0.9892[b]	11.2875	12.2767
Pooled SEM	0.38275	3.1306	3.2233
P-Value			
PA	0.5437	0.2371	0.1767
Vaccination	0.3132	0.0043	0.0027
Phytase	0.0400	0.8118	0.7292
PA*Vacc*Phytase	0.0145	0.4343	0.7993

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

[2] The arcsine transformation (arcsine √%) was on mortality percentages prior to analysis to determine P-values. Non-transformed percentages were used for averages.

Table 4.8 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on tibia ash of broilers (%)

	Day 7		Day 28	
Treatment	Ash	Weight	Ash (%)	Weight
Low PA	45.824	103.98[a]	53.4792	1513.52
High PA	45.363	96.9212[b]	53.3063	1486.11
Non-vaccinated	45.585	99.5369	53.5011	1540.11[a]
Vaccinated	45.603	101.37	53.2843	1459.51[b]
- Phytase	45.709	102.20	53.3455	1493.89
+ Phytase	45.478	98.7036	53.4400	1505.73
Pooled SEM	0.5362	2.00735	0.13515	20.97545
P-Value				
PA	0.5459	0.0155	0.3693	0.3590
Vaccination	0.9808	0.5218	0.2611	0.0085
Phytase	0.1232	0.2228	0.6226	0.6912

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.9 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on day 7 small intestinal pH of broilers

	Gizzard		Ileum	
Treatment	Proximal	Distal	Proximal	Distal
Low PA	2.1844[a]	2.5753	5.8839	6.7581[a]
High PA	2.0012[b]	2.4421	5.8184	6.5276[b]
Non-vaccinated	2.0933	2.6211[a]	6.0025[a]	7.0450[a]
Vaccinated	2.0923	2.3963[b]	5.6998[b]	6.2407[b]
- Phytase	2.0307[b]	2.4668	5.8320	6.6443
+ Phytase	2.1550[a]	2.5506	5.8703	6.6414
Pooled SEM	0.03966	0.06251	0.03394	0.07897
P-Value				
PA	0.0018	0.1369	0.1773	0.0432
Vaccination	0.9857	0.0134	< 0.0001	< 0.0001
Phytase	0.0302	0.3471	0.4283	0.9792

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.10 Effect of a live coccidial vaccination[1], dietary phytic acid concentration, or phytase on day 28 small intestinal pH of broilers

		Gizzard		Ileum	
Treatment		Proximal	Distal	Proximal	Distal
Low PA		2.5378[a]	2.9036	5.7789	6.7249
High PA		2.1947[b]	2.6566	5.7388	6.5766
Non-vaccinated		2.5129[a]	2.8086	5.7567	6.6856
Vaccinated		2.2195[b]	2.7516	5.7610	6.6159
- Phytase		2.4427	2.7959	5.7954	6.6878
+ Phytase		2.2897	2.7643	5.7222	6.6136
Pooled SEM		0.08245	0.09647	0.02877	0.06320
Low PA	- Phytase	2.6039	2.9697	5.8683[a]	6.8486
	+ Phytase	2.4717	2.8375	5.6894[b]	6.6011
High PA	- Phytase	2.2815	2.6221	5.7225[ab]	6.5271
	+ Phytase	2.1078	2.6911	5.7550[ab]	6.6261
Pooled SEM		0.11660	0.13645	0.04068	0.08936
P-Value					
PA		0.0045	0.0750	0.3277	0.1021
Vaccination		0.0144	0.6775	0.9161	0.4387
Phytase		0.1943	0.8176	0.0768	0.4093
PA*phytase		0.8592	0.4636	0.0117	0.0570

[a-b] Means with different superscripts within the same column differ significantly ($P \leq 0.05$)

[1]Coccivac-B, Schering-Plough Animal Health Corporation

Table 4.11 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on day 7 AA digestibility of broilers (g/g)

Treatment	HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS	MET
Low PA	0.8650[a]	0.9044[a]	0.7913[a]	0.8196[a]	0.8341[a]	0.8474[a]	0.8474[a]	0.9147[a]	0.9346
High PA	0.8418[b]	0.8898[b]	0.7538[b]	0.7895[b]	0.8079[b]	0.8226[b]	0.8225[b]	0.9013[b]	0.9266
Non-Vaccinated	0.8641[a]	0.9049[a]	0.7904[a]	0.8225[a]	0.8350[a]	0.8487[a]	0.8479[a]	0.9174[a]	0.9378[a]
Vaccinated	0.8427[b]	0.8893[b]	0.7546[b]	0.7866[b]	0.8070[b]	0.8213[b]	0.8220[b]	0.8986[b]	0.9234[b]
- Phytase	0.8601	0.8982	0.7815	0.8128	0.8281	0.8398	0.8396	0.9102	0.9336
+ Phytase	0.8467	0.8961	0.7635	0.7963	0.8139	0.8302	0.8303	0.9058	0.9276
Pooled SEM	0.00559	0.00425	0.00876	0.00870	0.00666	0.00608	0.00621	0.00376	0.00309
P-Value									
PA	0.0071	0.0228	0.0058	0.0222	0.0103	0.0080	0.0090	0.0188	0.0770
Vaccination	0.0123	0.0158	0.0079	0.0075	0.0066	0.0040	0.0069	0.0017	0.0030
Phytase	0.1034	0.7269	0.1590	0.1938	0.1469	0.2740	0.2993	0.4181	0.1775

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 4.12 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on day 7 AA digestibility of broilers (g/g)

Treatment	ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low PA	0.8404[a]	0.8865[a]	0.8319[a]	0.8122[a]	0.8474[a]	0.8398[a]	0.8516[a]	0.8543[a]
High PA	0.8149[b]	0.8661[b]	0.8020[b]	0.7809[b]	0.8221[b]	0.8139[b]	0.8247[b]	0.8299[b]
Non-Vaccinated	0.8401[a]	0.8866[a]	0.8338[a]	0.8096[a]	0.8505[a]	0.8376[a]	0.8511[a]	0.8548[a]
Vaccinated	0.8153[b]	0.8660[b]	0.8001[b]	0.7836[b]	0.8189[b]	0.8161[b]	0.8252[b]	0.8294[b]
- Phytase	0.8321	0.8789	0.8184	0.8051	0.8406	0.8323	0.8444	0.8473
+ Phytase	0.8232	0.8737	0.8154	0.7881	0.8288	0.8215	0.8319	0.8370
Pooled SEM	0.00646	0.00509	0.00813	0.00756	0.00625	0.00621	0.00616	0.00605
P-Value								
PA	0.0103	0.0091	0.0158	0.0073	0.0086	0.0070	0.0050	0.0089
Vaccination	0.0121	0.0087	0.0074	0.0232	0.0015	0.0220	0.0065	0.0067
Phytase	0.3444	0.4781	0.7964	0.1236	0.1939	0.2325	0.1621	0.2427

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 4.13 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on day 28 AA digestibility of broilers (g/g)

Treatment	HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS	MET
Low PA	0.8485[b]	0.8865[b]	0.7708[b]	0.7941[b]	0.8130[b]	0.8442[b]	0.8324[b]	0.8888[b]	0.9295[b]
High PA	0.8768[a]	0.9076[a]	0.7988[a]	0.8307[a]	0.8483[a]	0.8690[a]	0.8606[a]	0.9128[a]	0.9411[a]
Non-Vaccinated	0.8553	0.8912	0.7753	0.8053	0.8264	0.8491	0.8398	0.8972	0.9320
Vaccinated	0.8699	0.9029	0.7943	0.8195	0.8349	0.8641	0.8532	0.9044	0.9386
- Phytase	0.8518[b]	0.8871[b]	0.7727	0.7981[b]	0.8145[b]	0.8449[b]	0.8334[b]	0.8924[b]	0.9332
+ Phytase	0.8735[a]	0.9070[a]	0.7969	0.8266[a]	0.8468[a]	0.8683[a]	0.8596[a]	0.9092[a]	0.9374
Pooled SEM	0.00616	0.00454	0.00838	0.00756	0.00753	0.00669	0.00685	0.00421	0.00304
P-Value									
PA	0.0035	0.0031	0.0262	0.0022	0.0029	0.0149	0.0076	0.0005	0.0123
Vaccination	0.1063	0.0811	0.1207	0.1954	0.4291	0.1273	0.1800	0.2347	0.1360
Phytase	0.0199	0.0048	0.0523	0.0136	0.0057	0.0206	0.0122	0.0096	0.3322

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 4.14 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on day 28 AA digestibility of broilers (g/g)

Treatment	ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low PA	0.8074[b]	0.8722[b]	0.8133[b]	0.7863[b]	0.8479[b]	0.8330[b]	0.8334[b]	0.8376[b]
High PA	0.8386[a]	0.8939[a]	0.8333[a]	0.8171[a]	0.8712[a]	0.8576[a]	0.8541[a]	0.8632[a]
Non-Vaccinated	0.8153	0.8759	0.8110[b]	0.7927	0.8526	0.8357	0.8395	0.8433
Vaccinated	0.8308	0.8902	0.8356[a]	0.8108	0.8665	0.8549	0.8480	0.8576
- Phytase	0.8076[b]	0.8727[b]	0.8132[b]	0.7896[b]	0.8506	0.8353[b]	0.8334[b]	0.8394[b]
+ Phytase	0.8384[a]	0.8934[a]	0.8334[a]	0.8138[a]	0.8684	0.8554[a]	0.8341[a]	0.8614[a]
Pooled SEM	0.00718	0.00520	0.00672	0.00829	0.00652	0.00661	0.00617	0.00628
P-Value								
PA	0.0053	0.0068	0.0460	0.0147	0.0184	0.0145	0.0265	0.0082
Vaccination	0.1402	0.0643	0.0160	0.1363	0.1434	0.0504	0.3395	0.1199
Phytase	0.0057	0.0094	0.0436	0.0500	0.0651	0.0415	0.0265	0.0209

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).

Table 4.15 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on intestinal histology of 7-day-old broilers[1]

Treatment		Duo VH[2]	Duo CD[3]	Duo VCR	Jej VH	Jej CD	Jej VCR	Ile VH	Ile CD	Ile VCR
Low PA		1.4732[a]	0.1532[a]	9.7456	0.7185	0.1363	5.3718	0.5086	0.1339[a]	3.8765[b]
High PA		1.4025[b]	0.1453[b]	9.7310	0.7039	0.1289	5.5132	0.4938	0.1200[b]	4.1561[a]
Non-Vaccinated		1.3861[b]	0.1368[b]	10.1802[a]	0.7417	0.1241[b]	5.9916[a]	0.4956	0.1190[b]	4.1888[a]
Vaccinated		1.4896[a]	0.1617[a]	9.2965[b]	0.6807	0.1411[a]	4.8934[b]	0.5067	0.1350[a]	3.8438[b]
- Phytase		1.4481	0.1549[a]	9.4226[b]	0.7257	0.1380[a]	5.3554	0.5016	0.1349[a]	3.7856[b]
+ Phytase		1.4275	0.1436[b]	10.0540[a]	0.6967	0.1271[b]	5.5295	0.5007	0.1191[b]	4.2470[a]
Pooled SEM		0.018955	0.002585	0.1577	0.0190	0.00335	0.1239	0.00937	0.00288	0.0746
- Phytase	-[4]	1.3595[b]	0.1403	9.7212	0.7347	0.1250	5.8824	0.4941	0.1231	4.0324
	+[5]	1.5368[a]	0.1695	9.1241	0.7167	0.1511	4.8285	0.5092	0.1466	3.5388
+ Phytase	-	1.4127[b]	0.1333	10.6392	0.7486	0.1231	6.1008	0.4971	0.1148	4.3452
	+	1.4423[ab]	0.1539	9.4689	0.6448	0.1310	4.9583	0.5043	0.1233	4.1488
Pooled SEM		0.02680	0.00366	0.2230	0.0269	0.00473	0.1751	0.01326	0.00408	0.1055
P-Value										
PA		0.0105	0.0357	0.9481	0.5901	0.1226	0.4222	0.2688	0.0011	0.0101
Vaccination		0.0003	<0.0001	0.0002	0.0275	0.0006	<0.0001	0.4035	0.0002	0.0017
Phytase		0.4447	0.0029	0.0062	0.2864	0.0239	0.3239	0.9432	0.0003	<0.0001
Phytase*Vaccination		0.0077	0.2496	0.2034	0.1171	0.0584	0.8010	0.7667	0.0696	0.1638

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Each vaccination and phytic acid combination represents the means of 12 replications.
[2] VH = villus height (mm)
[3] CD = crypt depth (mm)
[4] Non-vaccinated birds.
[5] Vaccinated birds.

Table 4.16 Effect of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on intestinal histology of 28-day-old broilers[1]

Treatment	Duo VH[2]	Duo CD[3]	Duo VCR	Jej VH	Jej CD	Jej VCR	Ile VH	Ile CD	Ile VCR
Low PA	2.3672	0.2464	10.1389	1.6051	0.1972	8.5867	0.8712	0.1743	5.1416
High PA	2.2620	0.2501	9.9752	1.5429	0.1895	8.5399	0.8749	0.1617	5.5182
Non-Vaccinated	2.2831	0.2227[b]	11.3891[a]	1.6108	0.1656[b]	9.9917[a]	0.8552	0.1622	5.3550
Vaccinated	2.3461	0.2739[a]	8.7250[b]	1.5371	0.2212[a]	7.1349[b]	0.8909	0.1737	5.3048
- Phytase	2.3503	0.2689[a]	9.4825	1.6229	0.1983	8.6324	0.9172[a]	0.1727	5.4482
+ Phytase	2.2789	0.2277[b]	10.6315	1.5251	0.1884	8.4942	0.8288[b]	0.1633	5.2116
Pooled SEM	0.04716	0.0109	0.42265	0.0373	0.00691	0.25655	0.02170	0.00480	0.18240
P-Value									
PA	0.1196	0.8077	0.7851	0.2429	0.4384	0.8980	0.9045	0.0682	0.1493
Vaccination	0.3481	0.0015	<0.0001	0.1674	<0.0001	<0.0001	0.2484	0.0964	0.8463
Phytase	0.2887	0.0095	0.0591	0.0687	0.3170	0.7046	0.0054	0.1700	0.3626
PA*Phytase*Vac	0.7784	0.2156	0.2779	0.0793	0.3499	0.0383	0.6228	0.0252	0.0331

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Each vaccination and phytic acid combination represents the means of 12 replications.
[2] VH = villus height (mm)
[3] CD = crypt depth (mm)

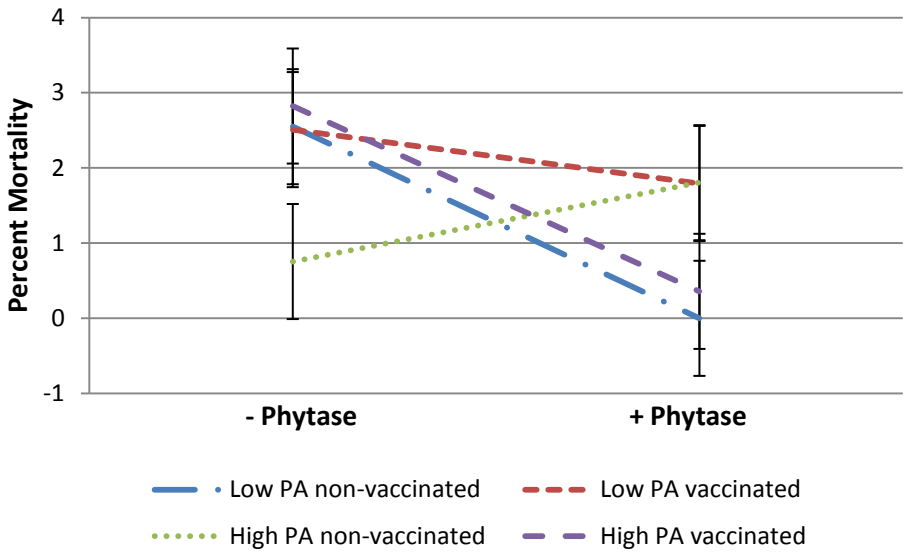

Figure 4.1 Three-way interaction (P = 0.0145) of a live coccidiosis vaccination (Coccivac-B, Schering-Plough Animal Health), dietary phytic acid concentration, and phytase on d 7 mortality of broilers (%). The arcsine transformation was used on mortality percentages prior to analysis to determine P-values. Non-transformed percentages were used for averages.

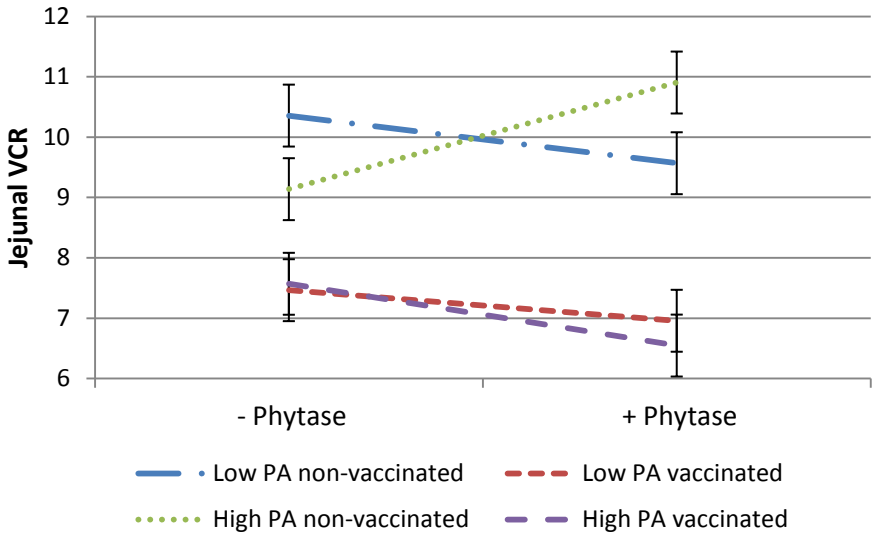

Figure 4.2 Three-way interaction (P = 0.0383) of a live coccidiosis vaccination (Coccivac-B, Schering-Plough Animal Health), dietary phytic acid concentration, or phytase on jejunal villus height to crypt depth ratio (VCR) of 28-day-old broilers.

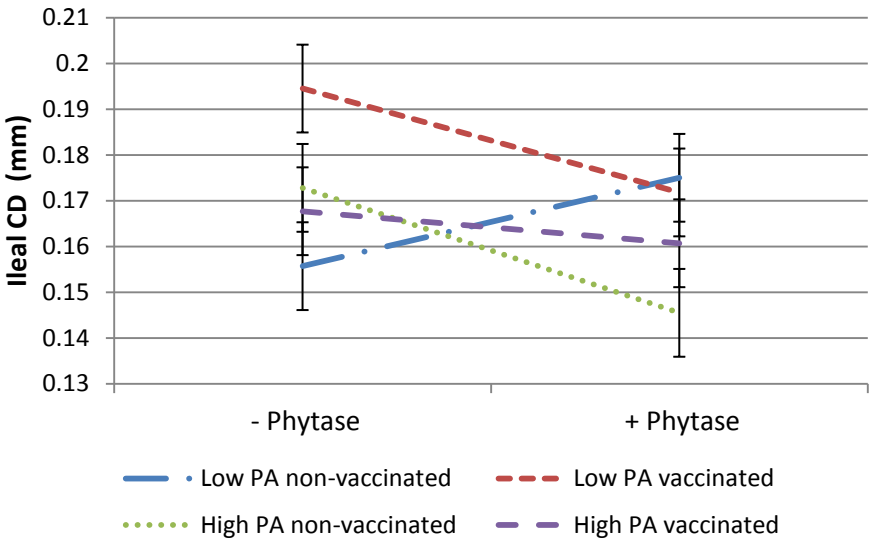

Figure 4.3 Three-way interaction (P = 0.00959) of a live coccidiosis vaccination (Coccivac-B, Schering-Plough Animal Health), dietary phytic acid concentration, or phytase on ileal crypt depth (CD) of 28-day-old broilers.

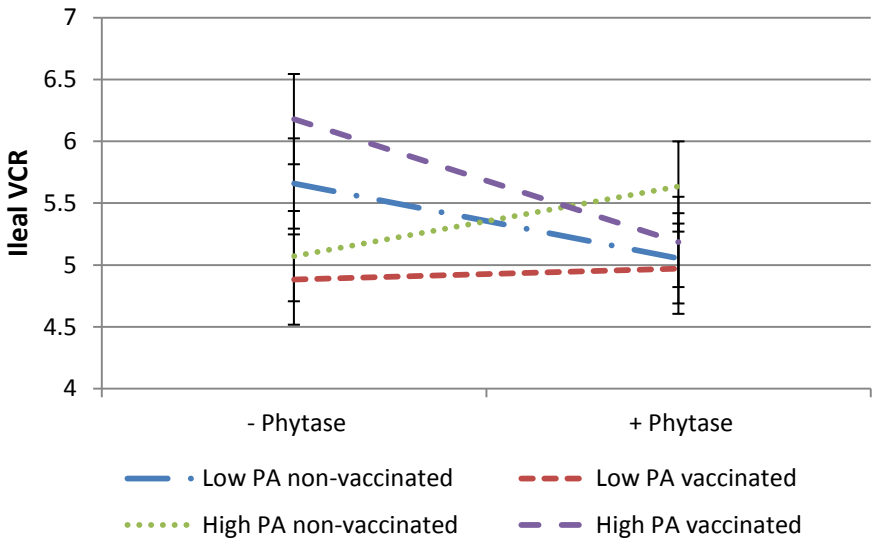

Figure 4.4 Three-way interaction (P = 0.0331) of a live coccidiosis vaccination, dietary phytic acid concentration, or phytase on ileal villus height to crypt depth ratio (VCR) of 28-day-old broilers.

CHAPTER V

EPILOGUE

In the poultry industry, improvements in nutrition have had a dramatic impact on the efficiency and productivity of the broiler. With continual changes to dietary and health regulations, consumer preferences, feed ingredients and additives, and much more, it is the goal of research scientists to strive to improve the health and productivity of the bird to benefit the producer, consumer, and ultimately advance the field of science. While improving nutrition is important, it is equally important to understand how nutrition plays a role in other aspects of bird management. For example, how the nutritional needs of birds change under disease or stress conditions or how poor nutrition influences susceptibility to disease.

One area of research that has received much attention recently is the health of the intestinal tract. Considering 20% of a bird's energy goes towards maintenance of the GIT, factors such as disease or diet that could disrupt the natural healthy balance of the intestine become extremely important avenues to explore to insure optimal health and efficient production of birds. One such dietary factor examined in this dissertation that has been found to disrupt normal intestinal health, digestion of nutrients, and exert other anti-nutritional consequences is phytate. Within the past twenty years, the exogenous enzyme phytase has become commercially available to limit some of the negative effects of phytate on bird performance. It was initially introduced to improve availability of P in poultry diets as a means to decrease feed costs and reduce environmental pollution caused by poultry litter high in P. Now phytase is included in nearly half of all poultry diets because of its extra-phosphoric advantages of improved bird performance as well as advantages in intestinal health.

As mentioned earlier, it should be the goal of poultry nutritionist to not only improve the nutrition of birds, but to understand the other factors of poultry management that ultimately have an effect on nutrition. Because a healthy intestine is necessary for adequate nutrient digestion, enteric diseases such as coccidiosis that compromise the integrity of the intestinal tract are of great importance when considering how to improve intestinal health through nutrition. Equally important, is the way nutritional strategies affect disease control methods. In the past, in-feed coccidiostat medication was the primary method used to control coccidiosis. However, as consumer preferences change, vaccinating broilers against coccidiosis is becoming increasingly more common. Unfortunately, the use of live oocyst vaccines is associated with an initial set-back in performance. Therefore, it was the aim of this dissertation to determine the combined effect of dietary phytic acid and a live coccidial oocyst vaccine on intestinal health and bird performance. The second experiment evaluated the addition of exogenous phytase as a nutritional strategy to improve bird response to vaccination and dietary phytic acid.

In the first experiment, the goal was to determine if the negative effects on performance from dietary phytic acid are exacerbated when birds are vaccinated with a live coccidial oocyst vaccine. It was found that the combined effects of both treatments lowered feed intake and weight gain in birds. It was originally hypothesized that negative performance caused by both treatments would be a result of altered intestinal integrity. However, the combined effect of these treatments on intestinal morphology was not apparent. Perhaps phytate and protein solubility play a greater role in poor performance and mortality than does intestinal morphology. Though not directly measured, the high Ca: P ratios in the diet could have influenced phytate solubility and the phytic acid: protein ratio may have lowered protein solubility. Even so, the negative

effect that vaccination has on feed intake, weight gain, and mortality of birds with higher phytic acid diets should not be overlooked.

In the second experiment, the combined effects of high phytic acid and vaccination on feed intake and body weight gain were not seen. Perhaps the lowered Ca: P ratio allowed for greater solubility of the phytate complex and subsequently greater nutrient digestion. As a result, the addition of phytase did not improve bird performance. Still there was an improvement in intestinal morphology and apparent IAAD, especially of younger birds, with the addition of phytase. Typically, with the formulation of diets containing phytase, the level of certain nutrients in the diets is reduced in order to see advantages in performance. In the present experiment, if the level of nutrients were reduced, an advantage in intestinal health and bird performance would have most likely been seen with the addition of phytase.

Due to the dynamic nature of the intestine, complex life cycle of *Eimeria* in the intestinal tract, and variety of anti-nutritional influences phytic acid has on intestinal morphology and nutrient digestibility, it is difficult to determine specific mechanisms by which performance is altered. Additionally, it is equally complex to elucidate the influence phytase has on improving intestinal health and the conditions required for a noticeable improvement in performance. Even so, this dissertation has attempted demonstrate the combined effect of dietary phytic acid during a live coccidial oocyst vaccine on bird performance and intestinal morphology and digestibility. Furthermore, it has shown the influences that diet formulation has on bird health during a coccidiosis vaccination and how using nutritional means, such as addition of phytase, can improve certain aspects of bird health.

As mentioned before, as nutritional advances are made in the poultry industry, it is important to determine how these advances can improve bird performance under a variety of conditions to stay relevant to commercial poultry production. As production systems, regulations, breeds, and ingredient options change, it is the responsibility of poultry scientists to determine how to best use the available resources for the optimal advancement of the poultry industry.

APPENDIX A

Table A.1 Effect of a live coccidial vaccination and dietary phytic acid concentration on goblet cell number per villus area[1] of 7-day-old broilers

Treatment		Duodenum	Jejunum	Ileum
Low PA		6.4538	7.0342	7.0778
Med PA		6.3874	6.9869	7.0612
High PA		6.3831	6.8918	6.9952
Pooled SEM		0.04710	0.05272	0.05248
Non-Vaccinated		6.4301	7.0058	7.0475
Vaccination		6.3860	6.9361	7.0420
Pooled SEM		0.03846	0.04304	0.04285
Low PA	-[2]	6.5523	7.1200	7.1747
	+[3]	6.3553	6.9484	6.9810
Med PA	-	6.3817	7.0441	7.0228
	+	6.3931	6.9297	7.0996
High PA	-	6.3564	6.8534	6.9450
	+	6.4097	6.9302	7.0453
Pooled SEM		0.06661	0.07455	0.07421
P-Value				
PA		0.4984	0.1633	0.5052
Vaccination		0.4222	0.2587	0.9279
PA*Vaccination		0.1448	0.2299	0.1008

[1] Values natural log transformed prior to analysis.
[2] Non-vaccinated birds.
[3] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.2 Effect of a live coccidial vaccination and dietary phytic acid concentration on goblet cell number per villus area[1] of 21-day-old broilers

Treatment		Duodenum	Jejunum	Ileum
Low PA		6.4299	6.8216	7.2032[a]
Med PA		6.2762	6.8230	6.9595[b]
High PA		6.2867	6.8324	7.0239[ab]
Pooled SEM		0.05201	0.06832	0.06672
Non-Vaccinated		6.3611	6.9801[a]	7.0869
Vaccination		6.3008	6.6713[b]	7.0374
Pooled SEM		0.04247	0.05578	0.05447
Low PA	-[2]	6.3966	7.0649	7.1916
	+[3]	6.4633	5.5784	7.2148
Med PA	-	6.3589	6.9873	7.0100
	+	6.1934	6.6588	6.9090
High PA	-	6.3279	6.8882	7.0593
	+	6.2456	6.7767	6.9886
Pooled SEM		0.07356	0.09662	0.09435
P-Value				
PA		0.0771	0.9926	0.0366
Vaccination		0.3207	0.0003	0.5239
PA*Vaccination		0.2890	0.1625	0.7914

[a-b] Means within the same column with different superscripts differ significantly ($P \leq 0.05$).
[1] Values natural log transformed prior to analysis.
[2] Non-vaccinated birds.
[3] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.3 Treatment means for feed intake resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vaccination	d 0-7	d 7-28	d 0-28
Low	0	-[1]	133.14	1936.74	2069.89
Low	0	+[2]	137.61	1771.79	1909.40
Low	1000	-	130.45	1985.86	2116.31
Low	1000	+	136.10	1835.83	1971.93
High	0	-	129.05	1951.13	2080.18
High	0	+	132.01	1891.08	2023.08
High	1000	-	128.57	1994.70	2123.28
High	1000	+	134.38	1859.54	1993.92
	Pooled SEM		1.552	61.655	62.061
P-Value					
PA*Phytase*Vaccination			0.7049	0.6076	0.6165

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.4 Treatment means for body weight resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vaccination	d 0	d 7	d 28
Low	0	-[1]	38.74	138.46	1440.41
Low	0	+[2]	39.04	146.08	1361.43
Low	1000	-	38.69	139.11	1491.29
Low	1000	+	38.93	146.26	1397.17
High	0	-	38.53	135.56	1427.40
High	0	+	39.05	140.98	1387.33
High	1000	-	38.64	136.63	1494.36
High	1000	+	39.10	141.53	1398.86
	Pooled SEM		0.180	1.340	29.450
P-Value					
PA*Phytase*Vaccination			0.9857	0.9913	0.6304

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.5 Treatment means for body weight gain resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vaccination	d 0-7	d 7-28	d 0-28
Low	0	-[1]	99.70	1303.38	1403.09
Low	0	+[2]	107.04	1215.91	1322.95
Low	1000	-	100.42	1353.03	1453.45
Low	1000	+	107.34	1250.97	1358.31
High	0	-	97.02	1292.59	1389.61
High	0	+	101.92	1246.57	1348.49
High	1000	-	97.99	1358.08	1456.07
High	1000	+	102.44	1256.04	1358.48
	Pooled SEM		1.332	29.178	29.413
P-Value					
PA*Phytase*Vaccination			0.9916	0.6175	0.6200

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.6 Treatment means for feed conversion resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vaccination	d 0-7	d 7-28	d 0-28
Low	0	-[1]	1.3359	1.4869	1.4762
Low	0	+[2]	1.2862	1.4557	1.4416
Low	1000	-	1.3002	1.4694	1.4577
Low	1000	+	1.2684	1.4629	1.4477
High	0	-	1.3314	1.5139	1.5007
High	0	+	1.2955	1.5156	1.4986
High	1000	-	1.3147	1.4684	1.4579
High	1000	+	1.3123	1.4740	1.4623
	Pooled SEM		0.01587	0.03324	0.03049
P-Value					
PA*Phytase*Vaccination			0.7274	0.8253	0.8337

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.7 Treatment means for mortality (%) resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vaccination	d 0-7	d 7-28	d 0-28
Low	0	-[1]	2.5489	4.6256	5.7756
Low	0	+[2]	2.5100	26.7433	20.0678
Low	1000	-	0.0	4.9222	3.4978
Low	1000	+	1.7933	18.2044	14.0522
High	0	-	0.7556	2.9767	3.7322
High	0	+	2.8238	8.6637	11.4862
High	1000	-	1.8044	2.7511	4.5556
High	1000	+	0.3589	19.2722	19.6311
	Pooled SEM		0.76535	6.26021	6.4455
P-Value					
PA*Phytase*Vaccination			0.0145	0.2709	0.7993

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.8 Treatment means for tibia ash resulting from dietary phytic acid concentration, phytase, and vaccination combination

			Day 7		Day 28	
PA	Phytase	Vaccination	Ash (%)	Weight (g)	Ash (%)	Weight (g)
Low	0	-[1]	45.87	105.93	53.51	1550.33
Low	0	+[2]	44.79	104.44	53.37	1485.56
Low	1000	-	46.95	105.18	53.58	1572.56
Low	1000	+	45.69	100.37	53.46	1445.63
High	0	-	44.39	92.59	53.38	1518.85
High	0	+	47.79	105.83	53.13	1420.83
High	1000	-	45.13	94.44	53.54	1518.70
High	1000	+	44.14	94.82	53.19	1486.04
	Pooled SEM		1.072	4.014	0.2703	41.944
P-Value						
PA*Phytase*Vaccination			0.1700	0.4042	0.8648	0.2867

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.9 Treatment means for d 7small intestinal pH resulting from dietary phytic acid concentration, phytase, and vaccination combination

			Gizzard		Ileum	
PA	Phytase	Vaccination	Proximal	Distal	Proximal	Distal
Low	0	-[1]	2.1500	2.7333	5.9889	7.0378
Low	0	+[2]	2.1678	2.4333	5.6789	6.4756
Low	1000	-	2.1578	2.6722	6.0711	7.1011
Low	1000	+	2.2622	2.4622	5.7967	6.4178
High	0	-	1.9411	2.3756	6.0078	7.1389
High	0	+	1.8637	2.3250	5.6525	5.9250
High	1000	-	2.1244	2.7033	5.9422	6.9022
High	1000	+	2.0756	2.3644	5.6711	6.1444
Pooled SEM			0.07930	0.12504	0.06787	0.15789
P-Value						
PA*Phytase*Vaccination			0.7962	0.2887	0.8009	0.2010

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.10 Treatment means for d 28small intestinal pH resulting from dietary phytic acid concentration, phytase, and vaccination combination

			Gizzard		Ileum	
PA	Phytase	Vaccination	Proximal	Distal	Proximal	Distal
Low	0	-[1]	2.7772	2.9528	5.8350	6.8700
Low	0	+[2]	2.4306	2.9867	5.9017	6.8272
Low	1000	-	2.5222	2.8006	5.6594	6.6533
Low	1000	+	2.4211	2.8744	5.7194	6.5489
High	0	-	2.4656	2.7667	5.7900	6.5667
High	0	+	2.0975	2.4775	5.6550	6.4875
High	1000	-	2.2867	2.7144	5.7422	6.6522
High	1000	+	1.9289	2.6678	5.7678	6.6000
Pooled SEM			0.16485	0.19295	0.05753	0.12635
P-Value						
PA*Phytase*Vaccination			0.6157	0.7118	0.3081	0.8050

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.11 Treatment means for d 7 apparent ileal amino acid digestibility resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vacc	HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS	MET
Low	0	-[1]	0.8728	0.9090	0.7855	0.8383	0.8485	0.8573	0.8575	0.9228	0.9420
Low	0	+[2]	0.8608	0.8960	0.7600	0.8115	0.8295	0.8372	0.8368	0.9078	0.9293
Low	1000	-	0.8785	0.9180	0.7605	0.8360	0.8495	0.8665	0.8668	0.9270	0.9432
Low	1000	+	0.8480	0.8948	0.7090	0.7925	0.8090	0.8287	0.8287	0.9013	0.9240
High	0	-	0.8588	0.8980	0.8013	0.8160	0.8253	0.8405	0.8385	0.9118	0.9370
High	0	+	0.8480	0.8898	0.7793	0.7853	0.8090	0.8243	0.8258	0.8985	0.9263
High	1000	-	0.8463	0.8948	0.8145	0.7998	0.8168	0.8305	0.8290	0.9080	0.9290
High	1000	+	0.8140	0.8767	0.7700	0.7570	0.7805	0.7950	0.7968	0.8870	0.9140
Pooled SEM			0.0112	0.0085	0.0175	0.0174	0.0133	0.0122	0.0124	0.0075	0.0062
P-Value											
PA*Phytase*Vaccination			0.9251	0.9836	0.9442	0.9239	0.9686	0.9656	0.9494	0.8889	0.8985

PA	Phytase	Vacc	ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low	0	-	0.8475	0.8940	0.8403	0.8223	0.8603	0.8448	0.8608	0.8635
Low	0	+	0.8288	0.8770	0.8115	0.8057	0.8375	0.8340	0.8408	0.8455
Low	1000	-	0.8600	0.9020	0.8590	0.8278	0.8665	0.8558	0.8708	0.8715
Low	1000	+	0.8252	0.8730	0.8167	0.7932	0.8253	0.8247	0.8342	0.8368
High	0	-	0.8323	0.8788	0.8220	0.8038	0.8435	0.8313	0.8463	0.8480
High	0	+	0.8198	0.8658	0.8000	0.7888	0.8213	0.8190	0.8300	0.8320
High	1000	-	0.8205	0.8715	0.8138	0.7845	0.8317	0.8188	0.8268	0.8363
High	1000	+	0.7873	0.8482	0.7723	0.7468	0.7918	0.7868	0.7958	0.8035
Pooled SEM			0.0129	0.0102	0.0163	0.0151	0.0125	0.0124	0.0123	0.0121
P-Value										
PA*Phytase*Vaccination			0.8977	0.9520	0.8973	0.9125	0.9832	0.9888	0.9603	1.0000

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.12 Treatment means for d 28 apparent ileal amino acid digestibility resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vacc	HIS	ARG	THR	VAL	ILE	LEU	PHE	LYS	MET
Low	0	-[1]	0.8293	0.8698	0.7488	0.7723	0.7930	0.8283	0.8142	0.8763	0.9258
Low	0	+[2]	0.8368	0.8748	0.7550	0.7740	0.7888	0.8307	0.8160	0.8765	0.9255
Low	1000	-	0.8508	0.8895	0.7722	0.7998	0.8223	0.8425	0.8332	0.8928	0.9265
Low	1000	+	0.8772	0.9120	0.8070	0.8303	0.8480	0.8752	0.8660	0.9097	0.9403
High	0	-	0.8665	0.8978	0.7863	0.8183	0.8383	0.8545	0.8480	0.9075	0.9383
High	0	+	0.8745	0.9060	0.8007	0.8280	0.8380	0.8660	0.8553	0.9095	0.9433
High	1000	-	0.8748	0.9078	0.7938	0.8308	0.8520	0.8713	0.8638	0.9123	0.9375
High	1000	+	0.8913	0.9188	0.8145	0.8458	0.8650	0.8843	0.8755	0.9220	0.9455
Pooled SEM			0.0123	0.0091	0.0168	0.0151	0.0151	0.0134	0.0137	0.0084	0.0061
P-Value											
PA*Phytase*Vaccination			0.7659	0.5709	0.6430	0.5880	0.6975	0.4547	0.5005	0.7085	0.5280

PA	Phytase	Vacc	ASP	GLU	SER	GLY	ALA	PRO	TYR	TOTAL
Low	0	-	0.7820	0.8558	0.7922	0.7635	0.8355	0.8160	0.8205	0.8203
Low	0	+	0.7890	0.8620	0.8028	0.7690	0.8372	0.8250	0.8182	0.8240
Low	1000	-	0.8130	0.8707	0.8085	0.7908	0.8445	0.8288	0.8337	0.8385
Low	1000	+	0.8458	0.9002	0.8498	0.8220	0.8743	0.8623	0.8612	0.8677
High	0	-	0.8255	0.8808	0.8168	0.8100	0.8603	0.8413	0.8465	0.8520
High	0	+	0.8338	0.8923	0.8410	0.8160	0.8695	0.8588	0.8485	0.8615
High	1000	-	0.8405	0.8965	0.8265	0.8065	0.8700	0.8568	0.8573	0.8623
High	1000	+	0.8545	0.9063	0.8490	0.8360	0.8850	0.8738	0.8640	0.8770
Pooled SEM			0.0144	0.0104	0.0134	0.0166	0.0130	0.0132	0.0123	0.0126
P-Value										
PA*Phytase*Vaccination			0.6271	0.4035	0.4010	0.9621	0.5517	0.5100	0.4806	0.5737

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.13 Treatment means for d 7 small intestinal morphology resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vacc	Duo VH[2]	Duo CD[3]	Duo VCR	Jej VH	Jej CD	Jej VCR	Ile VH	Ile CD	Ile VCR
Low	0	-[1]	1.4047	0.1430	9.8801	0.7311	0.1249	5.8445	0.5044	0.1293	3.9215
Low	0	+[2]	1.5758	0.1760	9.0312	0.6907	0.1510	4.6719	0.5149	0.1542	3.4225
Low	1000	-	1.4503	0.1363	10.6576	0.7590	0.1268	6.0010	0.5003	0.1205	4.1521
Low	1000	+	1.4620	0.1574	9.4136	0.6932	0.1423	4.9696	0.5147	0.1317	4.0098
High	0	-	1.3143	0.1377	9.5623	0.7384	0.1250	5.9203	0.4838	0.1169	4.1433
High	0	+	1.4977	0.1630	9.2169	0.7427	0.1512	4.9851	0.5035	0.1391	3.6551
High	1000	-	1.3751	0.1303	10.6207	0.7382	0.1195	6.2006	0.4939	0.1091	4.5383
High	1000	+	1.4227	0.1504	9.5242	0.5963	0.1198	4.9469	0.4939	0.1149	4.2878
Pooled SEM			0.0379	0.0052	0.3153	0.0318	0.0067	0.2476	0.0187	0.0058	0.1491
P-Value											
PA*Phytase*Vaccination			0.8257	0.6498	0.6912	0.2677	0.4218	0.5141	0.6575	0.8647	0.7784

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.

Table A.14 Treatment means for d 28 small intestinal morphology resulting from dietary phytic acid concentration, phytase, and vaccination combination

PA	Phytase	Vacc	Duo VH[2]	Duo CD[3]	Duo VCR	Jej VH	Jej CD	Jej VCR	Ile VH	Ile CD	Ile VCR
Low	0	-[1]	2.4849	0.2424	10.9231	1.7379	0.1706	10.3567[a]	0.8765	0.1557	5.6590
Low	0	+[2]	2.4389	0.2856	8.8014	1.5904	0.2300	7.4644[bcd]	0.9084	0.1945	4.8827
Low	1000	-	2.2220	0.1961	11.8437	1.5194	0.1613	9.5678[ab]	0.8693	0.1750	5.0542
Low	1000	+	2.3230	0.2615	8.9872	1.5727	0.2268	6.9577[cd]	0.8306	0.1718	4.9704
High	0	-	2.2072	0.2745	9.6845	1.5878	0.1826	9.1378[abc]	0.8718	0.1728	5.0719
High	0	+	2.2701	0.2730	8.5211	1.5754	0.2100	7.5706[bcd]	1.0123	0.1677	6.1793
High	1000	-	2.2181	0.1776	13.1051	1.5983	0.1477	10.9046[a]	0.8031	0.1455	5.6349
High	1000	+	2.3526	0.2754	8.5901	1.4099	0.2179	6.5467[d]	0.8124	0.1607	5.1868
Pooled SEM			0.0943	0.0218	0.8452	0.0735	0.0138	0.51290	0.0434	0.0095	0.36484
P-Value											
PA*Phytase*Vaccination			0.7784	0.2156	0.2779	0.0793	0.3499	0.0383	0.6228	0.0252	0.0331

[1] Non-vaccinated birds.
[2] Vaccinated birds, Coccivac-B, Schering-Plough.