

THE EFFECTS OF POTENTIAL DIETARY PREBIOTICS AND GRAIN PARTICLE SIZE
ON THE GROWTH PERFORMANCE AND INTESTINAL MICROFLORA OF YOUNG
POULTRY

BY

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DISSERTATION

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Abstract

Experiments were conducted to evaluate the effects of grain particle size and several potential prebiotic feed additives when fed for varying lengths of time on growth performance, nutrient utilization, and select cecal and ileal microbial populations in young chicks. In addition, some of these dietary additives were also evaluated for their ability to ameliorate the negative effects of an *Eimeria acervulina* coccidiosis infection on the above response variables. In the first study, large particle corn, whole sorghum, and Grobiotic (GB) were evaluated in a corn-soybean meal diet with or without the addition of distillers dried grains with solubles (DDGS). In general, the results showed that larger particle sizes from large ground corn or whole sorghum, as well as the addition of GB, can be supplemented to a corn-SBM or corn-SBM DDGS diet with no adverse effect on growth performance and nutrient digestibility when fed from 0 to 21 d of age. Also, relative gizzard weights can be significantly increased ($P < 0.05$) when large ground corn, whole sorghum, or DDGS is included in young chick diets. The second study evaluated the effects of fine or large ground corn, whole sorghum, or whole wheat in the presence or absence of an acute *E. acervulina* infection in chicks fed a corn-soybean meal diet. The results of this study indicated that feeding large ground corn, whole wheat, or whole sorghum in the presence or absence of a coccidial challenge significantly increased ($P < 0.05$) relative gizzard weights and decreased *E. coli* populations, but had no consistent effects on overall growth performance or responses to *E. acervulina* infection. The next study evaluated the effects of short-term feeding (3, 7, or 21 d) of GB, Dairylac-80, and lactose in both a corn-soybean meal diet and a casein-dextrose diet, as well as the effects of these additives in the presence or absence of an acute *E. acervulina* infection. The results of this study indicated that an *E. acervulina* infection reduced chick performance, ME_n, and AA digestibility ($P < 0.05$), and the addition of GB,

Dairylac-80, and lactose were not effective in alleviating the negative effects of a coccidiosis infection. In the absence of a coccidiosis infection, the results indicated that the feeding of GB, Dairylac-80, and lactose may yield some beneficial responses in chick growth performance, especially when added to a casein-dextrose diet; however, the responses were not consistent. Also, short-term feeding of various potential prebiotics, especially those which contain some lactose, can beneficially influence cecal microbial populations in young chicks, particularly elevated levels of bifidobacteria and lactobacilli and decreased levels of *E. coli*, even when given to the chick for a period of only 3 or 7 d. The final study evaluated the prebiotic potential and the effects of short-term feeding (3, 7, or 21 d) of Elusieve fiber (higher fiber fraction obtained from DDGS), Temulose products (high mannan by-products from the lumber industry), and Alternan (oligosaccharides produced from alternansucrase catalysis) in a corn-soybean meal and casein-dextrose diet. Temulose and partially hydrolyzed Temulose had no consistent effects on weight gain, feed intake, or feed efficiency over all time periods. The addition of partially hydrolyzed Temulose for 3 d did increase populations of bifidobacteria and lactobacilli ($P < 0.10$) when compared to the original, nonhydrolyzed Temulose; however, at 7 d both compounds decreased lactobacilli populations. Diets containing Elusieve fiber and Alternan also had no consistent effects on growth performance when added to a corn-soybean meal diet or casein-dextrose diet. Bifidobacteria were decreased ($P < 0.10$) when 2% Alternan was added to both types of diets. Overall, this study also indicated that short-term feeding of various potential prebiotics can beneficially influence cecal microbial populations in young chicks even when given for a period of only 3 or 7 d.

Dedication

This thesis is dedicated to my husband, Derek, and my wonderful son, Parker. Without your support and love, I could not have accomplished many of the things I have. I love both of you.

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Chapter I

Introduction and Literature Review

After the discovery that low concentrations of antibiotics included in livestock diets had a beneficial effect on production efficiency (Jukes et al., 1950; Moore et al., 1946), the use of antibiotic growth promoters (AGPs) became a common practice in the United States and other countries around the world, benefiting both the livestock industry and consumers. However, since the widespread usage of AGPs began, there has been increased concern about the development of antibiotic resistance. The methods that are used in feeding antibiotics to livestock, such as sub-therapeutic dosage, mass treatment during disease outbreaks, and long-term administration, are proposed to elevate the risk for selection of antibiotic-resistant microbes (Mateu and Martin, 2001; Witte, 1998), and this resistance could possibly be transferred to human pathogens (Casewell et al., 2003). Recent consumer concerns about this transmission of antibiotic resistance and political pressures have resulted in the precautionary prohibition of adding AGPs to livestock diets in the European Union. In the United States, there has been very little regulatory action taken regarding the use of AGPs (Dibner and Richards, 2005), but since the ban in the European Union, this practice has come under intense scrutiny. The latter has led to a new urgency in the search for possible alternatives, such as new feed additives or modifications.

Regardless of why bacteria become resistant to antibiotics or whether the use of AGPs does, indeed, increase development of resistance, alternative dietary strategies need to be found that can maintain the level of uniform performance seen when using low levels of antibiotics, while limiting the outbreak of disease. In order to understand how these new strategies may be

potential alternatives to AGPs, the role of antibiotics and microbiota within the chicken's gastrointestinal tract needs to be examined.

Antibiotic Growth Promoters

Antibiotics exert a number of therapeutic effects on the gastrointestinal tract (GIT) of animals, with the majority of these being associated with the microbial population established within. To prevent disease outbreaks and promote growth, low, sub-therapeutic concentrations of antibiotics are often added to the diets of livestock. The precise mechanisms of how antibiotics promote growth are not fully understood, but the main effects are focused around the microbiota within the GIT (Gaskins et al., 2002), since some of these antibiotics are not absorbed and early studies demonstrated that oral antibiotics do not promote the growth of germ-free animals (Coates et al., 1955; 1963). It has been proven that microbiota do provide real benefits to the monogastric animal such as production of B vitamins and protection from pathogenic bacteria through competitive exclusion, but it is often forgotten that these benefits come at a cost. Bedford (2000), Dibner and Richards (2005), and Niewold (2007) proposed that microbiota within the GIT exert their negative effects on animal performance by:

- competing with the host animal for nutrients
- producing microbial metabolites that suppress growth and increase gut epithelial cell turnover
- inducing an ongoing immune response which causes a reduction in appetite and an increase in muscle catabolism
- stimulating inflammatory cells within the GIT
- causing disease
- and decreasing the ability of the intestine to absorb nutrients.

To reduce the competition for nutrients, AGPs are added to prevent excessive growth of pathogenic microbiota; thus, reducing the overall microbial load within the GIT. The best demonstration of this is in the observation that oral antibiotics administered to germ-free animals do not elicit enhanced growth (Coates et al., 1963), while inoculating germ-free animals with GIT bacteria depresses growth (Coates, 1980). This was also shown in a study performed by Muramatsu et al. (1994) that compared the microbial influence on apparent metabolizable energy intake and body weight in germ-free and conventional chicks. They found that when conventional chicks and germ-free chicks were fed a semi-purified diet containing no AGPs, the conventional chicks had a lower body weight (100 vs. 105 g) and had higher ($P < 0.01$) energy utilization from the diet (12.4 vs. 11.5 kJ/kg).

In addition to competition, when pathogenic strains of bacteria increase beyond acceptable concentrations, they can begin to produce toxic compounds that can stimulate an immune response within the host. For example, *Campylobacter jejuni* and *Escherichia coli* have the ability to produce bacterial toxins called cytolethal distending toxins, that result in swelling and eventual cell destruction of cultured mammalian cells (Elwell and Dreyfus, 2000; Lara-Tejero and Galan, 2000). Some pathogens also can control nuclear factor – kappa B (NF- κ B) activation which is a major contributor to the gut inflammatory response (Karin and Ben-Neriah, 2000). Once this immune response is initiated, macrophages and epithelial cells release proinflammatory cytokines and anti-inflammatory cytokines. Upon release of these cytokines, nutrients and energy are shifted away from the growth of the animal towards production of acute phase proteins (Gruys et al., 2006). It has also been shown that a high concentration of ammonia, which is produced from amino acid deamination and urea hydrolysis, depresses growth due to disturbances in the mucosa cycle and increased gut epithelial cell turnover (Pond and Yen, 1987;

Veldman and Aar, 1997; Visek, 1978). This high rate of cell turnover is accompanied by an increased rate of metabolism and protein synthesis within the gut, which can account for 23 to 36% of the whole body expenditure (Cant et al., 1996; Summers, 1991). Also, with an ongoing immune response, there can be a reduction in appetite, which leads to a further breakdown of muscle tissue to provide a supply of energy and amino acids to the animal since these nutrients are not being ingested (Biggs, 2006).

Thus, the primary reason that AGPs are included in livestock diets is to reduce pathogen carriage and inhibit subsequent subclinical infections, thus reducing the metabolic costs of immune system activation (Niewold, 2007). For example, when *Clostridium perfringens*, a bacterium commonly found in the GIT of poultry, grows excessively, a disease called necrotic enteritis can result. The increased growth of this organism leads to toxin production, which, in turn, results in gut mucosal lesions (Parish, 1961). If untreated, the disease leads to intestinal inflammation, diarrhea, increased mortality, growth depression, and liver condemnation at slaughter (Craven, 2000). It can also be associated with other diseases such as gas edema disease, gizzard erosions, and gangrenous dermatitis (Craven, 2000), and manifests into secondary infections during times of coccidiosis infection. Outbreaks of necrotic enteritis and growth of *Clostridium perfringens* are minimized when low concentrations of AGPs are included in the diet, which, in turn, results in unhindered growth of the animal (Collier et al., 2003; George et al., 1982).

Digestion and absorption of nutrients within the GIT also are affected by several physicochemical conditions, including viscosity and pH of the chyme, and these factors may affect the intestinal microbiota and the need for AGPs. Increased intestinal viscosity can contribute to decreased nutrient absorption by the intestine (Jeurissen et al., 2002). When

intraluminal viscosity is increased, the thickness of the unstirred water layer covering the mucosa cells increases, thus reducing the rate of nutrient absorption (Johnson and Gee, 1981). Increased digesta viscosity also may interfere with digestion and absorption of nutrients by decreasing their interaction with pancreatic enzymes and bile acids (Edwards et al., 1988), as well as the movement of nutrients towards the mucosal surface (Fengler and Marquardt, 1988). The reduced mixing and passage rate decreases luminal oxygenation, which allows for a more favorable environment for the increased growth of microbial populations due to an increased residence time (Bedford, 1996).

Due to the increased viscosity and reduced rate of nutrient absorption, more starch and protein reach the hindgut where they can be fermented and provide energy for microbiota that would normally not have access to them. Increasing the flow of these fermentable substrates provides more opportunities for an increase in pathogenic bacteria. One particular bacterium that thrives in protein-rich environments is *Clostridium perfringens*. The increase in this proteolytic bacterium, as well as the general microbial population, stimulates an increase in mucus production by goblet cells within the GIT. This changing environment can select for mucolytic bacteria and thereby serve as an initiation step in the development of necrotic enteritis (Collier et al., 2003; Jeurissen et al., 2002). This proinflammatory-mediated increase in mucus production combined with an increase in fluid secretion and rate of passage, which characterizes intestinal inflammatory responses, could select for fast-growing pathogenic bacteria (Collier et al., 2003).

While digesta viscosity is important, it is not the only factor responsible for differences in nutrient digestibility. The pH of the GIT decreases as the chyme passes from the crop into the proventriculus and gizzard and then becomes less acidic as the digesta progresses down the length of the small intestine (Jeurissen et al., 2002). This variation in pH may be important in

terms of nutrient absorption and gut barrier functions, and also in relation to mineral absorption. Langhout (1998) demonstrated the former when he reported increases in broiler performance and nutrient digestibility when there was a reduction in gastric pH, due to an increased retention time of feed in the gizzard. In addition to the beneficial effect of reduced gastric pH on nutrient digestibility, a reduced pH may also have an antimicrobial effect and limit the growth of microbes in the GIT, particularly pathogenic species.

In order to compensate for the decrease in the ability to absorb nutrients and the increased competition for nutrients with the ever-growing microbial populations, the body increases its production of digestive enzymes, which causes the pancreas to hypertrophy and the intestine to lengthen and increase in weight (Bedford, 2000). Maintaining intestinal health is dependent on the optimal balance between proliferation, maturation, and apoptosis of epithelial cells (Jeurissen et al., 2002); thus, as the intestine increases in length, the rate of enterocyte migration from the intestinal crypts to the villus tip is increased, and the enterocytes are required to become active before they have reached maturity (Silva and Smithard, 1996). The growth of the intestine actually limits enterocyte absorption of nutrients due to the lack of sufficient enzyme activity (Bedford, 2000) and more energy must be diverted away from growth to the replacement of epithelial cells (Tannock, 1997). Also, when the inflammatory response is initiated due to the increasing pathogenic microbial populations, an accumulation of inflammatory cells occurs within the mucosa, leading to a thicker intestinal wall (Niewold, 2007). The addition of AGPs can lead to a reduction in gut weight, including thinner intestinal villi and total gut wall (Coates et al., 1955), which may lead to enhanced nutrient digestibility.

The use of AGPs affects the body in many beneficial ways and improves the growth and efficiency of feed utilization by the host animal. The increased public and political pressure to

remove AGPs from the diets of livestock creates many new challenges for producers and researchers. Producers will ultimately have to decide upon an alternative(s) that fits well with their nutritional and management practices already in place or that alternative might not be economically viable. Alternatives also need to be found that can maintain the level of uniform performance seen when using low-level antibiotics, while limiting the outbreak of disease. Two particular alternatives are compounds which exhibit prebiotic-type effects and feeding whole or large particle grains. Prebiotic-type products such as oligosaccharides may limit the growth of potentially pathogenic bacteria within the GIT while promoting the growth of beneficial bacteria. The addition of larger grain particles sizes to the diet of broiler chickens may increase gizzard size and increase the digestibility of the diet, thus limiting the presence of excess nutrients reaching the hindgut, and, in turn, limit the growth of pathogenic bacteria. Increased gizzard size and increased retention time of feed in the gizzard may also reduce the amount of viable pathogenic bacteria present in the digesta.

Prebiotics

Introduction

One of the most important aspects to consider when removing AGPs from the diet of livestock is how the microbial populations within the GIT will be controlled since one of the main modes of action of AGPs is to inhibit subclinical infections. Enteric diseases caused by pathogenic bacteria lead to lost productivity, increased morbidity and mortality, and microbial contamination of the poultry products that are intended for human consumption. Numerous studies have shown that commensal microbiota possess the ability to inhibit pathogens, mainly through competitive exclusion. Certain changes in the intestinal environment can increase the host's risk to developing infection, and prebiotics can contribute to increased resistance to

infection (Rolfe, 2000). However, in order to understand how prebiotics exert their effects on the bacterial populations within the GIT, a closer inspection of the environment within the intestinal tract needs to be initiated.

Bacteria

Microbes have been around since the beginning of the earth and they can be found almost everywhere. They are found throughout the entire gastrointestinal tract of the chicken, from the mouth to the cloaca. The exact composition of this microbiota is hard to define, but it is assumed that hundreds of species colonize the GIT, with the majority being anaerobic bacteria (Zhu et al., 2002). They can be found attached to epithelial surfaces, deep within the crypts of the mucosa, and within the lumen attached to or freely floating among the digesta (Jeurissen et al., 2002). Initial inoculation of the GIT with beneficial bacteria from the environment is very important, since the chick's digestive tract is sterile before hatching. There is no limit on the growth of the first bacteria to enter at this early stage and, hopefully, beneficial bacteria will be the first to colonize rapidly to prevent the establishment of harmful microbial species. The populations that are present vary both qualitatively and quantitatively from one region to another and are influenced by changes in available substrates, temperature, and pH.

First, the crop is essentially a storage organ that regulates the supply of food to the proventriculus and gizzard. Few types of bacteria are present in this area, but the predominant organisms are lactobacilli, especially *Lactobacillus salivarius*, and streptococci (Hinton et al., 2000; Huyghbaert, 2003; Sarra et al., 1985). These organisms adhere to the epithelial surface where they form an almost complete layer (Vispo and Karasov, 1997). Through both bacteriostatic and bacteriocidal effects, mainly the production of lactic acid which lowers the pH to 5.0, the lactobacilli are capable of controlling populations of *Escherichia coli* and *Clostridium*

perfringens, while inhibiting the growth of *Bacteroides* spp. (Fuller, 1977). Some starch digestion occurs in the crop through bacterial degradation (Champ et al., 1981), while some sucrose degradation apparently occurs by endogenous enzymes (Pritchard, 1972). However, due to the acidic pH, extensive digestion of starch and sucrose cannot occur here.

The proventriculus and gizzard present a relatively inhospitable environment for bacteria due to the very acidic pH values ranging from 1 to 5, and any microbial survival within these regions is due to acid tolerance (Mead, 1997); therefore, feed residues leaving the gizzard are significantly depopulated of bacteria. However, some species of lactobacilli are able to maintain populations of up to 10^8 per gram of contents (Smith, 1965). These bacteria are thought to be able to survive here because they attach themselves to the mucous layer which can somewhat shelter them from the ambient pH, and because microbial ureases can produce ammonia which counteracts the acidity in the surrounding microenvironment. Also, uneven mixing of the digesta allows areas of high pH to persist (Karasov and Hume, 1995) where microbes may be able to survive.

The duodenum is another site where bacterial populations tend to be limited. Due to the rapid rate of food passage and, thus, the short residence of time of digesta, and the more acidic pH of digesta leaving the proventriculus and gizzard, the lumen of the duodenum is an unsuitable environment for the colonization of microbes (Vispo and Karasov, 1997). The aerobic and anaerobic bacteria counts from the jejunum and ileum of the chicken are very similar, with *Bacteriodaceae*, *Staphylococcus*, *Streptococcus*, *Lactobacillus* spp., and *Escherichia coli* dominating (Lu et al., 2003). Some obligate anaerobes also are present and these include *Eubacterium*, *Propionibacterium*, *Clostridium*, and *Fusobacterium* spp. Strict anaerobes comprise approximately 9 to 39% of the total number of bacteria present, and there is great

diversity among them in the proximal small intestine (Salanitro et al., 1978). It is here, since it is the major site of nutrient absorption, that pathogenic bacteria can have their greatest effect on growth depression by producing toxic compounds, competing with the host for utilization of essential nutrients, and suppressing organisms that synthesize vitamins or other host growth factors (Mead, 1997).

The ceca of adult chickens provide a relatively stable environment for microorganisms, so they contain a larger and more diverse bacterial population than other parts of the GIT. It has been found that young broiler chickens less than 2 weeks of age lack this established anaerobic environment of older chickens, but this stable environment occurs once facultative anaerobes have become established (Salanitro et al., 1978). After this period of 2 weeks, the predominant organisms are obligate anaerobic bacteria that occur in the lumen at concentrations of 10^{10} to 10^{11} per gram of wet weight (Vispo and Karasov, 1997). Barnes et al. (1972; 1979) isolated several hundred bacterial strains from cecal samples and found that *Peptostreptococcus*, *Coprococcus*, and *Streptococcus* spp. accounted for approximately 28% of the total anaerobes, while *Eubacterium* spp. accounted for 16%. *Clostridium* spp. occurred at concentrations of 10^9 per gram or higher and accounted for 5% of the total anaerobes isolated, while *Bifidobacterium* spp., a beneficial bacterium, accounted for approximately 9% (Barnes et al., 1972, 1979).

Although facultative anaerobes only predominate in the ceca during the first 2 weeks after hatching (Mead and Adams, 1975), these bacteria are consistently present throughout the life of the bird. For example, Barnes et al. (1972) isolated coliforms (mainly *Escherichia coli*), and lactobacilli at $10^6 - 10^8$ per gram and enterococci at $10^5 - 10^7$ per gram.

The majority of the predominant anaerobes present in the ceca are saccharolytic, or able to ferment glucose, and many of them also have the ability to ferment lactose, which is of interest

because the bird itself lacks lactase activity (Mead, 1989). Since diet plays an important role in determining the composition of the gut microbiota, incorporating feed ingredients such as prebiotics, mainly in the form of nondigestible carbohydrates, into the diet can possibly attain and maintain a desirable microbial balance in the absence of AGPs.

Gibson and Roberfroid (1995) defined a prebiotic as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance. Normally, prebiotics contain some type of a non-digestible carbohydrate fraction (Rehman et al., 2009). Although some carbohydrates can reach the hindgut without being digested, most of them are not selective in the bacterial populations that they affect (Wang and Gibson, 1993); thus, do not meet the proposed definition. However, selective stimulation occurs with certain types of oligosaccharides because they are readily fermented by what is considered to be beneficial types of bacteria, mainly lactobacilli and bifidobacteria, and are not used as effectively by potentially pathogenic bacterial species. Bacteria which have a preferential food substrate readily available have a proliferative advantage over other bacteria. One such group of oligosaccharides, fructooligosaccharides (FOS), are the most common prebiotic studied and they have been shown to positively affect the growth of selective bacteria.

Fructooligosaccharides

The general term 'FOS' includes all non-digestible oligosaccharides composed of fructose and glucose units, but specifically FOS refers to short chains of fructose units bound by β (2-1) linkages attached to a terminal glucose unit (Swanson and Fahey, 2002). The predominant FOS that have been studied are inulin, oligofructose, and short-chain

fructooligosaccharides (scFOS). Generally, FOS range in length from 2 to 60 fructose units with or without a glucose molecule residing at the end of each fructose chain (Flickinger et al., 2003).

Due to the β (2-1) linkages, these compounds are resistant to hydrolytic digestion in the upper part of the intestinal tract of monogastric animals (Fishbein et al., 1988), making them highly digestible substrates for fermentation by cecal microbes, and any caloric contribution to the host is obtained from lactate and short-chain fatty acids (SCFA) produced by these microbes (Ellegard et al., 1997). Lactate, which is produced from saccharolytic bacteria such as lactobacilli and bifidobacteria, decreases the pH in the lumen, thus creating an unfavorable environment for the growth of pathogenic organisms that are not acid tolerant such as *Salmonella* spp., *E. coli*, or *Clostridium perfringens*. The main SCFAs that are produced from bacterial fermentation are acetate, propionate, and butyrate, and they can provide several benefits to the host animal.

FOS have been postulated to enhance growth responses in livestock. Ammerman et al. (1988) investigated the growth response of broiler chickens to diets containing 2.5 or 5.0 g/kg supplemental oligofructose when fed for 46 days. The addition of oligofructose did not result in a significant increase in body weights, but both concentrations of oligofructose improved feed efficiency compared to the control containing no oligofructose. In a second study performed by Ammerman et al. (1989), higher concentrations of oligofructose (3.75 or 7.5 g/kg) were included in the diet to see if there were similar effects on growth performance. The authors found that at 3.75 g/kg oligofructose, broilers had increased weight gain, increased hot and chilled carcass weights, and a higher percentage of breast meat. For 7.5 g/kg oligofructose, there was also increased weight gain but none of the other effects were observed. Wu et al. (1999) also observed beneficial effects on body weight gain and feed efficiency when 2.5 or 5.0 g/kg FOS

were included in the diet. In a study performed by Xu et al. (2003), the authors found that the addition of 4.0 g/kg FOS significantly increased average daily weight gain of broilers, while diets containing 2.0 or 8.0 g/kg FOS had no significant effect. Feed to gain ratios were also decreased in the chicks fed 2.0 and 4.0 g/kg FOS, while there was no improvement when 8.0 g/kg FOS was supplemented. Other authors, however, have reported no effects of FOS on growth in poultry (Stanczuk et al., 2005; Waldroup et al., 1993), which indicates that the effects on growth performance may not be consistent.

The primary benefit of FOS, however, is that they selectively stimulate indigenous populations of bifidobacteria and lactobacilli, which are considered beneficial species (Yang et al., 2009), usually at the expense of *E. coli* and *Salmonella* species. Bifidobacteria and lactobacilli are selectively enhanced because of their ability to ferment FOS due to the presence of β -fructosidase (Swanson and Fahey, 2002). These results can be achieved with very low dosages in several species. For example, Niness (1999) reported that oligofructose intakes ranging from 5 to 20 g per day in humans resulted in altered colonic microbiota, especially bifidobacteria populations. In a study conducted with male beagles fed 3, 6, or 9 g/kg supplemental oligofructose for 18 days, the dogs fed the highest concentration of oligofructose tended ($P < 0.10$) to have higher numbers of bifidobacteria when compared to the negative control group (Flickinger et al., 2002). This was also shown in a study conducted with rats where higher doses of 6% oligofructose or scFOS included in the diet showed an increase in bifidobacteria populations (Campbell et al., 1997). Sparkes et al. (1998) were one of the first to examine the effects of FOS in cats. When cats were fed a diet containing 7.5 g/kg FOS, increased lactobacilli and bacteroides concentrations were seen compared to the control group, and *Clostridium perfringens* and *E. coli* concentrations tended to decrease. In broiler chickens fed

diets containing 0, 2, 4, or 8 g/kg FOS up to 49 days, the cecal concentrations of bifidobacteria were increased when birds were fed 4 g/kg FOS, and concentrations of lactobacilli were increased, whereas concentrations of *E. coli* were decreased when birds were fed 2 or 4 g/kg FOS compared with the control (Xu et al., 2003). There was no effect observed on cecal microbes when 8 g/kg FOS were fed.

Mannanoligosaccharides

Mannanoligosaccharides (MOS) are another type of oligosaccharide that may positively influence gut microbial populations and immune function. These compounds are special when compared to other oligosaccharides because of their proposed mode of action when influencing microbial populations in the GIT. Mannose, the main component of MOS, is a unique sugar because many enteric bacteria have receptors that bind to it (Griggs and Jacob, 2005). These receptors, called Type-1 fimbriae, are involved in the attachment of bacteria to host cells, and this attachment is critical for pathogens if they want to establish colonization in the host. Mannanoligosaccharides contain a high affinity ligand for bacteria and offer a competitive binding site, so pathogens, which possess these fimbriae, attach to the MOS instead of attaching to the intestinal wall and, therefore, move through the intestine without colonization (Newman, 1994). *Escherichia coli*, *Salmonella* spp. and clostridia are a few types of pathogenic bacteria that exhibit this mannan-binding behavior. In several experiments conducted with broiler chickens, it was determined that supplemental MOS increased lactobacilli and bifidobacteria populations in the ceca compared to diets containing no antibiotic growth promoters (Baurhoo et al., 2007; Fernandez et al., 2002). Mannnanoligosaccharide supplementation of turkey poults increased the concentration of bifidobacteria, while *Clostridium* spp. were decreased from 4.22

log₁₀ CFU/g in the control to 2.98 log₁₀ CFU/g in the MOS supplemented diet (Finucane et al., 1999).

Mannanoligosaccharides may also have an effect on growth performance, along with the intestinal changes discussed above. Based on a meta-analysis from 1993-2003 of 44 broiler pen research trials where MOS was fed as a supplement, Hooge (2004a) concluded that birds fed MOS (ranged from 0.05 to 0.3% of the diet) showed improved growth performance and feed conversion ratios in 79.5% of cases compared with those fed diets containing no antibiotic growth promoters. In a similar meta-analysis conducted by the same author, turkeys fed MOS as a supplement showed increased body weight in 70.4% of cases and reduced mortality in 56.3% of cases (Hooge, 2004b). In contrast, studies by Stanczuk et al. (2005) and Waldroup et al. (2003) reported no significant changes in body weight gain or feed conversion when MOS was added to the diet of either turkeys or broilers, respectively.

Glucooligosaccharides and Galactooligosaccharides

Two other types of oligosaccharides with prebiotic activity are glucooligosaccharides and galactooligosaccharides. These compounds have been studied very little in livestock.

Glucooligosaccharides are synthesized from the transfer of glucose molecules from sucrose to maltose by the enzymatic action of glucosyltransferase, which is derived from *Leuconostoc mesenteroides* (Vallete et al., 1993). Galactooligosaccharides are typically generated from lactose by the enzymatic action of β -galactosidase, an enzyme synthesized by *Aspergillus oryzae* (Kan et al., 1989). The concentrations of SCFAs produced by fermentation of these compounds are similar to that of FOS.

Glucooligosaccharides and galactooligosaccharides positively affect beneficial types of intestinal bacteria, including bifidobacteria and lactobacilli. *In vitro* and *in vivo* studies

performed by Djouzi et al. (1995) with gnotobiotic rats demonstrated that glucooligosaccharides served as a substrate for enhanced growth of bacteroides, bifidobacteria, and *C. butyricum* strains. Transgalactooligosaccharides (TOS) have been patented in Japan and are marketed as bifidogenic agents for humans. *In vivo* experiments utilizing rats inoculated with human microbiota and fed 5% TOS resulted in a significant increase in total cecal anaerobes including lactobacilli and bifidobacteria (Rowland and Tanaka, 1993). Smiricky-Tjardes et al. (2003) obtained similar results when 6% TOS was fed to pigs.

Lactose

A final potential prebiotic-type compound for poultry is lactose. Lactose cannot be considered a “true” prebiotic by definition; however, lactose fits within the prebiotic concept for poultry because birds cannot digest it because of their lack of lactase activity (Denbow, 2000); therefore, it becomes an available substrate for microflora in the hindgut. Initially, the interest in the prebiotic effect of lactose was focused on reducing *Salmonella* colonization in the bird (Corrier et al., 1990; Hinton et al., 1990); however, more recent research has evaluated its potential to improve growth rate and efficiency. Douglas et al. (2003) demonstrated that inclusion of 2 or 4% galactose or lactose to the diet increased weight gain from 0 to 21 days post-hatch in broiler chicks. Simoyi et al. (2006) reported that when turkeys were fed diets containing lactose levels from 0 to 8%, body weight gain significantly improved after a six-week feeding period, with the optimal response at the 2% inclusion level. McReynolds et al. (2007) evaluated the effects of dietary lactose levels ranging from 0 to 4.5% on the control of necrotic enteritis in broiler chicks. All of the control birds (100%) challenged with *C. perfringens* had intestinal lesions compared to only 30% of the birds fed 2.5% lactose.

The use of prebiotics to modulate microbial populations, as well as overall gut health, has been studied exclusively in human and companion animal nutrition. The use of these compounds in livestock has gained increased interest because of their potential prebiotic properties and their potential to maintain gut health and animal performance in the absence of AGPs. More research needs to be performed, however, to fully understand how these additives may improve gut health and maintain performance.

Whole or Large Particle Grains

The health aspect of consuming whole grains in humans has long been understood, while the effects in livestock species, in particular poultry, are still unclear and rather variable. Initially, countries such as Europe, Australia, and Canada included whole grains in poultry diets as a way to reduce feed costs. By including locally grown grains, producers could reduce or eliminate the costs of shipping and grinding the ingredients (Cumming, 1994). Besides the reduction in feed costs, it has also been hypothesized that feeding whole grains or larger particle sizes may improve the health of the bird, as well as strongly influence gizzard size and function (Amerah et al., 2007; 2008; Gabriel et al., 2006). The majority of the research on feeding whole grains has been conducted in Europe. The diets that generally are used in these countries are based on wheat, sorghum, barley, and oats. Wheat is the most commonly consumed, comprising 66-75% of the total (Slavin, 2004). However, poultry diets in the US are primarily based on corn; so by comparison, feeding large particle size corn may produce benefits similar to the effects seen in whole grain feeding (Parsons et al., 2006).

Engberg et al. (2004) investigated the effects on broiler performance and microbial composition when broilers were fed increasing concentrations of whole wheat as they aged. From 15 to 21 d of age, the birds were fed 10% whole wheat; from 22 to 28 d, 25% whole wheat

was included; and from 29 to 42 d of age, the birds were fed 30% whole wheat in a wheat-soybean meal based diet. At 28 d, the birds fed the whole wheat had higher body weights when compared to birds fed no whole wheat, but this effect was not observed at 42 d. Over the 42 d experiment, feed conversion improved from 1.74 g/kg in the negative control diet to 1.64 g/kg in the whole wheat diet. The authors found no differences in *C. perfringens*, coliform bacteria, or anaerobic bacteria throughout the GIT; however, there was an increase in lactobacilli populations (8.28 vs. 7.53 log₁₀ CFU/g) in the distal small intestine when compared to the negative control diet. When a higher inclusion rate of 40% whole wheat was evaluated by Gabriel et al. (2003a), the authors found that weight gain was increased when whole wheat was fed; however, no effect on feed efficiency was seen. The authors also noted an increase in gizzard weight and a lower pH of the gizzard contents in the birds fed whole wheat. Taylor and Jones (2004) conducted two trials to investigate the effects of either 20% whole wheat or 20% whole barley on performance characteristics in broilers. No difference was observed in the growth of the birds fed either whole grain when compared to the control. There was a significant improvement in feed conversion from 22 to 42 d, but this result was not seen for the entire experiment. The authors also noted an increase in gizzard weight and a lower pH of the gizzard contents in the birds fed whole wheat.

Parsons et al. (2006) evaluated the effects of fine (781 µm), small (950 µm), medium (1,042 µm), and large (1,109 µm) ground corn particle sizes on 3 to 6 week old broiler performance. The authors found that feeding larger particle corn significantly reduced feed efficiency when compared to broilers fed any of the other corn sizes. Also, gizzard weight as a percent of live body weight increased ($P = 0.0001$) as dietary corn particle size increased. Similarly, Healy (1992) found gizzard weights increased from 20.9 g/kg of body weight when chicks were consuming 300 µm ground corn to 24.4 g/kg of body weight when chicks were

consuming 900 μm ground corn. Lott et al. (1992) and Douglas et al. (1990) reported that feeding larger ground corn sizes significantly reduced body weight and feed efficiency when compared to finer ground corn. In contrast, Nir et al. (1994) reported that at 21 d of age, chicks fed medium or coarse ground corn sizes were heavier and exhibited better feed efficiency than those fed a finer diet. So, overall, feeding larger particle corn has no consistent effects on either weight gain or feed efficiency across all experiments.

To maximize the possible effects on intestinal health and gizzard size and function that may occur from feeding larger particle sizes, it may be beneficial to expose chicks to diets containing large particle sizes as early as possible. If gizzard size and activity level are increased at a younger age, this may help to improve the digestibility of nutrients and inactivate potential microbial pathogens. Nir et al. (1994) found that when day-old chicks were fed coarse and medium corn particles, gizzard weight at 21 days increased by 26 to 41% when compared to chicks fed fine particles. Also, because nutrient digestion in chicks is suboptimal until about 14 days of age (Batal and Parsons, 2002; Noy and Skylan, 1999), improving nutrient digestion during the first few weeks of life may be an important step to improving weight gain potential to market weight. With larger particle sizes, there is increased retention time of feed in the gizzard and this can lead to increased enzymatic digestion and starch digestibility (Hetland et al., 2002). Since the gizzard is a major regulator of intestinal motility and enzymatic secretion, Nir et al. (1995) suggested that larger particles are also better suited to the intestinal tract because they stimulate gizzard size and peristalsis more than smaller particles do. Digestion of larger particle sizes is slower within the gizzard and small intestine; thus, feed passage rate is slowed, resulting in more reflux of intestinal contents and increased exposure time of nutrients to digestive enzymes, which in turn, may improve energy and nutrient utilization (Nir et al., 1995).

Diet plays a very important role in determining the composition of the indigenous gut microbiota and their effect on the host animal (Swanson and Fahey, 2002). Incorporating larger particle sizes into the diet may also have an indirect effect on reducing enteric disease problems in poultry by altering the microbial populations within the gastrointestinal tract. More specifically, there needs to be an increase in the concentrations of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, and/or a decrease in the concentrations of pathogenic bacteria, such as *Clostridium perfringens* and *E. coli*. The stimulation of gizzard development through increased grinding activity leads to an improvement in gut motility (Ferket, 2000), and increases the secretion of hydrochloric acid into the gizzard and intestine, ultimately reducing pH. This, in combination with the increased retention time of feed in the gizzard, may have an antimicrobial effect because pathogenic bacteria entering the gastrointestinal tract via the feed have a greater chance of being inactivated by the highly acidic environment (Engberg et al., 2002; Naughton and Jensen, 2001). Therefore, the gizzard can be regarded as a barrier in preventing pathogenic bacteria from entering the distal intestinal tract (Engberg et al., 2004). Overall, including larger particle sizes of grains in broiler chicken diets may provide a method to potentially improve intestinal health, particularly when antibiotic growth promoters are not included in the diet.

Coccidiosis Infection in Poultry

One of the most common pathogenic organisms entering the GIT of poultry are coccidia. In chickens, this organism can produce a disease ranging from a light infection with no adverse effects on health or growth performance to a more severe clinical infection which causes high incidences of mortality and morbidity (Williams, 1999). Thus, coccidiosis is a very important disease in poultry and is recognized as the parasitic disease that has the greatest economic impact

on the poultry producer (Allen and Fetterer, 2002). The U.S. broiler industry is estimated to lose \$350 million annually from coccidial infections in floor reared poultry, while losses worldwide are estimated to be greater than \$1 billion (Jenkins et al., 2010). These estimates included the costs of prophylactic medication for broilers and broiler-breeders, alternative treatments if the medications fail, and losses due to mortality, morbidity, and poor growth and feed conversion of the birds (Allen and Fetterer, 2002).

Seven species of *Eimeria* (*E. acervulina*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. mitis*, *E. brunetti*, and *E. praecox*) have been recognized to infect chickens (Shirley, 1986), and the disease is generally characterized by diarrhea, enteritis, intestinal tissue damage, and general unthriftiness of the bird. The epithelial tissue damage present can also interfere with food digestion and nutrient absorption and, ultimately, growth rate. For example, Larbier et al. (1974) showed that metabolizable energy in chicks which were inoculated with 1.5×10^6 *E. acervulina* oocysts decreased in infected chicks in comparison to non-infected chicks (2,260 kcal/kg vs. 3,060 kcal/kg). Persia et al. (2006) also showed that an acute infection of chicks with *E. acervulina* oocysts (5×10^5) resulted in large and significant ($P < 0.05$) reductions in metabolizable energy and amino acid digestibility in comparison to sham inoculated birds. Coccidial infections in poultry have also been shown to decrease growth by 15 to 20 % in mild infections and as much as 30 to 40% in more severe infections (Willis and Baker, 1981).

In addition to decreased nutrient absorption, intestinal tissue damage can expose the bird to bacterial infections, like *Clostridium* and *Salmonella*. Indeed, necrotic enteritis, an intestinal disease caused by *C. perfringens*, often occurs as the most common secondary infection due to coccidiosis. The presence of this bacterium in poultry constitutes a risk for human health as *C.*

perfringens is one of the most frequently isolated bacterial pathogens in foodborne disease outbreaks (Buzby and Roberts, 1997).

Previous limited research has indicated that the type of dietary ingredients may have an effect on chick responses to a coccidial infection. Cumming (1987) reported that feeding chicks whole grains decreased oocyst output and lowered mortality when chickens were infected with *E. tenella*, *E. acervulia*, and *E. maxima*. However, several other studies have reported either detrimental effects (Banfield et al., 1999; 2002; Gabriel et al., 2003b) or no effects of whole grain feeding (Banfield and Forbes, 2001; Waldenstedt et al., 1998) on coccidial infection. These contrasting results may be due to differences in parasite dose, age of introduction of whole grain, quantity and type of whole grain, and age of inoculation.

Omega-3 fatty acids have also been shown to be effective against coccidiosis infections. In a series of experiments testing different sources of omega-3 fatty acids during an *E. tenella* infection (Allen et al., 1997; 1996a,b), broilers were fed diets supplemented with various concentrations of fish oil, flaxseed oil, or flaxseed from 1- 3 weeks of age. Lesion scores were reduced by both 5 and 10% fish oil and 10% flaxseed oil. In another experiment (Allen et al., 1998), supplementation of fish oils as low as 2.5% reduced lesion scores by 30%. In this series of experiments, there were no effects on weight gain. The feeding of 8 ppm of γ -tocopherol, which is found in seed oils such as flax, wheat, corn, and soybeans also significantly improved chick weight gain and reduced lesions scores and oocyst output during an *E. maxima* infection, but had no effect when chicks were infected with *E. tenella* (Allen et al., 1998). Persia et al. (2006) also observed improved performance when 10% fish meal, which is high in omega-3 fatty acids, was added to diets of chicks infected with *E. acervulina*.

Although the effects of antibiotics or anticoccidials on coccidiosis have been studied extensively, there is still little or no knowledge on the effects of prebiotics or whole or large particle grains on coccidiosis when antibiotics are not included in the diet. Therefore, with the fear of increasing microbial resistance and governmental regulations, it is important to better understand the influence these dietary ingredients may have on the severity of coccidial infection in growing chicks. Feeding of whole or large particle gains may ameliorate the effects of a coccidiosis challenge by increasing the size and activity of the gizzard, thus increasing the secretion of hydrochloric acid and ultimately reducing pH, which may act as a barrier to the coccidiosis. It is also of interest to determine if certain prebiotic-type products ameliorate any effects on growth performance and nutrient digestibility of birds challenged with *E. acervulina* coccidial infection. These products are of interest because they have shown some positive results on growth, nutrient utilization, and cecal microbial populations of chicks when added to a corn-soybean meal diet. They may have even greater beneficial effects in the presence of a coccidiosis infection by reducing the proliferation of secondary infections of undesirable bacteria such as clostridia.

Conclusions and General Objectives

Recent consumer concerns about the transmission of antibiotic resistance to human pathogens and the complete prohibition of AGP use in Europe has resulted in a new urgency in the search for alternative feed additives which can improve the growth and maintain the health of poultry in the absence of AGPs. Feeding prebiotic-type compounds and whole or large particle grains may be possible alternatives to AGPs. In order for these compounds to be viable alternatives, they will need to improve intestinal health by supporting the growth of beneficial microbial populations (*Lactobacillus* and *Bifidobacterium* spp.), while having no negative effects

on growth or nutrient digestibilities. In addition, if these prebiotic-type compounds can elicit a positive response on gut health and intestinal microbiota even when being fed for short time periods or during a coccidiosis infection, they may give producers an economically viable way to maintain the health of their flock. The overall objective of this thesis is to evaluate the effects of grain particle size and several potential prebiotic feed additives when fed for varying lengths of time on growth performance, nutrient digestibility, and cecal microbial populations in young chicks. In addition, these dietary additives were also evaluated for their ability to ameliorate the negative effects of a coccidiosis infection (*Eimeria acervulina*) on the above response variables.

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Chapter II

The Effects of Large Ground Corn, Whole Sorghum, and Grobiotic® on Growth Performance, Nutrient Digestibility, and Microbial Populations in Chicks Fed Diets With and Without Distillers Dried Grains with Solubles (DDGS)

Abstract

Two experiments were conducted to evaluate the effects of large particle corn, whole sorghum, and Grobiotic (GB) in a corn-soybean meal (SBM) diet with or without the addition of distillers dried grains with solubles (DDGS). The first experiment evaluated diets containing fine ground corn, large ground corn, and 20% whole sorghum with or without the addition of 15% DDGS. The second experiment evaluated diets containing fine ground corn, large ground corn, large ground corn + 5% GB, and fine or large ground corn plus 15% DDGS and 5% GB. Weight gain (Experiment 1) and feed efficiency (Experiment 1 and 2) were decreased ($P < 0.05$) during the first week for chicks fed the diets containing large ground corn or 20% whole sorghum in a corn-SBM diet (no DDGS), but these results did not persist in commercial broiler chicks throughout the remainder of the 21 d experiment. When 15% DDGS was included in the diet this depression in growth was not seen. In the second experiment, weight gain was increased ($P < 0.05$) after the first week for chicks fed diets containing 5% GB and 15% DDGS when compared to their respective controls. In Experiment 1, the diet containing 20% whole sorghum yielded the highest ME_n value ($P < 0.05$) at both age periods when compared to all other treatments. In Experiment 2, all diets containing 5% GB yielded significantly higher ME_n values ($P < 0.05$) at 7 d when compared to diets containing no GB. The effects of diet on AA digestibility were generally small and inconsistent in both experiments. In Experiment 1, chicks fed the large ground corn or whole sorghum had significantly increased ($P < 0.05$) relative gizzard weights

compared to chicks fed the fine ground corn. Also, chicks fed the diets containing DDGS had increased relative gizzard weights ($P < 0.05$) when compared to chicks given diets containing no DDGS. In Experiment 2, no significant differences were detected among any of the dietary treatments for cecal bifidobacteria and lactobacilli; however, there was a significant decrease in cecal *E. coli* when large ground corn, 5% GB, and 15% DDGS was fed. These results indicate that larger particle sizes from large ground corn or whole sorghum, as well as the addition of GB, can be supplemented to a corn-SBM or corn-SBM DDGS diet with no adverse effect on growth performance and nutrient digestibility when fed from 0 to 21 d of age. Also, relative gizzard weights can be significantly increased when large ground corn or sorghum is included in very young chick diets, which may have beneficial effects on intestinal health.

Introduction

As discussed earlier, including whole grains in poultry diets is typically used as a way to reduce feed processing costs. By using whole grains or larger particle sizes, producers can reduce the amount of energy that is needed to grind the ingredients to smaller particle sizes. Recently, it has been hypothesized that feeding whole grains or larger particle sizes may provide benefits other than reduced feed costs, such as improving the health of the bird, strongly influencing gizzard size and function, and indirectly reducing enteric disease problems by altering the microbial populations within the gastrointestinal tract (Amerah et al., 2007; 2008; Gabriel et al., 2006). Typically, many of the diets that have been used are based on wheat, sorghum, barley, and oats. However, poultry diets in the United States are primarily based on corn because of its wide availability; so it may be possible that feeding larger particle size corn can also produce benefits which have been seen with whole grain feeding (Parsons et al., 2006). Sorghum is the

other most commonly used grain in poultry diets in the United States, but little research has been conducted to evaluate feeding whole sorghum to poultry.

Because of its wide availability in the United States and high starch content, corn has also become very desirable for use in ethanol production. In the last decade, ethanol production from corn has seen an exponential increase, resulting in a proportional increase in the production of the corn co-product distiller's dried grain with solubles (DDGS). In the past, DDGS was mainly fed to cattle and was used at only low inclusion levels in poultry and swine diets because of its high fiber content (Shurson, 2002). However, with the substantial increase in DDGS supply and the price of corn increasing, DDGS is now commonly used in poultry diets. Only a limited number of studies have been published concerning the effects of supplementing large particle sizes in a corn-soybean meal diet, and none have been published examining the effects in diets containing DDGS. Therefore, one of the objectives of this study was to evaluate large particle corn and sorghum when included in poultry diets containing DDGS.

In addition to larger particle sizes, incorporating certain prebiotics into the diet may also beneficially alter microbial populations in the gut, as well as possibly improving growth performance. Grobiotic (GB; International Ingredient Corporation, St. Louis, MO) is a prebiotic-type product that contains a proprietary mixture of partially-autolysed yeast, yeast extract, dried whey solubles, and citric acid fermentation solubles. The product provides yeast cell wall and yeast soluble components, lactose, and other fermentable carbohydrates. Previous research conducted by Douglas et al. (2003) reported that when concentrations of 5 or 6% GB were fed to commercial broiler chicks, an improvement in growth was achieved for chicks 2 weeks of age or younger. Persia et al. (2006) reported approximately a 6% increase in body weight gain when 2 to 6% GB was included in the diet of chicks. Biggs and Parsons (2008) also reported beneficial

effects of GB on cecal microbial populations when included in a corn-soybean meal diet. The explanation for the positive effects of GB is unknown, but part of the effect could be due to its lactose and yeast content. No previous studies have been published concerning the effects of a combination of GB and large particle corn in diets with and without DDGS.

Therefore, the objectives of this study were to evaluate large particle corn, whole sorghum, and GB in a corn-soybean meal diet with or without the addition of DDGS and examine the effects of these diets on growth performance, metabolizable energy, and amino acid digestibility at different ages, as well as the effect on 21-day gizzard weights, pH, and cecal microbial populations of bifidobacteria, lactobacilli, and *E. coli*. We hypothesize that including larger particle sizes or whole grains in the diet of young chicks, as well as the addition of GB, will have no adverse effects on growth performance and may possibly improve performance. It is expected that including larger particle sizes in the diet will increase gizzard size, as well as decrease gizzard pH. An increase in gizzard size and a reduction in pH may have an indirect effect on reducing populations of *E. coli*. Also, with an increase in gizzard size and possible slowing of feed passage rate, energy and amino acid digestibility will be increased because of increased exposure time of nutrients to digestive enzymes.

Materials and Methods

Animals and Husbandry

The University of Illinois Institutional Animal Care and Use Committee approved all procedures. Experiment 1 was conducted with our laboratory strain New Hampshire x Columbian male crossbred chicks, while Ross x Ross 308 commercial male broiler chicks were used in the second experiment. Chicks were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally controlled room with light provided

continuously. The wire floors of the batteries were left soiled from a previous experiment in an attempt to increase the chicks' exposure to a bacterial challenge. Both feed and water were provided ad libitum. Diets were formulated to meet or exceed NRC (1994) nutrient requirements for young chicks. At hatch, chicks were weighed, wing-banded, and allotted to pens and dietary treatments so that the initial mean weight was similar among treatment groups.

Metabolizable Energy and Amino Acid Analysis

Metabolizable energy (ME_n) and amino acid (AA) digestibility were evaluated using the following procedures. Chromic oxide was included in all diets at 0.3% as an indigestible marker. Excreta from each replicate pen were collected over a 24-hour period on either day 7 and 21 post-hatch and freeze-dried. Feed and excreta samples from d 7 and 21 were analyzed for gross energy using an adiabatic bomb calorimeter. Amino acid concentrations [method 982.30E (a, b, c); (AOAC International, 2006)] and chromium [method 990.08; (AOAC International, 2006)] in the feed and excreta samples and nitrogen analyses [method 990.03; (AOAC International, 2006)] were determined at the University of Missouri-Columbia Experiment Station Chemical Laboratory. The ME_n of the diets was calculated using the equation described by Hill and Anderson (1958). Apparent digestibility of amino acids was calculated using the equation described by Adedokun et al. (2007).

Gizzard Weight and pH Determination

Gizzards were collected from the chicks according to the following procedures. At the end of the experiment, birds were euthanized via CO₂ inhalation. After the chicks were euthanized, the gizzards were extracted and the surrounding fat removed. The gizzards were then opened, the contents were removed, and the gizzard was rinsed and blotted dry. The gizzards were weighed and expressed as a percentage of body weight. The gizzard contents from each of

2 or 3 birds were pooled together to provide two subsamples per replicate for pH determination. A total of 1 g of gizzard contents was taken from each pooled sample and added to 9 mL of distilled water and shaken to provide an aqueous solution. The pH probe then was placed directly into the aqueous solution and the pH recorded.

Microbial Populations

In assays where microbial populations were evaluated, the following procedures were used. At the end of the feeding period, chicks were euthanized via CO₂ inhalation and cecal contents were extracted from all birds. The contents of one cecum from each bird were used to determine percent dry matter of cecal contents, while the content of the other cecum was utilized for DNA extraction followed by quantitative PCR (qPCR) techniques described by Middelbos et al. (2007) with minor adaptations. Cecal DNA was extracted from freshly collected samples that had been stored at -20°C until analysis using a QIAmp DNA Stool Mini-kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacterium* spp., *Lactobacillus* spp., and *E. coli*. Briefly, a 10 µl final volume contained 5 µl 2X SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted cecal DNA. Standard curves were obtained by previous harvesting of pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution and amplified with the cecal DNA to create triplicate standard curves. Colony forming units (CFU) in each dilution were determined by plating on specific agars: *Lactobacillus* genus on Difco Lactobacilli MRS broth (Becton Dickinson Company, Sparks, MD), *Bifidobacterium* genus on

Difco Reinforced Clostridial Medium (Becton Dickinson Company) and *E. coli* on Luria-Bertani Medium [10 g/l tryptose, 5 g/l yeast extract, 5 g/l NaCl (pH = 7)]. The calculated log CFU/mL of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate CFU/g of dry cecal contents.

Experiment 1

The objective of this experiment was to evaluate the effects of large ground corn and whole sorghum on growth performance, ME_n, AA digestibility, and gizzard weight and pH in chicks fed diets with or without distillers dried grains with solubles (DDGS). Five pens of 5 male New Hampshire x Columbian chicks were allowed access to 1 of 6 diets from 0 to 21 days of age (Table 2.1): 1) fine ground corn-soybean meal (SBM) diet; 2) large ground corn-SBM meal; 3) 20% whole sorghum; 4, 5, 6) As diets 1-3 + 15% DDGS. Corn was ground through a hammer mill using 1.59 and 9.52 mm screen sizes. This provided corn particle sizes with mean geometric diameters (GMD) of 557 (± 0.133) and 1,387 μm (± 0.223), respectively. The GMD was determined according to the American Society of Agricultural Engineers (2003). The diets were formulated to contain 23% crude protein and an average of 3,046 kcal/kg ME_n / kg. The ME_n values assigned to the whole sorghum and DDGS were 3,288 and 2,480 kcal/kg, respectively (NRC, 1994). Chick weight gain and feed intake were recorded at the end of wk 1 and 3 and feed efficiency (gain:feed) was calculated for each pen replicate.

Experiment 2

The objective of this experiment was to evaluate the effects of GB and large ground corn on growth performance, ME_n, AA digestibility, and cecal microbial populations in commercial chicks fed diets containing DDGS. Five pens of 8 male Ross x Ross 308 commercial broiler chicks were allowed access to 1 of 5 diets from 0 to 21 days of age (Table 2.2): 1) fine ground

corn-soybean meal (SBM) diet; 2) large ground corn-SBM meal; 3) large ground corn + 5% GB; 4) fine ground corn + 15% DDGS + 5% GB; or 5) large ground corn + 15% DDGS + 5% GB. The diets were formulated to contain 22.5% crude protein and 3,051 kcal/kg ME_n / kg. It was assumed that the ME_n of GB was 2000 kcal/kg. The ME_n value for GB is an arbitrary estimated value based on the gross energy assuming that the dairy fraction would be poorly digested and that the yeast and dried fermentation extracts would be moderately to well digested. Corn was ground using the same screen sizes as in Experiment 1. Chick weight gain and feed intake were recorded and feed efficiency was calculated for each pen replicate.

Statistical Analysis

Data were subjected to the general linear models (GLM) procedure of SAS (SAS Institute Inc., 1990) for completely randomized designs. If the F-test for treatment effect was significant in the ANOVA, differences among treatment means were determined using the least significant difference test (Carmer and Walker, 1985). The effect of DDGS was also determined with single degree of freedom contrast statements comparing chicks fed diets with DDGS to chicks fed diets without DDGS. Differences in growth performance, gizzard characteristics, ME_n, and AA digestibility were considered significant when the $P < 0.05$, while differences in cecal microbial populations were considered significant when the $P < 0.10$.

Results and Discussion

Experiment 1

Weight gain (Table 2.3) was decreased ($P < 0.05$) during the first week for crossbred chicks fed the diet containing large ground corn (1,387 μm) when compared to the fine ground corn (557 μm) diet, but was similar among all the other treatments. This difference was not maintained throughout the remainder of the experiment; however, Jacobs et al. (2010) also

reported that feeding chicks diets with a larger ground corn size (1,210 or 1,387 μm) during the first week reduced weight gain when compared to diets that contained finer ground corn (557 or 858 μm). It may be that particle sizes that are larger than 1,000 μm are too large for young chicks (less than 7 d old) to use efficiently because of the physical limitation of consuming the feed particles or their gizzards are physically unable to break down the large particles (Cosava and Forbes, 1996). Consequently, feed passage through the gizzard may be slowed (Lott et al., 1992), resulting in less than maximal growth performance. However, when 15% DDGS was included in the diet, in addition to the large ground corn, this depression in growth was not seen. Previous studies also reported no differences in weight gain when 15% DDGS was included in a typical corn-SBM diet from day of hatch (Lumpkins et al., 2004; Wang et al., 2007a,b). In the current study, chicks may have consumed more of the finer ground portion of the diet during the first week due to physical limitation of feed size, resulting in less corn and more soybean meal being consumed, and reduced energy intake. It may be that the addition of the DDGS, in combination with the finer ground soybean meal, resulted in the chicks consuming more energy that was used to support growth.

Feed intake (Table 2.3) was increased ($P < 0.05$) from 8 to 21 and 0 to 21 d when 20% whole sorghum was included in either the corn or DDGS diets. Nir et al. (1990) and Portella et al. (1988) and reported that as birds become older, their preference for larger particle size feed increases. The increase in feed intake observed in the above two treatments supports the previous studies that the birds selected and consumed more of the whole sorghum particles as they became older. However, feed intake of the chicks consuming any of the diets containing DDGS was decreased ($P < 0.05$) from 0 to 7 and 0 to 21 d when compared to diets containing no DDGS. This result is in contrast to Lumpkins et al. (2004) and Wang et al. (2007a,b) who showed no

differences in feed intake when up to 15% DDGS was included in the diet of young chicks. In the current study, feeding large or whole grains in combination with the higher fiber DDGS may have limited feed intake due to gut capacity or the ME_n value assigned to the DDGS may have been too low.

Feed efficiency (Table 2.3) for the chicks fed large ground corn (1,387 µm) or 20% whole sorghum in a corn-SBM diet (no DDGS) was numerically reduced during the first week and was significantly decreased ($P < 0.05$) during the 8 to 21 d and 0 to 21 d time periods. Nir et al. (1994) also showed similar results in which birds receiving a coarser particle size diet from hatch to day 7 of age had reduced feed efficiency compared to those fed a smaller particle size diet. These effects became even more pronounced during the second and third week of life. In addition, the decrease in feed efficiency may be due to an increase in the energy needed for the growth of the gizzard and the increased grinding needed to reduce the particle size of whole or large grain for passage into the duodenum (Hetland et al., 2002). Over all time periods, the diets containing DDGS had increased ($P < 0.05$) feed efficiency when compared to diets containing no DDGS, again suggesting that the ME_n value assigned to the DDGS may have been too low.

The results for ME_n by age and diet for Experiment 1 are summarized in Table 2.4. The ME_n of all diets increased significantly ($P < 0.05$) from 7 to 21 d of age. At both 7 and 21 days of age, the diet containing 20% whole sorghum yielded the highest ME_n value ($P < 0.05$) when compared to all other treatments. This is in contrast with Biggs and Parsons (2009) who reported that inclusion of 10 to 20% whole sorghum had little or no effect on ME_n at 7 and 21 d. In the current study, it was observed that the chicks on the 20% whole sorghum treatment seemed to be selecting and consuming more of the whole sorghum portion of the diet instead of the ground portion; thus, they may have been consuming more energy than the chicks on the other

treatments (i.e., the ME_n of sorghum is higher than the ME_n of the total diet). In addition, high consumption of the whole sorghum may have resulted in a slower passage rate of large feed particles through the gizzard, resulting in increased exposure time to hydrolytic digestion and improved energy digestibility.

As was observed with the ME_n of the diets, the amino acid digestibility increased for all amino acids at 21 d when compared to values at 7 d (Table 2.5). These results are similar to previous results reported by our lab, which indicated that the ability of the chick to utilize energy and AA increases until the chick is approximately two weeks of age, then plateaus, suggesting that digestibility has reached its maximum at this time (Batal and Parsons, 2002). The effects of diet on AA digestibility were generally small and inconsistent. The diet containing 20% whole sorghum and 15% DDGS generally showed a small reduction ($P < 0.05$) in the digestibility of arginine, lysine, and threonine at 7 days of age when compared to the fine ground corn diet, but yielded similar values for the remaining amino acids. At 21 days of age, the diets containing either 20% whole sorghum alone or in combination with 15% DDGS showed significantly decreased ($P < 0.05$) AA digestibility values when compared to the fine ground corn diet. Biggs and Parsons (2009) reported no significant differences in AA digestibility when chicks received either 10 or 20% whole sorghum. During the 8 to 21 d period, the chicks in these treatments may have been selecting more of the whole sorghum out of the feed trough, which may have resulted in decreased apparent AA digestibility because of reduced protein and AA intake. Any selection of larger particles and subsequent effects on nutrient digestibility discussed above may possibly be reduced by pelleting the diets rather than feeding the diets in mash form as was done in the present study.

At 21 days of age, chicks fed the large ground corn or whole sorghum had significantly increased ($P < 0.05$) relative gizzard weights compared to chicks fed the fine ground corn (Table 2.6). These results are similar to Biggs and Parsons (2009) and Jacobs et al. (2010), who found that when 1-d-old chicks were fed large ground corn and whole sorghum, relative gizzard weights increased by 19 to 47% when compared to chicks fed fine particles. Chicks fed the combination of 20% whole sorghum and 15% DDGS diet had the largest increase in relative gizzard weight. The diets containing DDGS also had increased relative gizzard weights ($P < 0.05$) when compared to diets containing no DDGS. This may have been due to the higher fiber content of the DDGS. According to Urriola et al. (2010), DDGS from modern ethanol plants contains more neutral detergent fiber (37.4 to 44.4%), acid detergent fiber (9.7 to 12.9%), crude fiber (6.1 to 7.4%), and total dietary fiber (28.7 to 34.9%) than the grains used for the fermentation. Previous studies have shown that ingredients high in dietary fiber increase relative gizzard weights in young broilers (Jimenez-Moreno et al., 2009; 2010). In contrast to gizzard weight, there was no significant effect of diet on gizzard pH at 21 days (Table 2.6).

Experiment 2

Weight gain (Table 2.7) was increased ($P < 0.05$) during the 8 to 21 and 0 to 21 d time periods for commercial broiler chicks fed the fine ground corn diet containing 5% Grobionic (GB) and 15% DDGS when compared to the control fine ground corn diet. This same result was also seen when the large ground corn diet containing 5% GB and 15% DDGS was compared to the control large ground corn diet. The increased growth may have been due to the GB or DDGS or both. Previous research conducted by Douglas et al. (2003) reported that when concentrations of 5 or 6% GB were fed to commercial broiler chicks, a significant increase ($P < 0.05$) in body weight gain was achieved during the first 2 weeks post-hatch. Persia et al. (2006) reported

approximately a 6% increase in body weight gain when 2 to 6% GB was included in the diet of chicks. The diets containing DDGS had increased weight gain ($P < 0.01$) for the 8 to 21 and 0 to 21 d time periods when compared to diets containing no DGGS. In contrast, Lumpkins et al. (2004) and Wang et al. (2007a,b) showed no differences in weight gain when 15% DDGS was included in a typical corn-SBM diet. The results from Experiment 1 are also in agreement with the latter study, so the 5% GB rather than the DDGS may have been largely responsible for the increase in weight gain seen in the current experiment.

Feed intake (Table 2.7) over all time periods was not affected by any dietary treatment. Previous studies conducted by Biggs and Parsons (2008), Lumpkins et al. (2004), and Wang et al. (2007a,b) are in agreement with the results in this experiment. Feed efficiency (Table 2.7) for the chicks fed large ground corn (1,387 μm) alone or in combination with 5% GB in a corn-SBM diet was reduced ($P < 0.05$) during the first week, but this result did not persist throughout the remainder of the experiment. As discussed earlier, Jacobs et al. (2010) and Nir et al. (1994) also reported that feeding chicks diets with larger ground corn sizes during the first week reduced feed efficiency when compared to diets that contained finer ground corn. The reason for the decrease in feed efficiency may be due to an increase in the energy needed for the growth of the gizzard and the increased grinding to reduce the particle size for passage into the intestinal tract (Hetland et al., 2002).

As observed in Experiment 1, the ME_n of all diets increased significantly ($P < 0.05$) from 7 to 21 d of age (Table 2.4). At 7 days of age, all diets containing 5% GB yielded significantly higher ME_n values ($P < 0.05$) when compared to diets containing no GB. At 21 days, however, only the diet containing large ground corn, 5% GB, and 15% DDGS still continued to show a significantly increased ($P < 0.05$) ME_n value when compared to three of the other treatments.

These results are in contrast to Biggs and Parsons (2008) who reported that diets containing 4% GB resulted in a reduction ($P < 0.05$) in ME_n at 7, 14, and 21 d, and a diet containing 6% GB reduced ME_n at 14 and 21 d when compared to a basal diet. The difference between the two studies may be due to an overestimation of the ME_n value of the GB that was used in the formulation of the diets in the previous study. A ME_n value of 2,000 kcal/kg was used for GB in the current study, while a TME_n value of 2,960 kcal/kg determined using adult roosters was used in the Biggs and Parsons (2008) study.

As was observed with the ME_n of the diets, the amino acid digestibility increased for all amino acids at 21 d when compared to values at 7 d (Table 2.8). The effects of diet on AA digestibility were generally small and inconsistent. The diet containing large ground corn alone generally showed a small increase ($P < 0.05$) in the AA digestibility of arginine, isoleucine, and lysine at 7 days when compared to the fine ground corn diet, but all values were similar at 21 days. The diet containing fine ground corn in combination with 5% GB and 15% DDGS showed similar AA digestibility values compared to the fine ground corn diet at 7 days of age, but values for all AA were significantly reduced ($P < 0.05$) at 21 days of age. Biggs and Parsons (2008) also reported a reduction in AA digestibility at 21 d when 4 and 6% GB was included in the diet of young chicks. In Experiment 1, the diet containing fine ground corn in combination with 15% DDGS also showed a reduction in most AA at 21 days when compared to the fine ground corn control diet. However, the differences in AA coefficients in the current study were usually only decreased 3 to 4 percentage units, which suggests that any effect in AA digestibility is small.

The effects of diet on cecal microbial populations are presented in Table 2.9. Although no significant differences were detected among any of the dietary treatments for bifidobacteria and lactobacilli, there was a significant decrease in *E. coli* populations when the diet containing LGC

+ 5% GB + 15% DDGS was fed. The lack of response to GB for bifidobacteria and lactobacilli were unexpected as previous studies conducted in our lab have shown that there was a linear increase in lactobacilli and bifidobacteria when 2 to 6% GB was included in a corn-SBM diet (Biggs and Parsons, 2008), and a significant increase in lactobacilli populations when the largest ground corn size of 1,387 μm was included in the diet (Jacobs et al., 2010).

From these two experiments, we can conclude that larger particle sizes from large ground corn or whole sorghum, as well as the addition of GB, can be supplemented to a corn-SBM or corn-SBM DDGS diet with no adverse effect on weight gain when fed from 0 to 21 d of age. Feed efficiency may be depressed when large ground corn or whole sorghum is included in the diet during the first week of life, however, so particle sizes smaller than 1,387 μm may be preferred during this time. This feed efficiency effect seems to be ameliorated, however, when 15% DDGS is added in addition to the large particle sizes. Dietary ME_n may be increased when GB and 20% whole sorghum are fed. The dietary supplements generally had little or no effect on AA digestibility. Relative gizzard weights can be significantly increased when large ground corn or sorghum is included in young chick diets, which may have beneficial effects on intestinal health.

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Tables

Table 2.1. Nutritional composition (%) of chick diets used in Experiment 1¹.

Ingredient	Corn-SBM	Corn-DDGS	Whole Sorghum	Sorghum-DDGS
Corn ²	49.00	40.56	35.46	24.60
Soybean meal	38.73	33.15	37.25	31.74
Whole Sorghum	-	-	20.00	20.00
DDGS ²	-	15.00	-	15.00
Soybean oil	5.00	5.50	3.00	4.50
Limestone	1.31	1.44	1.44	1.53
Dicalcium phosphate	1.75	1.52	1.51	1.33
Salt	0.40	0.40	0.40	0.40
Vitamin mix ³	0.20	0.20	0.20	0.20
Mineral mix ⁴	0.15	0.15	0.15	0.15
DL-Methionine	0.19	0.14	0.19	0.15
Choline chloride	0.10	0.10	0.10	0.10
Dextrose	0.00	1.00	-	-
Solka Floc ⁵	2.87	1.54	-	-
Chromic Oxide	0.30	0.30	0.30	0.30
Calculated Analysis:				
Crude protein, %	23.1	23.2	23.0	23.0
ME _n , kcal/kg	3,052	3,044	3,043	3,044
Calcium, %	1.00	1.00	1.00	1.00
Nonphytate P, %	0.45	0.45	0.45	0.45

¹ SBM = soybean meal; DDGS = distillers dried grains with solubles.

² Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 µm, respectively.

³ Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

⁴ Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄•7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

⁵ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 2.2. Nutritional composition (%) of chick diets used in Experiment 2¹.

Ingredient	Corn-SBM	LGC-GB	Corn-GB-DDGS
Corn ²	45.10	45.10	39.60
Soybean meal	38.13	38.13	30.60
DDGS	-	-	15.00
Grobiotic	-	5.00	5.00
Soybean oil	5.50	5.50	5.50
Limestone	1.33	1.33	1.41
Dicalcium phosphate	1.76	1.76	1.56
Salt	0.40	0.40	0.40
Vitamin mix ³	0.20	0.20	0.20
Mineral mix ⁴	0.15	0.15	0.15
DL-Methionine	0.21	0.21	0.18
Choline chloride	0.10	0.10	0.10
Dextrose	2.90	-	-
Solka Floc ⁵	3.92	1.82	-
Chromic Oxide	0.30	0.30	0.30
Calculated Analysis:			
Crude protein, %	22.5	22.5	22.5
ME _n , kcal/kg	3,051	3,051	3,051
Calcium, %	1.00	1.00	1.00
Nonphytate phosphorus, %	0.45	0.45	0.45

¹ SBM = soybean meal; LGC = large ground corn; GB = Grobiotic; DDGS = distillers dried grains with solubles.

² Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 μ m, respectively.

³ Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μ g; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

⁴ Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄•7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

⁵ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 2.3. Effect of diet on performance of chicks fed fine and large particle corn, whole sorghum, and 15% distillers dried grains with solubles (DDGS) in Experiment 1^{1,2}.

	Fine ground corn ³	Large ground corn ³	20% whole sorghum	FGC + 15% DDGS [*]	LGC + 15% DDGS [*]	20% sorghum + 15% DDGS [*]	Pooled SEM
Weight gain							
(g/chick)							
0 to 7 d	59 ^a	52 ^b	57 ^{ab}	56 ^{ab}	60 ^a	59 ^a	2.2
8 to 21 d	353	336	341	357	351	346	7.4
0 to 21 d	411 ^{ab}	388 ^b	398 ^{ab}	414 ^a	411 ^{ab}	405 ^{ab}	8.3
Feed Intake							
(g/chick)							
0 to 7 d	132 ^{ab}	134 ^{ab}	146 ^a	114 ^b	123 ^{ab}	122 ^{ab}	8.4
8 to 21 d	559 ^{bc}	580 ^{ab}	601 ^a	541 ^c	548 ^{bc}	608 ^a	13.1
0 to 21 d	691 ^{bcd}	714 ^{abc}	747 ^a	654 ^d	671 ^{cd}	730 ^a	16.1
Gain/feed							
(g/kg)							
0 to 7 d	453 ^{ab}	397 ^b	388 ^b	503 ^a	491 ^a	494 ^a	31.9
8 to 21 d	630 ^a	581 ^b	567 ^b	662 ^a	640 ^a	570 ^a	15.7
0 to 21 d	596 ^{ab}	544 ^c	533 ^c	634 ^a	612 ^a	556 ^{bc}	15.2

^{a-d} Means within a row with no common superscript differ significantly ($P < 0.05$).

^{*} There was a significant effect ($P < 0.05$) of DDGS on feed intake (0 to 7 d, 0 to 21 d) and gain/feed (all age periods) based on a single degree of freedom contrast.

¹ Means represent 5 replicates of 5 chicks each per treatment; New Hampshire x Columbian males.

² FGC = fine ground corn; DDGS = distillers dried grains with solubles; LGC = large ground corn.

³Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 μm , respectively for the fine and large ground corn.

Table 2.4. Effect of diet and age on ME_n (kcal/kg DM) in Experiments 1 and 2.

Dietary treatment ¹	Days of age		Pooled SEM
	7	21	
Experiment 1 ² :			
Fine ground corn ³	2,845 ^{bz}	3,380 ^{cdy}	56.8
Large ground corn ³	2,895 ^{bz}	3,369 ^{dy}	56.0
20% whole sorghum	3,184 ^{az}	3,567 ^{ay}	23.5
FGC + 15% DDGS	2,955 ^{bz}	3,476 ^{by}	47.3
LGC + 15 % DDGS	2,984 ^{bz}	3,399 ^{cdy}	25.6
20% whole sorghum + 15 % DDGS	2,971 ^{bz}	3,411 ^{cy}	57.3
Pooled SEM	64.6	13.9	
Experiment 2 ⁴ :			
Fine ground corn ³	2,479 ^{cz}	3,197 ^{by}	38.8
Large ground corn ³	2,563 ^{bcz}	3,148 ^{by}	43.0
LGC + 5% Grobiotic (GB)	2,735 ^{az}	3,130 ^{by}	64.8
FGC + 5% GB+ 15% DDGS	2,659 ^{abz}	3,215 ^{aby}	36.4
LGC + 5% GB + 15% DDGS	2,806 ^{az}	3,303 ^{ay}	41.1
Pooled SEM	55.7	33.7	

^{a-d} Means within a column (diet effect) and experiment with no common superscript differ significantly ($P < 0.05$).

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ FGC = fine ground corn; DDGS = distillers dried grains with solubles; LGC = large ground corn.

² Means represent 5 replicates of 5 New Hampshire x Columbian male chicks each per treatment.

³ Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 μm , respectively for the fine and large ground corn.

⁴ Means represent 5 pens of 8 chicks each per treatment; Ross x Ross 308 commercial males.

Table 2.5. Effect of diet and age on selected amino acid digestibility (%) in Experiment 1.

Amino acid and dietary treatment ^{1,2}	Days of age		Pooled SEM
	7	21	
Arginine			
Fine ground corn	89 ^z	94 ^y	0.7
Large ground corn	89 ^z	94 ^y	0.6
20% whole sorghum	88 ^z	92 ^{y*}	0.6
FGC + 15% DDGS	88 ^z	92 ^{y*}	0.4
LGC + 15% DDGS	88 ^z	93 ^y	0.8
20% whole sorghum + 15% DDGS	86 ^{z*}	92 ^{y*}	1.0
Pooled SEM	0.9	0.3	
Cysteine			
Fine ground corn	76 ^z	85 ^y	1.2
Large ground corn	73 ^z	85 ^y	1.2
20% whole sorghum	73 ^z	83 ^{y*}	1.4
FGC + 15% DDGS	73 ^z	83 ^{y*}	1.1
LGC + 15% DDGS	74 ^z	85 ^y	1.1
20% whole sorghum + 15% DDGS	71 ^z	83 ^{y*}	1.5
Pooled SEM	1.7	0.6	
Isoleucine			
Fine ground corn	83 ^z	91 ^y	0.9
Large ground corn	83 ^z	91 ^y	0.9
20% whole sorghum	82 ^z	90 ^y	0.6
FGC + 15% DDGS	82 ^z	90 ^y	0.6
LGC + 15% DDGS	80 ^z	90 ^y	1.1
20% whole sorghum + 15% DDGS	80 ^z	90 ^y	0.9
Pooled SEM	1.1	0.4	
Lysine			
Fine ground corn	85 ^z	91 ^y	0.8
Large ground corn	84 ^z	91 ^y	0.8
20% whole sorghum	83 ^z	89 ^{y*}	0.7
FGC + 15% DDGS	82 ^z	88 ^{y*}	0.6
LGC + 15% DDGS	81 ^{z*}	90 ^y	1.0
20% whole sorghum + 15% DDGS	79 ^{z*}	88 ^{y*}	1.1
Pooled SEM	1.1	0.4	

Table 2.5 con't

Methionine

Fine ground corn	85 ^z	94 ^y	1.5
Large ground corn	86 ^z	95 ^y	1.0
20% whole sorghum	85 ^z	93 ^y	0.9
FGC + 15% DDGS	84 ^z	94 ^y	0.9
LGC + 15% DDGS	85 ^z	95 ^y	0.8
20% whole sorghum + 15% DDGS	82 ^z	93 ^y	1.8
Pooled SEM	1.6	0.4	

Threonine

Fine ground corn	79 ^z	87 ^y	0.9
Large ground corn	75 ^z	87 ^y	0.9
20% whole sorghum	75 ^z	84 ^{y*}	1.0
FGC + 15% DDGS	74 ^{z*}	84 ^{y*}	0.7
LGC + 15% DDGS	75 ^z	87 ^y	1.1
20% whole sorghum + 15% DDGS	72 ^{z*}	84 ^{y*}	1.6
Pooled SEM	1.5	0.5	

Valine

Fine ground corn	80 ^z	90 ^y	1.0
Large ground corn	78 ^z	90 ^y	0.7
20% whole sorghum	78 ^z	87 ^{y*}	1.1
FGC + 15% DDGS	78 ^z	88 ^{y*}	0.6
LGC + 15% DDGS	76 ^z	89 ^y	1.1
20% whole sorghum + 15% DDGS	79 ^z	89 ^y	1.2
Pooled SEM	1.3	0.6	

*Means within a column for each amino acid (diet effect) are significantly ($P < 0.05$) different than the fine (557 μ m) ground corn diet.

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 5 replicates of 5 chicks each per treatment.

² FGC = fine ground corn; DDGS = distillers dried grains with solubles; LGC = large ground corn.

Table 2.6. Effect of dietary treatments on 21-day gizzard weight (% of body weight) and gizzard pH of chicks in Experiment 1^{1,2}.

	Weight ³	pH
Fine ground corn	1.90 ^c	3.04
Large ground corn	2.47 ^b	3.37
20% whole sorghum	2.46 ^b	3.25
FGC+ 15% DDGS	1.95 ^c	3.06
LGC + 15% DDGS	2.46 ^b	3.04
20% whole sorghum + 15% DDGS	2.73 ^a	3.35
Pooled SEM	0.06	0.13

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 5 replicates of 5 chicks each per treatment.

² FGC = fine ground corn; DDGS = distillers dried grains with solubles; LGC = large ground corn.

³There was a significant effect ($P < 0.05$) of DDGS based on a single degree of freedom contrast.

Table 2.7. Effect of diet on performance of chicks fed fine and large particle corn, Grobiotic, and 15% DDGS in Experiment 2¹.

	Fine ground corn ²	Large ground corn ²	LGC+ 5% GB ³	FGC + 5% GB + 15% DDGS ³	LGC + 5% GB + 15% DDGS ³	Pooled SEM
Weight gain						
(g/chick)						
0 to 7 d	98 ^{ab}	89 ^{ab}	85 ^b	105 ^a	98 ^{ab}	5.8
8 to 21 d	642 ^{bc}	625 ^c	684 ^{ab}	697 ^a	692 ^{ab}	17.4
0 to 21 d	741 ^{bc}	714 ^c	769 ^{abc}	801 ^a	790 ^{ab}	20.0
Feed Intake						
(g/chick)						
0 to 7 d	130	132	125	138	132	5.8
8 to 21 d	1,016	1,038	1,035	1,074	1,068	35.0
0 to 21 d	1,146	1,170	1,160	1,212	1,200	38.8
Gain/feed						
(g/kg)						
0 to 7 d	759 ^a	667 ^b	669 ^b	761 ^a	742 ^a	20.0
8 to 21 d	635	604	666	650	649	23.4
0 to 21 d	649	611	667	661	659	19.8

^{a-c} Means within a row with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 5 replicates of 8 chicks each per treatment: Ross x Ross 308 commercial broiler males.

² Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 μm , respectively.

³ LGC = large ground corn; GB = Grobiotic; FGC = fine ground corn; DDGS = distillers dried grains with solubles.

Table 2.8. Effect of diet and age on selected amino acid digestibility (%) in Experiment 2.

Amino acid and dietary treatment ^{1,2}	Days of age		Pooled SEM
	7	21	
Arginine			
Fine ground corn	87 ^z	92 ^y	0.6
Large ground corn	90 ^{z*}	92 ^y	0.4
LGC + 5% Grobiotic (GB)	89 ^z	92 ^y	0.7
FGC + 5% GB + 15% DDGS	86 ^z	89 ^{y*}	0.5
LGC + 5 % GB + 15% DDGS	88 ^z	91 ^y	0.7
Pooled SEM	0.7	0.5	
Cysteine			
Fine ground corn	69 ^z	82 ^y	1.3
Large ground corn	73 ^z	82 ^y	1.3
LGC + 5% Grobiotic (GB)	72 ^z	80 ^{y*}	1.6
FGC + 5% GB + 15% DDGS	71 ^z	79 ^{y*}	1.1
LGC + 5 % GB + 15% DDGS	75 ^{z*}	82 ^y	1.3
Pooled SEM	1.8	0.6	
Isoleucine			
Fine ground corn	78 ^z	87 ^y	1.0
Large ground corn	82 ^{z*}	87 ^y	0.7
LGC + 5% Grobiotic (GB)	81 ^z	86 ^y	1.1
FGC + 5% GB + 15% DDGS	77 ^z	83 ^{y*}	0.8
LGC + 5 % GB + 15% DDGS	80 ^z	85 ^y	1.3
Pooled SEM	1.2	0.8	
Lysine			
Fine ground corn	80 ^z	87 ^y	0.9
Large ground corn	84 ^{z*}	88 ^y	0.6
LGC + 5% Grobiotic (GB)	82 ^z	87 ^y	1.1
FGC + 5% GB + 15% DDGS	78 ^z	83 ^{y*}	0.7
LGC + 5 % GB + 15% DDGS	81 ^z	85 ^{y*}	1.2
Pooled SEM	1.0	0.8	
Methionine			
Fine ground corn	86 ^z	92 ^y	0.8
Large ground corn	85 ^z	91 ^y	1.0
LGC + 5% Grobiotic (GB)	85 ^z	90 ^y	1.1
FGC + 5% GB + 15% DDGS	85 ^z	89 ^{y*}	0.6
LGC + 5 % GB + 15% DDGS	87 ^z	91 ^y	0.9
Pooled SEM	1.1	0.6	

Table 2.8 con't

Threonine

Fine ground corn	70 ^z	81 ^y	1.2
Large ground corn	73 ^z	81 ^y	0.9
LGC + 5% Grobionic (GB)	71 ^z	79 ^y	1.8
FGC + 5% GB + 15% DDGS	69 ^z	77 ^{y*}	0.9
LGC + 5 % GB + 15% DDGS	73 ^z	79 ^y	1.6
Pooled SEM	1.6	0.9	

Valine

Fine ground corn	75 ^z	84 ^y	1.0
Large ground corn	79 ^z	85 ^y	0.7
LGC + 5% Grobionic (GB)	78 ^z	83 ^y	1.3
FGC + 5% GB + 15% DDGS	74 ^z	81 ^{y*}	0.8
LGC + 5 % GB + 15% DDGS	78 ^z	83 ^y	1.5
Pooled SEM	1.3	0.9	

*Means within a column for each amino acid (diet effect) are significantly ($P < 0.05$) different than the fine (557 μm) ground corn diet.

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 5 replicates of 8 chicks each per treatment.

² LGC = large ground corn; FGC = fine ground corn; DDGS = distillers dried grains with solubles.

Table 2.9. Effect of diet on select cecal microbial populations in Experiment 2.

Dietary treatment ^{1,2}	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>
			(log ₁₀ CFU/g DM)
Fine ground corn	6.39	9.01	9.09 ^a
Large ground corn	6.55	8.80	8.93 ^a
LGC + 5% Grobionic	6.37	9.10	8.53 ^{ab}
FGC + 5% Grobionic + 15% DDGS	6.11	8.85	7.68 ^{ab}
LGC + 5% Grobionic + 15 % DDGS	6.36	9.32	7.40 ^b
Pooled SEM	0.22	0.30	0.62

^{a-b} Means within a column with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 5 replicates of 8 chicks each per treatment.

² LGC = large ground corn; FGC = fine ground corn; DDGS = distillers dried grains with solubles.

Chapter III

The Effects of Whole or Large Particle Grains and *Eimeria acervulina* Infection on Growth Performance, Nutrient Digestibility, and Microbial Populations in Young Chicks

Abstract

Two experiments were conducted to evaluate the effects of fine and large particle corn, whole sorghum, and whole wheat in the presence and absence of an acute *Eimeria acervulina* infection in chicks fed a corn-soybean meal diet. In the first experiment, the *E. acervulina* infection was generally not effective in reducing growth performance, so a second experiment using newly prepared oocysts was performed. The *E. acervulina* infection in the second experiment significantly reduced chick growth performance, ME_n, and AA digestibility ($P < 0.05$). However, the addition of large ground corn, whole sorghum, or whole wheat to the diet had no consistent effects on growth performance and did not ameliorate the negative effects of the coccidiosis infection. In both experiments, chicks fed the large ground corn, whole sorghum, or whole wheat had significantly increased ($P < 0.05$) relative gizzard weights when compared to chicks fed the fine ground corn. Also, chicks infected with *E. acervulina* had increased relative gizzard and small intestinal weights ($P < 0.05$) when compared to non-infected chicks. In both experiments, the addition of whole wheat or sorghum resulted in a significant decrease ($P < 0.02$) in cecal lactobacilli populations. Also, the addition of the whole wheat, whole sorghum, and large ground corn and the presence of the *E. acervulina* infection all decreased ($P < 0.05$) *E. coli* populations in both experiments. The results of this study indicated that feeding large ground corn, whole sorghum, or whole wheat in the presence or absence of a coccidiosis infection significantly increased relative gizzard weights and decreased *E. coli* populations, but had no consistent effects on overall growth performance or responses to *E. acervulina* infection.

Introduction

The health aspect of consuming whole grains in humans has long been understood, while the effects in livestock species, in particular poultry, are still unclear and rather variable. Initially, countries such as Europe, Australia, and Canada included whole grains in poultry diets as a way to reduce feed costs. However, it has also been hypothesized that feeding whole grains or larger particle sizes may improve the health of the bird, mainly due to increasing gizzard size and activity (Amerah et al., 2007; 2008; Gabriel et al., 2006). Feeding whole grains may also stimulate gastric functions including the secretion of hydrochloric acid, which could possibly have an antimicrobial effect by reducing pH and limiting the growth of pathogenic bacteria while promoting the growth of beneficial populations (Engberg et al., 2002).

It is especially of interest to determine if larger particle sizes can elicit a positive response when the bird has a challenge to its immune system, such as during an acute coccidiosis infection. In chickens, coccidiosis can range from a light infection with no adverse effects on health or growth performance (coccidiasis) to a more severe clinical infection which causes high incidences of mortality and morbidity (Williams, 1999). However, the most common form, especially from an economic standpoint, is coccidiosis which produces a subclinical infection, resulting in reduced weight gain and feed efficiency by the bird (Williams, 1999). In addition, interactions can occur between coccidiosis and other poultry diseases, mainly those caused by various pathogenic bacteria, leading to secondary infections. By feeding whole or large particle grains, the effects of a coccidiosis challenge, especially those produced from a subclinical infection, may be ameliorated. With larger particle sizes, the stimulation of gizzard development through increased grinding activity leads to an improvement in gut motility (Ferket, 2000) and increases the secretion of hydrochloric acid into the gizzard and intestine, ultimately reducing

pH. This may have an antimicrobial effect because pathogenic organisms entering the gastrointestinal tract via the feed or environment have a greater chance of being inactivated by the highly acidic environment (Engberg et al., 2002; Naughton and Jensen, 2001). Therefore, the gizzard can be regarded as a barrier in preventing pathogenic organisms, such as coccidia or pathogenic bacteria which may cause possible secondary infections, from entering the intestinal tract (Engberg et al., 2004). In addition, digestion of larger particle sizes is slower within the gizzard; thus, feed passage rate is slowed, resulting in more reflux of intestinal contents and increased exposure time of nutrients to digestive enzymes, which may improve nutrient utilization (Nir et al., 1995). This may be especially important during times of a coccidiosis infection where intestinal tissue damage can interfere with food digestion and nutrient absorption.

Therefore, the objectives of this study were to evaluate the effects of large particle corn and whole grains (sorghum and wheat) on growth performance, gizzard characteristics, and intestinal microflora during an *Eimeria acervulina* coccidiosis infection. We hypothesize that including larger particle sizes or whole grains in the diet of young chicks will have no adverse effects on growth performance and may possibly improve performance. It is expected that including larger particle sizes in the diet will increase gizzard size, as well as decrease gizzard pH. This may have an indirect effect on reducing the severity of a coccidiosis infection, as well as reducing the pathogenic populations of microbiota within the gastrointestinal tract. Also, with an increase in gizzard size and possible slowing of feed passage rate, energy and amino acid digestibility will be increased because of increased exposure time of nutrients to digestive enzymes.

Materials and Methods

Animals and Husbandry

The University of Illinois Institutional Animal Care and Use Committee approved all procedures. Both experiments were conducted with Ross x Ross 308 commercial male broiler chicks. Chicks were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally controlled room with light provided continuously. Both feed and water were provided ad libitum. Diets were formulated to meet or exceed all NRC (1994) nutrient requirements for young chicks 0 to 3 weeks of age. At hatch, chicks were weighed, wing-banded, and allotted to pens and dietary treatments so that the initial mean weight was similar among treatment groups.

Metabolizable Energy and Amino Acid Analysis

Metabolizable energy (ME_n) and amino acid (AA) digestibility were evaluated using the following procedures. Chromic oxide was included in all diets at 0.3% as an indigestible marker. Excreta from each replicate pen was collected over a 24-hour period on d 14 and 21 post-hatch and freeze-dried. Feed and excreta samples from d 14 and 21 were analyzed for gross energy using an adiabatic bomb calorimeter. Amino acid concentrations [method 982.30E (a,b,c); (AOAC International, 2006)] and chromium [method 990.08; (AOAC International, 2006)] in the feed and excreta samples and nitrogen analyses [method 990.03; (AOAC International, 2006)] were determined at the University of Missouri-Columbia Experiment Station Chemical Laboratory. The ME_n of the diets was calculated using the equation described by Hill and Anderson (1958). Apparent digestibility of amino acids was calculated using the equation described by Adedokun et al. (2007).

Gizzard Weight and pH Determination

Gizzards were collected from the chicks according to the following procedures. At the end of the experiment, birds were euthanized via CO₂ inhalation. After the chicks were euthanized, the gizzards were extracted and the surrounding fat removed. The gizzards were then opened, the contents were removed, and the gizzard was rinsed and blotted dry. The gizzards were weighed and expressed as a percentage of body weight. The gizzard contents from each of 2 or 3 birds were pooled together to provide two subsamples per replicate for pH determination. A total of 1 g of gizzard contents was taken from each pooled sample and added to 9 mL of distilled water and shaken to provide an aqueous solution. The pH probe then was placed directly into the aqueous solution and the pH recorded.

Microbial Populations

At the end of the feeding period (22 d of age), chicks were euthanized via CO₂ inhalation and ileal and cecal contents were extracted from all birds. For cecal samples, the contents of one cecum from each bird were used to determine percent dry matter of cecal contents, while the content of the other cecum was utilized for DNA extraction followed by quantitative PCR (qPCR) techniques described by Middelbos et al. (2007) with minor adaptations. For ileal samples, 0.5 g of distal ileal contents from each bird were pooled and used to determine percent dry matter of contents, while the remainder of the contents were pooled and utilized for DNA extraction. Cecal or ileal DNA was extracted from freshly collected samples that had been stored at -20°C until analysis using a QIAmp DNA Stool Mini-kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacterium* spp., *Lactobacillus* spp., and *E. coli*.

Briefly, a 10 µl final volume contained 5 µl 2X SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted cecal DNA. Standard curves were obtained by previous harvesting of pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution and amplified with the cecal DNA to create triplicate standard curves. Colony forming units (CFU) in each dilution were determined by plating on specific agars: *Lactobacillus* genus on Difco Lactobacilli MRS broth (Becton Dickinson Company, Sparks, MD), *Bifidobacterium* genus on Difco Reinforced Clostridial Medium (Becton Dickinson Company) and *E. coli* on Luria-Bertani Medium [10 g/l tryptose, 5 g/l yeast extract, 5 g/l NaCl (pH = 7)]. The calculated log CFU/mL of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate CFU/g of dry cecal or ileal contents.

Procedures for Coccidiosis Infection

For coccidiosis infection treatments, sporulated *Eimeria acervulina* oocysts (Animal Parasitic Diseases Laboratory, USDA, Beltsville, MD) were diluted with distilled water 1 day prior to inoculation. Chicks were orally gavaged with either 0.5 mL of sporulated oocysts in solution (1×10^6 oocysts/dose) or distilled water (sham inoculations) on day 8 of age (post-inoculation d 0) using 1 cc syringes. Body weights and feed intake were measured at the beginning of the experiment, as well as on day 0, 5, 7, 10, and 14 post-inoculation.

Experiment 1

A 4 x 2 factorial arrangement of treatments was utilized to evaluate the effects of fine and large particle corn, whole sorghum, and whole wheat in the presence and absence of an acute *E. acervulina* infection. Four pens of 5 male Ross x Ross 308 commercial broiler chicks were

allowed access to 1 of 4 diets containing fine ground corn, large ground corn, 20% whole sorghum, or 20% whole wheat (Table 3.1) and inoculated with 0 or 1×10^6 sporulated oocysts on day 8 of age. Corn was ground through a hammer mill using 1.59 and 9.52 mm screen sizes to obtain the fine and large ground corn, respectively. The effects of diet and coccidiosis infection on growth performance, gizzard weight and pH, ME_n, AA digestibility, and cecal and ileal weight and microbial populations were determined. All diets were formulated to contain 23.1% crude protein and an average of 3067 kcal of ME_n / kg (Table 3.1). Chicks were fed the experimental diets from 0 to 22 days of age. Body weights and feed intake were measured at the beginning of the experiment, as well as on day 0, 5, 7, 10, and 14 post-inoculation.

Experiment 2

Upon evaluation of the performance data in Experiment 1, there was concern that the oocysts used were not producing as severe of an infection as needed, which was indicated by no statistical difference in weight gain between infected and control chicks fed the fine ground corn control diet ($P < 0.06$). Therefore, the objective of the second coccidiosis experiment was to evaluate the effects on growth performance, gizzard weight and pH, ME_n, AA digestibility, and cecal and ileal weight and microbial populations when newly prepared oocysts were used.

Statistical Analysis

Data were subjected to the general linear models (GLM) procedure of SAS (SAS Institute Inc., 1990) for completely randomized designs with a factorial arrangement of treatments. Data are presented as means with a pooled SEM. When the main effect of diet was significant, single degree of freedom contrast statements were used to compare chicks fed diets with large ground corn or whole grains to chicks fed diets without large ground corn or whole grains. Differences were considered significant when the $P < 0.05$.

Results and Discussion

Experiment 1

The acute *E. acervulina* model used in the current experiment was not effective in causing a sufficiently severe subclinical coccidiosis infection in chicks, which was indicated by no statistical difference ($P > 0.05$) in weight gain between the fine ground corn sham and inoculated treatments and no main effect of coccidial challenge or interaction for any of the time periods (Table 3.2). The main effect of a coccidial challenge did approach significance ($P < 0.06$) for the 7 to 10 d post-inoculation period. This result was unexpected as previous experiments conducted in our lab have shown that infection with an acute dosage of 1×10^6 *E. acervulina* oocysts or lower results in a 13 to 30% significant decrease ($P < 0.05$) in weight gain during the infection period (Perez et al., 2010; Persia et al., 2006; Willis and Baker, 1981a,b). In the current experiment, weight gain was reduced by 15% during the 7 to 10 d post-inoculation period; however, this was not significant. Diet also had no significant effect on weight gain during any of the experimental periods. Previous studies also reported no differences in weight gain when large ground corn, whole sorghum, and whole wheat were included in a corn-soybean meal diet (Biggs and Parsons, 2009; Hetland et al., 2002; Jacobs et al., 2010). In contrast, Hamilton and Proudfoot (1995) reported that birds fed mash diets containing coarse or very coarse corn particles showed improved weight gain compared to those containing fine particles. Nir et al. (1994b; 1995) showed similar results with sorghum grain. The results of the current study combined with earlier published studies show that the effects of large ground corn, whole sorghum, and whole wheat on weight gain of poultry may be inconsistent under research conditions.

The *E. acervulina* infection also had no significant effect on feed efficiency; however, there was an effect of diet during all age periods. Feed efficiency for all chicks fed large ground corn diets was increased ($P < 0.002$) during the 7 to 10 d post-inoculation period when compared to chicks fed fine ground corn diets as indicated by a single degree of freedom contrast. Nir et al. (1994a) also reported that at 21 d of age, chicks fed medium to large corn particles exhibited better feed efficiency than those fed a finer mash diet. In contrast, chicks fed diets containing 20% whole sorghum had decreased ($P < 0.01$) feed efficiency during the 0 to 7 and 0 to 14 d post-inoculation periods. Biggs and Parsons (2009) also reported that feeding chicks diets containing 20% whole sorghum decreased ($P < 0.05$) feed efficiency during the 8 to 21 d age period. The authors suggested that the decrease in feed efficiency was due to the increased energy that is needed for the growth of the gizzard and the increased grinding needed to reduce the particle size of the whole grain before it can pass into the duodenum.

At 21 days of age, diet was the only factor that had a significant effect on gizzard weight. Chicks fed the large ground corn, whole sorghum, or whole wheat had significantly higher ($P < 0.0001$) relative gizzard weights than chicks fed the fine ground corn (Table 3.3). These results are similar to Biggs and Parsons (2009) and Jacobs et al. (2010), who found that when chicks were fed large ground corn, whole wheat, and whole sorghum from day of hatch, relative gizzard weights increased by 13 to 47% when compared to chicks fed diets devoid of whole grains. Gizzard pH was not affected by diet or *E. acervulina* infection and ranged from 4.56 to 5.28 (Table 3.3).

At 21 days of age, chicks fed diets containing whole or large particle grains and infected with *E. acervulina* had a significant increase ($P < 0.005$) in cecal bifidobacteria populations (Table 3.4). This effect did not occur in chicks that were not infected, resulting in a significant

interaction between diet and infection ($P < 0.03$). Infected chicks fed diets containing whole sorghum or whole wheat also had reduced ($P < 0.001$) cecal lactobacilli populations when compared to non-infected chicks, but this result did not occur for chicks fed fine or large ground corn, resulting in another significant interaction between diet and *E. acervulina* infection. Baba et al. (1987) reported that cecal lactobacilli decreased significantly ($P < 0.05$) 7 to 10 d after inoculation with coccidiosis but was almost equal to uninfected groups 14 d after inoculation; however, the authors used *E. tenella* to infect chickens rather than *E. acervulina*. In contrast, Gabriel et al. (2006) reported that birds fed whole wheat and infected with *E. acervulina* had no difference in lactobacilli populations when compared to their uninfected counterparts. However, the authors used a dose of 2.5×10^5 sporulated oocysts and fed 40% whole wheat, whereas the current study used a higher dosage of 1×10^6 oocysts and only 20% whole wheat. Cecal *E. coli* populations were decreased ($P < 0.002$) in chicks fed whole or large particle grain diets regardless of infection status (Table 3.4). Neither diet nor coccidiosis infection affected cecal weight. For ileal microbial populations (Table 3.5), no effects of diet or infection were observed except that *E. acervulina* inoculated birds had reduced ($P < 0.0001$) ileal *E. coli* populations when compared to sham-inoculated birds regardless of diet. Gabriel et al. (2006) reported that birds fed a corn-soybean meal diet and infected with *E. acervulina* had no differences in excreta *E. coli* populations when compared to the non-infected controls, while birds fed a whole wheat diet and infected with *E. acervulina* had higher excreta *E. coli* counts compared to their non-infected counterparts. The latter results are opposite to those obtained in the current study for cecal *E. coli* counts. The reason for the disagreement between studies is unknown. In the current study, *E. acervulina* infection increased small intestine weight (Table 3.5). Allen (1984) also reported increased weights of all intestinal segments when birds were inoculated with 1×10^6

sporulated *E. acervulina* oocysts and attributed the increase to swelling and edema of intestinal tissue.

Experiment 2

Growth performance for chicks infected with *E. acervulina* in the current experiment was significantly reduced ($P < 0.0001$) in comparison with sham-inoculated chicks (Table 3.6). These reductions were largest during the early post-inoculation period (d 0 to 7) and became less severe with increasing days post-inoculation. The depression in chick growth performance was similar to previous studies where coccidiosis was either experimentally induced (Perez et al., 2010; Persia et al., 2006; Willis and Baker, 1981a,b) or naturally occurred in commercial poultry production systems (Haug et al., 2008; Williams, 2005). Weight gain ($P < 0.01$) and feed intake ($P < 0.03$) for non-infected chicks consuming the 20% whole sorghum diet was significantly increased during all age periods, while weight gain for non-infected chicks consuming the large ground corn was increased ($P < 0.01$) during the 7 to 10 and 0 to 14 d period. There were no significant effects of diet on feed efficiency.

Acute infection of chicks with *E. acervulina* resulted in large and significant reductions ($P < 0.0001$) in ME_n and AA digestibility at 14 d when compared to sham-inoculated chicks (Table 3.7). There was a significant diet x *E. acervulina* interaction at 14 d which occurred because the whole wheat diet reduced AA digestibility in infected chicks but not in non-infected chicks. These reductions in ME_n and AA digestibility became less severe by d 21 of age (14 d post-inoculation) when the effect of infection was only significant for Cys, Thr, and ME_n. The 14 d results are in agreement with previous studies that also reported significant reductions in both ME_n and AA digestibility during an *E. acervulina* infection (Persia et al., 2006; Ruff and Wilkins, 1980; Takhar and Farrell, 1979a,b), and is primarily attributed to reduced absorption

capabilities. Amino acid and ME_n values for non-infected chicks fed diets containing large ground corn, whole sorghum, or whole wheat were not statistically different. These results are similar to Biggs and Parsons (2009) and Jacobs et al. (2010), who reported no differences in ME_n and AA digestibility when chicks were fed large ground corn and whole sorghum from day of hatch. In contrast, Biggs and Parsons (2009), Preston et al. (2000), and Wu et al. (2004) reported that inclusion of whole wheat improved ($P < 0.05$) ME_n and AA digestibility. Thus, the responses to feeding large ground corn or whole grains continue to be variable among studies.

Acute *E. acervulina* infection and diet both had a significant main effect on gizzard weight and there was a significant interaction between the two treatment variables ($P < 0.01$). Chicks infected with *E. acervulina* had increased ($P < 0.0001$) relative gizzard weights when compared to non-infected chicks (Table 3.8). In addition, chicks fed the large ground corn, whole sorghum, or whole wheat had significantly increased ($P < 0.0001$) relative gizzard weights compared to chicks fed the fine ground corn; this effect was greater in the presence than in the absence of a coccidial infection, resulting in a significant interaction between diet and infection. The effects of diet on gizzard weight are similar to those obtained in Experiment 1. There was no significant main effect of diet or *E. acervulina* infection on gizzard pH (Table 3.8); however, there was a significant interaction between diet and infection. This interaction occurred because the whole sorghum diet reduced pH in infected chicks but increased pH in non-infected chicks.

At 21 days of age, diet was generally the only factor that had a significant effect on cecal microbial populations (Table 3.9). Chicks fed diets containing large ground corn had a significant decrease ($P < 0.03$) in cecal bifidobacteria and *C. perfringens* populations when compared to all other treatments. Jacobs et al. (2010) also showed a decrease in bifidobacteria populations when large ground corn sizes were included in the diet. Chicks fed diets containing

large ground corn, whole sorghum, or whole wheat also had reduced ($P < 0.02$) cecal lactobacilli and *E. coli* populations when compared to chicks fed fine ground corn. These responses are similar to those obtained in Experiment 1. In contrast, Engberg et al. (2004) reported that lactobacilli concentrations were 1 to 2 times higher in the ceca of birds fed whole wheat in comparison to those receiving no whole wheat.

For ileal microbial populations, the *E. acervulina* infection was generally the only factor that had a significant effect (Table 3.10). Bifidobacteria and lactobacilli populations were increased ($P < 0.04$; $P < 0.0007$) in infected birds when compared to non-infected birds; however, *C. perfringens* was also increased ($P < 0.04$) in infected birds, while *E. coli* populations were decreased ($P < 0.04$). Turk and LittleJohn (1987) also reported an increase in fecal lactobacilli during the period of 16 d post-inoculation; however, they used an *E. necatrix* infection, which is localized to the distal part of the duodenum and proximal part of the jejunum. Several other studies have also reported that an increase in *C. perfringens* may predispose chickens to necrotic enteritis, which often occurs as the most common secondary infection due to coccidiosis (Al-Sheikhly and Al-Saieg, 1980; Baba et al., 1997). The presence of this bacterium in poultry constitutes a risk for human health as *C. perfringens* is one of the most frequently isolated bacterial pathogens in foodborne disease outbreaks (Buzby and Roberts, 1997). The *E. acervulina* infection alone and the addition of large ground corn and whole wheat also increased ($P < 0.006$) small intestine weight, but there was no significant interaction between diet and *E. acervulina* infection (Table 3.10). The increase in intestinal weight for infected birds was similar to the results obtained in Experiment 1.

From these two experiments, we can conclude that an *E. acervulina* infection reduced chick performance, ME_n, and AA digestibility in Experiment 2, and the addition of large ground

corn, whole sorghum, or whole wheat were not effective in alleviating the negative effects of a coccidiosis infection. However, this may be dependent on the severity of the infection as the effects of *E. acervulina* on growth performance were inconsistent among experiments. In the absence of a coccidiosis infection, whole or large particle grains can be included in the diet of young chicks beginning from day of hatch without compromising growth performance and nutrient digestibility. In addition, relative gizzard weights were increased and cecal *E. coli* populations were decreased when large ground corn or whole grains were included in the diet, which may result in a healthier intestinal environment. Moreover, the increases in gizzard weights with the large ground corn and whole wheat or sorghum diets were greater in the presence than in the absence of a coccidiosis infection, indicating some particularly potential intestinal health benefits of feeding larger particle or whole grains in chicks infected or exposed to a coccidial challenge.

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Tables

Table 3.1. Nutritional composition (%) of chick diets used in Experiments 1 and 2.

Ingredient	Corn-SBM ¹	Whole Sorghum	Whole Wheat
Corn	49.00	29.60	29.60
Soybean meal	38.73	38.50	36.30
Whole Sorghum	-	20.00	-
Whole Wheat	-	-	20.00
Soybean oil	5.00	5.00	5.00
Limestone	1.31	1.31	1.31
Dicalcium phosphate	1.75	1.75	1.75
Salt	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15
DL-Methionine	0.19	0.19	0.19
Choline chloride	0.10	0.10	0.10
Dextrose	1.00	1.00	4.70
Solka Floc ⁴	1.87	1.50	-
Chromic Oxide	0.30	0.30	0.30
Calculated Analysis:			
Crude protein, %	23.1	23.1	23.1
ME _n , kcal/kg	3,068	3,070	3,064
Calcium, %	1.00	1.00	1.00
Nonphytate phosphorus, %	0.45	0.45	0.45

¹ Corn was ground through the following hammer mill screen sizes: 1.59 and 9.52 mm. This provided an average particle size of 557 and 1,387 μ m, respectively.

² Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μ g; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³ Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄•7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

⁴ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 3.2. Growth performance of chicks fed fine and large ground corn, whole sorghum, or whole wheat and inoculated with or without *Eimeria acervulina* oocysts in Experiment 1¹.

	Sham				<i>E. acervulina</i>				Pooled SEM	P- value ²		
	FGC ³	LGC ³	Sorghum	Wheat	FGC ³	LGC ³	Sorghum	Wheat		Diet	Ch	Inter.
Weight gain ⁴ (g/chick)												
PI d 0 to 7	234	226	221	229	218	213	229	208	13.17	0.92	0.27	0.71
PI d 7 to 10	144	158	148	147	123	147	150	134	8.17	0.11	0.06	0.49
PI d 0 to 14	545	569	523	537	512	543	552	526	24.52	0.67	0.52	0.66
Feed intake ⁴ (g/chick)												
PI d 0 to 7	310	299	323	310	284	283	328	289	14.53	0.11	0.18	0.75
PI d 7 to 10	193	191	216	192	171	182	208	181	10.66	0.04	0.11	0.90
PI d 0 to 14	739	750	780	734	697	725	775	714	29.78	0.21	0.28	0.94
Gain:feed ⁴ (g/kg)												
PI d 0 to 7	762	754	684	742	766	753	701	727	20.51	0.01	0.93	0.88
PI d 7 to 10	746	828	689	767	708	807	726	740	26.19	0.001	0.51	0.50
PI d 0 to 14	737	758	678	732	735	749	712	735	14.97	0.005	0.53	0.49

¹ Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age (post-inoculation (PI) day 0).

²Effect of diet, Sham vs. *E. acervulina* challenge (Ch), and diet x challenge interaction (Inter.).

³FGC = fine ground corn; LGC = large ground corn.

⁴Age periods: PI d 0 to 7 = day 8 to 15 of age; PI d 7 to 10 = day 15 to 18 of age; PI d 0 to 14 = day 8 to 22 of age.

Table 3.3. Effect of diet and *Eimeria acervulina* challenge on gizzard weight (% of body weight) and gizzard pH of chicks in Experiment 1¹.

	Weight	pH
Fine ground corn		
Sham	1.49	4.69
<i>E. acervulina</i>	1.52	4.56
Large ground corn		
Sham	2.07	4.88
<i>E. acervulina</i>	2.15	4.58
Whole sorghum		
Sham	2.10	5.28
<i>E. acervulina</i>	1.99	4.73
Whole wheat		
Sham	1.97	4.59
<i>E. acervulina</i>	2.03	4.93
Pooled SEM	0.05	0.27
	Probability	
Diet	<0.0001	0.58
<i>E. acervulina</i>	0.73	0.42
Diet x <i>E. acervulina</i>	0.26	0.43

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age.

Table 3.4. Effect of diet and *Eimeria acervulina* challenge on select cecal microbial populations and cecal weight in Experiment 1¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Cecal Wt. (% of BW ²)
	(log ₁₀ CFU/g DM)				
Fine ground corn					
Sham	6.91	9.74	10.97	6.71	1.01
<i>E. acervulina</i>	6.24	9.72	11.07	6.93	1.12
Large ground corn					
Sham	6.10	9.42	10.63	6.27	1.06
<i>E. acervulina</i>	7.15	9.99	10.46	6.47	0.96
Whole Sorghum					
Sham	6.85	10.43	10.62	5.90	1.00
<i>E. acervulina</i>	7.26	8.18	10.52	6.17	0.97
Whole wheat					
Sham	6.73	10.17	10.56	6.17	1.00
<i>E. acervulina</i>	7.08	7.58	10.00	6.10	1.01
Pooled SEM	0.21	0.42	0.17	0.30	0.07
	Probability				
Diet	0.10	0.16	0.002	0.09	0.78
<i>E. acervulina</i>	0.07	0.001	0.14	0.72	0.94
Diet x <i>E. acervulina</i>	0.005	0.001	0.28	0.66	0.57

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

² BW = body weight.

Table 3.5. Effect of diet and *Eimeria acervulina* challenge on select ileal microbial populations and small intestine weight in Experiment 1¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Intestine Wt. (% of BW ²)
	(log ₁₀ CFU/g DM)				
Fine ground corn					
Sham	6.23	9.92	9.96	6.29	3.19
<i>E. acervulina</i>	5.88	9.60	8.76	6.06	3.30
Large ground corn					
Sham	5.82	9.92	9.95	5.66	3.46
<i>E. acervulina</i>	5.61	9.77	8.81	6.49	4.23
Whole Sorghum					
Sham	5.73	9.81	9.79	6.32	3.46
<i>E. acervulina</i>	5.85	9.87	8.99	6.03	3.65
Whole wheat					
Sham	5.65	9.82	9.97	5.63	3.33
<i>E. acervulina</i>	5.34	9.27	8.42	5.64	4.02
Pooled SEM	0.22	0.20	0.25	0.25	0.23
	Probability				
Diet	0.11	0.41	0.85	0.12	0.08
<i>E. acervulina</i>	0.24	0.10	<0.0001	0.65	0.01
Diet x <i>E. acervulina</i>	0.70	0.48	0.54	0.12	0.36

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

² BW = body weight.

Table 3.6. Growth performance of chicks fed fine and large ground corn, whole sorghum, or whole wheat and inoculated with or without *Eimeria acervulina* oocysts in Experiment 2¹.

	Sham				<i>E. acervulina</i>				Pooled SEM	P- value ²		
	FGC ³	LGC ³	Sorghum	Wheat	FGC ³	LGC ³	Sorghum	Wheat		Diet	Ch	Inter.
Weight gain ⁴ (g/chick)												
PI d 0 to 7	243	265	282	243	147	147	157	121	9.17	0.003	<0.0001	0.39
PI d 7 to 10	150	177	183	164	113	110	127	101	7.06	0.008	<0.0001	0.17
PI d 0 to 14	559	643	648	592	399	393	436	364	21.59	0.01	<0.0001	0.24
Feed intake ⁴ (g/chick)												
PI d 0 to 7	328	339	374	331	262	264	254	257	16.73	0.61	<0.0001	0.37
PI d 7 to 10	209	238	253	224	188	180	192	168	9.08	0.03	<0.0001	0.12
PI d 0 to 14	797	866	921	821	680	678	741	655	27.9	0.009	<0.0001	0.58
Gain:feed ⁴ (g/kg)												
PI d 0 to 7	742	785	754	735	563	568	656	469	48.08	0.21	<0.0001	0.37
PI d 7 to 10	716	746	726	733	599	613	663	601	28.69	0.62	<0.0001	0.58
PI d 0 to 14	701	743	703	722	588	582	588	556	20.43	0.60	<0.0001	0.39

¹ Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age (post-inoculation (PI) day 0).

²Effect of diet, Sham vs. *E. acervulina* challenge (Ch), and diet x challenge interaction (Inter.).

³FGC = fine ground corn; LGC = large ground corn.

⁴Age periods: PI d 0 to 7 = day 8 to 15 of age; PI d 7 to 10 = day 15 to 18 of age; PI d 0 to 14 = day 8 to 22 of age.

Table 3.7. Effect of diet and *Eimeria acervulina* challenge on selected amino acid (AA) digestibility (%) and ME_n (kcal/kg DM) in Experiment 2¹.

Diet and AA or ME _n	Sham control		<i>E. acervulina</i>	
	Days of age			
	14	21	14	21
Fine ground corn				
Cysteine	76	78	50	76
Isoleucine	85	86	68	87
Lysine	85	86	69	89
Methionine	90	91	79	91
Threonine	79	81	61	80
Valine	84	85	66	85
ME _n	3,209	3,368	2,216	3,304
Large ground corn				
Cysteine	79	81	51	75
Isoleucine	88	87	69	86
Lysine	89	88	71	88
Methionine	92	91	78	92
Threonine	84	83	61	81
Valine	87	86	68	87
ME _n	3,288	3,374	1,909	3,273
Whole sorghum				
Cysteine	78	80	57	75
Isoleucine	87	88	73	87
Lysine	87	89	73	87
Methionine	91	92	80	90
Threonine	81	83	63	81
Valine	86	87	70	85
ME _n	3,442	3,533	2,325	3,393
Whole wheat				
Cysteine	78	80	42	76
Isoleucine	87	88	60	87
Lysine	87	87	61	87
Methionine	92	91	54	80
Threonine	81	82	59	85
Valine	86	86	59	85
ME _n	3,445	3,477	1,952	3,407

Table 3.7 con't

Day 14 probability				
	<u>Diet</u>	<u><i>E. acervulina</i></u>	<u>Diet x <i>E. acervulina</i></u>	<u>Pooled SEM</u>
Cysteine	0.06	<0.0001	0.08	2.0
Isoleucine	0.02	<0.0001	0.02	1.3
Lysine	0.02	<0.0001	0.03	1.4
Methionine	0.10	<0.0001	0.03	0.9
Threonine	0.08	<0.0001	0.07	1.4
Valine	0.02	<0.0001	0.02	1.3
ME _n	0.21	<0.0001	0.23	93.1

Day 21 probability				
	<u>Diet</u>	<u><i>E. acervulina</i></u>	<u>Diet x <i>E. acervulina</i></u>	<u>Pooled SEM</u>
Cysteine	0.97	0.0002	0.84	1.0
Isoleucine	0.12	0.94	0.49	0.5
Lysine	0.13	0.96	0.39	0.4
Methionine	0.61	0.90	0.19	0.4
Threonine	0.16	0.02	0.99	0.7
Valine	0.34	0.51	0.53	0.5
ME _n	< 0.0001	<0.0001	0.45	18.5

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age.

Table 3.8. Effect of diet and *Eimeria acervulina* challenge on gizzard weight (% of body weight) and gizzard pH of chicks in Experiment 2¹.

	Weight	pH
Fine ground corn		
Sham	1.55	3.97
<i>E. acervulina</i>	1.86	4.38
Large ground corn		
Sham	2.10	4.31
<i>E. acervulina</i>	2.69	3.81
Whole sorghum		
Sham	1.99	4.62
<i>E. acervulina</i>	2.59	4.04
Whole wheat		
Sham	1.91	3.97
<i>E. acervulina</i>	2.66	4.23
Pooled SEM	0.06	0.14
	Probability	
Diet	<0.0001	0.24
<i>E. acervulina</i>	<0.0001	0.28
Diet x <i>E. acervulina</i>	0.01	0.002

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age.

Table 3.9. Effect of diet and *Eimeria acervulina* challenge on select cecal microbial populations and cecal weight in Experiment 2¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Cecal Wt. (% of BW ²)
	(log ₁₀ CFU/g DM)				
Fine ground corn					
Sham	6.85	10.13	10.74	5.84	0.96
<i>E. acervulina</i>	7.09	10.53	11.21	6.21	1.10
Large ground corn					
Sham	6.44	8.74	10.22	5.57	1.01
<i>E. acervulina</i>	6.48	8.34	10.36	5.54	0.98
Whole Sorghum					
Sham	7.29	8.78	10.33	6.16	0.85
<i>E. acervulina</i>	7.03	9.39	10.67	6.18	1.00
Whole wheat					
Sham	6.64	9.11	10.37	5.72	1.02
<i>E. acervulina</i>	7.18	10.11	10.72	5.78	1.06
Pooled SEM	0.14	0.40	0.20	0.21	0.07
	Probability				
Diet	0.004	0.001	0.02	0.03	0.38
<i>E. acervulina</i>	0.18	0.17	0.03	0.49	0.15
Diet x <i>E. acervulina</i>	0.06	0.36	0.87	0.79	0.50

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

² BW = body weight.

Table 3.10. Effect of diet and *Eimeria acervulina* challenge on select ileal microbial populations and small intestine weight in Experiment 2¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Intestine Wt. (% of BW ²)
	log ₁₀ CFU/g DM				
Fine ground corn					
Sham	5.11	9.61	9.47	5.68	3.89
<i>E. acervulina</i>	5.45	10.04	8.66	5.92	5.09
Large ground corn					
Sham	5.33	8.92	9.99	5.25	4.27
<i>E. acervulina</i>	5.25	9.33	9.06	5.57	6.41
Whole Sorghum					
Sham	5.47	9.41	9.78	5.71	3.87
<i>E. acervulina</i>	5.50	9.90	9.63	5.80	6.03
Whole wheat					
Sham	5.08	9.00	9.64	5.16	4.26
<i>E. acervulina</i>	5.61	10.25	9.46	5.88	6.96
Pooled SEM	0.14	0.23	0.34	0.22	0.30
	Probability				
Diet	0.42	0.04	0.29	0.27	0.006
<i>E. acervulina</i>	0.04	0.0007	0.04	0.04	<0.0001
Diet x <i>E. acervulina</i>	0.12	0.24	0.54	0.55	0.12

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

² BW = body weight.

Chapter IV
The Effects of Short-term Feeding of Grobiotic[®], Dairylac-80, and Lactose on Growth Performance, Nutrient Digestibility, and Microbial Populations in Young Chicks

Abstract

Two experiments were conducted to evaluate the effects of short-term feeding of Grobiotic (GB), Dairylac-80, and lactose when fed to young chicks for 3, 7, or 21 d. In addition, one experiment was conducted to evaluate the effects of these additives in the presence and absence of an acute *Eimeria acervulina* infection. In Experiments 1 and 2, diets containing GB, Dairylac-80, and lactose had no consistent effects on growth performance whether they were fed in a corn-SBM diet or casein-dextrose diet. Chicks fed all casein-dextrose diets, regardless of supplementation, had increased weight gain and feed efficiency ($P < 0.05$) during the first week when compared to chicks fed the corn-SBM diet, as well as increased ME_n and AA digestibility. In both experiments, the addition of GB, Dairylac-80, and lactose resulted in a significant increase ($P < 0.05$) in cecal bifidobacteria populations even as early as 3 d of age. In addition, cecal lactobacilli increased ($P < 0.03$) and *E. coli* decreased ($P < 0.03$) when casein-dextrose diets were supplemented with GB, Dairylac-80, and lactose. The *E. acervulina* infection in the third experiment significantly reduced chick growth performance, ME_n, and AA digestibility ($P < 0.05$). However, the addition of GB, Dairylac-80, and lactose to the diet had no consistent effects on growth performance and did not ameliorate the negative effects of the coccidiosis infection. Overall, the results from this study indicated that very short-term feeding of various potential prebiotics for only 3 or 7 d, especially those which contain some lactose, had no negative effects on chick growth performance and can influence cecal microbial populations, particularly elevated levels of bifidobacteria and lactobacilli and decreased levels of *E. coli*.

Introduction

Rising concerns that the use of antibiotic growth promoters in livestock diets could possibly lead to antibiotic resistance to human microbial pathogens, coupled with the increasing pressure from consumers and politicians to reduce the use of antibiotic growth promoters in livestock diets, has prompted researchers and producers to investigate alternative feed additives that may maintain and improve animal health. Because microorganisms play such an important role in the health of the digestive tract and the health of the host animal, they can either have a beneficial or harmful effect on the host, depending on which species are present. Without the addition of antibiotic growth promoters, bacteria that could be considered pathogenic such as *Salmonella* spp., *Escherichia coli*, and *Clostridia perfringens* can proliferate and become dominant compared with other bacteria within the intestine (Niewold, 2007). Therefore, early establishment of beneficial microbial populations in the young chick is crucial in resisting colonization of pathogenic species, since chicks have a virtually sterile gastrointestinal tract upon hatch (Dibner and Richards, 2005). Altering the ingredients within a bird's diet can potentially influence the microbial populations in the gastrointestinal tract and their behavior. Incorporating compounds that exhibit prebiotic effects could possibly be one method to increasing these beneficial microbial populations, in addition to possibly increasing dietary energy utilization and nutrient digestibility.

As stated previously, Grobiotic (GB) is a prebiotic-type product that contains a proprietary mixture of partially-autolyzed yeast, yeast extract, dried whey solubles, and citric acid fermentation solubles. The product provides yeast cell wall and yeast soluble components, lactose, and other fermentable carbohydrates. Previous research conducted by Douglas et al. (2003) reported that when 5% GB was fed to commercial broiler chicks, a significant increase (*P*

< 0.05) in body weight gain was achieved during the first 2 weeks post-hatch. Persia et al. (2006) reported that inclusion of 2 to 6% GB in the diet of young chicks completely ameliorated the negative effects of coccidiosis infection in one experiment. Also, the addition of GB resulted in approximately a 6% increase in body weight gain of chicks that were not infected with coccidiosis. Altering the ingredients within a bird's diet can potentially influence the microbial populations in the gastrointestinal tract and their behavior. Biggs and Parsons (2008) showed that when 2 to 6% GB was included in a corn-soybean meal diet fed to young chicks, there was a significant linear increase in bifidobacteria and lactobacilli populations. Since GB contains lactose and research has demonstrated that supplementing lactose can alter the populations of lactobacilli and bifidobacteria (Morishita et al., 1982), it may exert similar effects. In addition, GB also contains yeast cell wall fractions, which have been shown to bind pathogenic bacteria (Oyofe et al., 1989; Spring et al., 2000) and inhibit the development of infection.

Another potential prebiotic compound is lactose. As mentioned previously, lactose is not a "true" prebiotic by definition, as enterocytes contain lactase; however, the lactase activity in birds is very low (Denbow et al., 2000), with 50% or more of ingested lactose reaching the ceca unchanged (Atkinson et al., 1957; Morishita et al., 1982) and acting as an available substrate for microbial fermentation. Lactose is believed to promote the growth of lactose-fermenting bacteria that either compete with pathogens for colonization or produce substances that are toxic to pathogens (Rehman et al., 2009). For example, research has demonstrated that supplementing lactose can alter the populations of lactobacilli and bifidobacteria (Morishita et al., 1982) and reduce cecal salmonella content (Chambers et al., 1997; Nisbet et al., 1993). This is possible because the chicken lacks lactase activity; therefore, the lactose can easily enter the ceca where the microbes present can ferment it (Siddons and Coates, 1972) and produce volatile fatty acids.

Most previous studies conducted with prebiotic-type compounds in poultry have involved feeding them for more than 21 days post-hatch, but it may be possible that feeding these potential prebiotics for shorter time periods may still improve microbial populations in the gastrointestinal tract, as well as lead to improvements in growth performance and nutrient utilization. Feeding these products for shorter time periods would be less expensive as cost is often the primary factor preventing the use of prebiotics in poultry diets. In addition, mortality is highest during the first week and short-term feeding of prebiotics may improve livability.

It is also of interest to determine if GB and lactose products exhibit any additional prebiotic effects on growth performance and nutrient digestibility when birds are challenged with *E. acervulina* coccidia infection. As mentioned earlier, Persia et al. (2006) reported that inclusion of 2 to 6% GB in the diet completely ameliorated the negative effects of coccidiosis infection in one experiment. Elmusharaf et al. (2006; 2007) also showed that chickens fed a diet supplemented with yeast cell wall, which is a component of GB, reduced the severity of infection due to coccidiosis. No studies to date have examined the effects of lactose on a coccidiosis infection; however, several researchers have demonstrated the effectiveness of lactose for reducing the cecal *Salmonella* and *C. perfringens* content of experimentally infected chicks (Chamber et al., 1997; Corrier et al, 1990; Nisbet et al., 1993; Takeda et al., 1995).

Therefore, the objectives of the following study were to evaluate the short-term feeding of GB, Dairy-lac-80, and lactose in both a corn-soybean meal diet and a casein-dextrose diet and examine the effects of these diets on growth performance at several ages, as well as the effects on nutrient digestibility, and ileal and cecal microbial populations of bifidobacteria, lactobacilli, *E. coli*, and *C. perfringens*. In addition, the effects of GB, Dairy-lac-80, and lactose on growth performance and intestinal characteristics during an *E. acervulina* coccidiosis infection will also

be determined. We hypothesize that feeding these compounds, even for short periods of 3 or 7 days, will improve beneficial microbial populations such as lactobacilli and bifidobacteria at the expense of *E. coli* and *C. perfringens* and will have no adverse effects on growth performance. Also, the addition of these potential prebiotics to the diet may have an indirect effect on reducing the severity of a coccidiosis infection.

Materials and Methods

Animals and Husbandry

The University of Illinois Institutional Animal Care and Use Committee approved all procedures. Experiment 1 and 2 were conducted with New Hampshire x Columbian male crossbred chicks, while Ross x Ross 308 commercial male broiler chicks were used in Experiment 3. Chicks were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally controlled room with light provided continuously. The wire floors of the batteries in Experiments 1 and 2 were left soiled from a previous experiment in an attempt to increase the chicks' exposure to a bacterial challenge. Both feed and water were provided ad libitum. Diets were formulated based on NRC (1994) feeding requirements for young chicks and were adequate in all nutrients unless otherwise noted. At hatch, chicks were weighed, wing-banded, and allotted to pens and dietary treatments so that the initial mean weight was similar among treatment groups.

Metabolizable Energy and Amino Acid Analysis

In assays where metabolizable energy (ME_n) and amino acid (AA) digestibility were evaluated, the following procedures were used. Chromic oxide was included in all diets at 0.3% as an indigestible marker. Excreta from each replicate pen were collected over a 24-hour period on either day 7, 14, and 21 post-hatch and freeze-dried. Feed and excreta samples from d 7, 14,

and 21 were analyzed for gross energy using an adiabatic bomb calorimeter. Amino acid concentrations [method 982.30E (a,b,c); (AOAC International, 2006)] and chromium [method 990.08; (AOAC International, 2006)] in the feed and excreta samples and nitrogen analyses [method 990.03; (AOAC International, 2006)] were determined at the University of Missouri-Columbia Experiment Station Chemical Laboratory. The ME_n of the diets was calculated using the equation described by Hill and Anderson (1958). Apparent digestibility of amino acids was calculated using the equation described by Adedokun et al. (2007).

Microbial Populations and pH

At the end of the feeding period, chicks were euthanized via CO₂ inhalation and ileal and cecal contents were extracted from all birds. For cecal samples, the contents of one cecum from each bird were used to determine percent dry matter of cecal contents and pH, while the contents of the other cecum were utilized for DNA extraction followed by quantitative PCR (qPCR) techniques described by Middelbos et al. (2007) with minor adaptations. For ileal samples, 0.5 g of distal ileal contents from each bird were pooled and used to determine percent dry matter of contents, while the remainder of the contents were pooled and utilized for DNA extraction. Cecal or ileal DNA was extracted from freshly collected samples that had been stored at -20°C until analysis using a QIAmp DNA Stool Mini-kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacterium* spp., *Lactobacillus* spp., and *E. coli*. Briefly, a 10 µl final volume contained 5 µl 2X SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted cecal DNA. Standard curves were obtained by previous

harvesting of pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution and amplified with the cecal DNA to create triplicate standard curves. Colony forming units (CFU) in each dilution were determined by plating on specific agars: *Lactobacillus* genus on Difco Lactobacilli MRS broth (Becton Dickinson Company, Sparks, MD), *Bifidobacterium* genus on Difco Reinforced Clostridial Medium (Becton Dickinson Company) and *E. coli* on Luria-Bertani Medium [10 g/l tryptose, 5 g/l yeast extract, 5 g/l NaCl (pH = 7)]. The calculated log CFU/mL of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate CFU/g of dry cecal or ileal contents.

Procedures for Coccidiosis Infection

For coccidiosis infection treatments, sporulated *Eimeria acervulina* oocysts (Animal Parasitic Diseases Laboratory, USDA, Beltsville, MD) were diluted with distilled water 1 day prior to inoculation. Chicks were orally gavaged with either 0.5 ml of sporulated oocysts in solution (1×10^6 oocysts/dose) or distilled water (sham inoculations) on day 8 of age (post-inoculation day 0) using 1 cc syringes. Body weights and feed intake were measured at the beginning of the experiment, as well as on day 0, 5, 7, 10, and 14 post-inoculation.

Experiment 1

The objective of this experiment was to evaluate the effects of short-term feeding of GB, Dairylac-80 (beta lactose enriched feed ingredient), and lactose in a corn-soybean meal diet on the growth performance and cecal microbial populations of young chicks. Four pens of 8 chicks were placed on 1 of 12 dietary treatments beginning at day of hatch: Treatments 1, 2, and 3 were a corn-soybean meal (SBM) control diet (Table 4.1) fed for 3, 7, or 21 d; 4, 5, and 6 were 5% GB fed for 3, 7, or 21 d; 7, 8, and 9 were 1.2% Dairylac-80 (80% lactose) fed for 3, 7, or 21 d; and

10, 11, and 12 were 1% crystalline lactose fed for 3, 7, or 21 d. All diets were formulated to contain 23.2% crude protein and 3,065 ME_n / kg. To keep the diets isocaloric, GB, Dairylac-80, and lactose were added to the control diet in place of dextrose and Solka Floc. It was assumed that the ME_n of GB was 2000 kcal/kg. The ME_n value for GB is an arbitrary estimated value based on the gross energy assuming that the dairy fraction would be poorly digested and that the yeast and dried fermentation extracts would be moderately to well digested. It was also assumed that Dairylac-80 and lactose would pass through the digestive system relatively undigested, so they were given an ME_n value of zero. The low levels of lactose or Dairylac-80 (equivalent to 1% lactose content) were chosen because high levels of lactose (> 5%) have been shown to increase the incidence of diarrhea in chicks (Waldroup et al., 1992) and a low level of 1% lactose would more likely be cost effective. Simoyi et al. (2006) also showed that feeding turkeys lactose levels of 0.5, 1, or 2% resulted in improved growth performance. When the end of a feeding period for GB, Dairylac-80, or lactose was reached, the treatments were ended at that time. Chick weight gain and feed intake were recorded at the beginning and the end of the 3, 7, or 21 d experimental feeding periods and feed efficiency was calculated for these time periods. The effect of diet on cecal microbial populations was also determined for each time period.

Experiment 2

The objective of this experiment was to evaluate the effects of short-term feeding of GB, Dairylac-80, and lactose in a casein-dextrose diet on the growth performance, ME_n, AA digestibility, and cecal microbial populations of young chicks. This diet was used because it contains little or no indigestible oligosaccharides whereas a corn-soybean meal diet, such as in Experiment 1, contains large amounts of indigestible oligosaccharides, mostly from the soybean meal, which could diminish and confound the effects of the potential prebiotic compounds. In

addition, the casein-dextrose diet has been shown to have very high ME_n and amino acid digestibility even in newly hatched chicks and it was reported to yield growth that was greater than that obtained from a corn-soybean meal diet during the first 7 d of life (Batal and Parsons, 2002). Four pens of 5, 10, or 15 chicks (depending on the age at collection) were placed on 1 of 15 dietary treatments beginning at day of hatch: Treatments 1, 2, and 3 were a casein-dextrose control diet (Table 4.1) fed for 3, 7, or 21 d; 4, 5, and 6 were 5% GB fed for 3, 7, or 21 d; 7, 8, and 9 were 1.2% Dairylac-80 (80% lactose) fed for 3, 7, or 21 d; 10, 11, and 12 were 1% crystalline lactose fed for 3, 7, or 21 d; and 13, 14, and 15 were a corn- soybean meal (SBM) control diet (Table 4.1) fed for 3, 7, or 21 d. GB, Dairylac-80, and lactose were added to the control casein-dextrose diet in place of Solka Floc. As in Experiment 1, it was assumed that the ME_n of GB was 2000 kcal/kg. When the end of a feeding period for GB, Dairylac-80, or lactose was reached, the treatments were ended at that time. Chick weight gain and feed intake were recorded at the beginning and the end of the experimental feeding periods and feed efficiency was calculated for these time periods. The effect of diet on cecal microbial populations was also determined for each time period.

Experiment 3

A 4 x 2 factorial arrangement of treatments was utilized to evaluate the effects of GB, Dairylac-80, and lactose in the presence and absence of an acute *E. acervulina* infection. Seven pens of 6 male Ross x Ross 308 commercial broiler chicks were allowed access to 1 of 4 diets containing control corn-SBM (Table 4.1) or the control diet supplemented with 5% GB, 1.2% Dairylac-80, or 1% crystalline lactose and inoculated with 0 or 1 x 10⁶ sporulated oocysts on day 8 of age. The effects of diet and coccidial infection on growth performance, ME_n, AA digestibility, cecal pH, and cecal and ileal weight and microbial populations were determined.

The diets were formulated to contain 23.2% crude protein and an average of 3065 kcal of ME_n / kg (Table 4.1) and were kept isocaloric by adjusting the content of dextrose and Solka Floc within each diet. Chicks were fed the experimental diets from 0 to 22 days of age. Body weights and feed intake were measured at the beginning of the experiment, as well as on day 0, 5, 7, 10, and 14 post-inoculation (d 8, 13, 15, and 22 of age).

Statistical Analysis

Analysis of variance was performed on means data from each pen using the general linear models (GLM) procedure of SAS (SAS Institute Inc., 1990) appropriate for completely randomized designs. If the F-test for treatment effect was significant in the ANOVA, differences among treatment means were determined using the least significant difference test (Carmer and Walker, 1985). The effects of dietary supplements were also determined with single degree of freedom contrast statements comparing chicks fed diets supplemented with GB, Dairylac-80, or lactose to chicks fed the control unsupplemented diet. Differences in growth performance, gastrointestinal characteristics, ME_n, and AA digestibility were considered significant when the $P < 0.05$, while differences in cecal and ileal microbial populations were considered significant when the $P < 0.10$. Experiment 3 was analyzed as a 4 x 2 factorial (4 diets with/without coccidiosis infection). When the main effect of diet was significant, single degree of freedom contrast statements were used to compare chicks fed dietary prebiotic supplements to chicks fed the unsupplemented control diet.

Results and Discussion

Experiment 1

Weight gain (Table 4.2) was generally not significantly affected by the supplementation of GB, Dairylac-80, or lactose for all three time periods when compared to the corn-SBM control

diet. These results are in agreement with Biggs and Parsons (2008) and Jacobs and Parsons (2009) who reported that feeding chicks 2 to 6% GB in a corn-SBM diet from day of hatch resulted in no significant increases in weight gain. In contrast, Douglas et al. (2003) and Persia et al. (2006) indicated that feeding chicks 2 to 6% GB resulted in significant ($P < 0.05$) increases in weight gain, especially during the first 2 wk post-hatch. In addition, Simoyi et al. (2006) and Torres-Rodriguez et al. (2007) reported that feeding turkey poults 0.1 to 8% lactose also improved weight gain from 0 to 6 wks of age. Thus, a consistent effect of GB and lactose products on growth does not occur in all experiments. There was a significant overall effect of dietary supplementation on both feed intake and feed efficiency at 3 and 7 d, respectively. When means were averaged using a single degree of freedom contrast statement for all supplemented treatments, feed intake was decreased ($P < 0.0002$; $P < 0.03$) when compared to the unsupplemented corn-SBM control, whereas feed efficiency was increased ($P < 0.0001$; $P < 0.01$); however, these effects did not continue to be significant for the remainder of the experiment. Douglas et al. (2003) also showed that when 5% GB was included in the diet of broiler chicks, feed efficiency from 0 to 7 d improved compared to the control diet. Simoyi et al. (2006) showed that a level of 1% lactose included in the diet of young turkeys improved feed efficiency when compared to diets containing no lactose.

At 3 and 7 d of age, there was an increase ($P < 0.001$) in cecal bifidobacteria for all GB, Dairy-lac-80, and lactose treatments when compared with the corn-SBM diet (Table 4.3). This is in agreement with previous studies conducted in our lab where concentrations of 5% GB resulted in an increase in bifidobacteria when fed for up to 7 d (unpublished data). Morishita et al. (1982) also showed increases in cecal bifidobacteria populations when lactose was included in the diet. There was an increase ($P < 0.10$) in *E. coli* populations at 3 d of age when both the 5% GB and

1.2% Dairylac-80 were fed, while feeding the 1% lactose diet resulted in a decrease ($P < 0.10$) in *E. coli* populations over all age periods. In contrast, Van der Wielen et al. (2002) reported increased *E. coli* populations when chicks were fed lactose; however, the authors fed 5% lactose compared to 1% lactose used in the current experiment. At 7 d of age, there was a decrease in lactobacilli ($P < 0.001$) for all dietary supplemented treatments, whereas there was no effect at 3 and 21 d. Morishita et al. (1982) also reported decreased lactobacilli populations in young chicks (5 and 14 d) when they were fed lactose from day of hatch. In contrast, previous studies conducted in our lab showed a significant linear increase ($P < 0.008$) in lactobacilli populations as 5% GB was included in the diet from 1 to 7 d of age (unpublished data). The variable results of the current study in combination with those from previous studies shows that the relationship between lactose level fed, microbial populations, and the environment within the ceca is a very complex one that requires further study.

Experiment 2

Weight gain (Table 4.4) was increased ($P < 0.05$) at 3 and 21 d for chicks fed the diet containing 5% GB when compared to the control casein-dextrose diet, while all other diets were not different from the control. When means were averaged using a single degree of freedom contrast statement for all supplemented treatments at 21 d, weight gain was increased ($P < 0.04$) when compared to the unsupplemented casein-dextrose control. In addition, all casein-dextrose diets, regardless of supplementation, had increased weight gain ($P < 0.0001$) during the first 3 d when compared to the corn-SBM diet; however, this did not persist throughout the remainder of the experiment. Batal and Parsons (2002) also reported that chicks fed a casein-dextrose diet immediately after hatching had significantly ($P < 0.05$) higher weight gains at 48 h post-hatching when compared to chicks fed a corn-SBM diet. At 7 and 21 d, feed intake was increased ($P <$

0.05) for the 5% GB treatment when compared to the casein-dextrose control. Feed efficiency for the chicks fed the corn-SBM diet was reduced ($P < 0.002$) compared to the casein-dextrose diets during the first week, but this result did not persist throughout the remainder of the experiment. This result is in agreement with Batal and Parsons (2002) who also showed that chicks fed a casein-dextrose diet had significantly ($P < 0.05$) higher feed efficiency than those fed a corn-SBM diet during the first week post-hatch. The results of the current experiment show that GB is able to increase growth of young chicks when added to a casein-dextrose diet and the combined results of Experiment 1 and 2 suggest that this type of diet is more sensitive than a corn-SBM diet for detecting this effect.

The ME_n of all diets increased significantly ($P < 0.05$) from 7 to 21 d of age (Table 4.5). At 7 days of age, all diets containing casein-dextrose yielded significantly higher ME_n values ($P < 0.05$) than the corn-SBM diet. This in agreement with Batal and Parsons (2002) who also showed increased ME_n values for a casein-dextrose diet when compared to a corn-SBM diet. In addition, these differences (400-484 kcal/kg) were much greater than the 99 kcal/kg difference based on calculated values (Table 4.1). The very high ME_n of the casein-dextrose diet indicates that glucose and casein are very well utilized by the young chick. At 21 days, all casein-dextrose diets still continued to show a significantly increased ($P < 0.05$) ME_n value when compared the corn-SBM diet, however, the differences were much smaller (150-200 kcal/kg). The supplementation of GB, Dairy-lac-80, or lactose to a casein-dextrose diet had no effect on ME_n when compared to the unsupplemented casein control diet.

As was observed with the ME_n of the diets, the amino acid digestibility increased for all amino acids at 21 d when compared to values at 7 d (Table 4.6). There were generally no differences in AA digestibility among the casein-dextrose treatments at 7 and 21 d. This result in

combination with no increase in ME_n suggests that the increase in weight gain which was observed in the 5% GB treatment is likely due to increased feed intake by the chicks rather than increased nutrient digestibility. When compared to the casein-dextrose control diet, AA digestibility was decreased for the corn-SBM diet. Again, this is in agreement with Batal and Parsons (2002) who showed increased AA digestibility values for a casein-dextrose diet compared to a corn-SBM diet, indicating that chicks have a high ability to absorb AA from highly digestible proteins even at a young age.

At 3 d of age, there was a significant increase ($P < 0.10$) in bifidobacteria when 5% GB and 1.2% DairyLac-80 were fed in comparison to the casein-dextrose control diet (Table 4.7). These results are similar to those obtained in Experiment 1. In addition, previous studies conducted in our lab where concentrations of 5% Grobiotic were added to a corn-SBM diet or a casein-dextrose diet also reported an increase ($P < 0.05$) in bifidobacteria populations (Biggs and Parsons, 2008; Jacobs et al. (unpublished data)). At 3 and 21 d of age, there was also an increase ($P < 0.03$) in lactobacilli for all supplemented treatments when compared with the casein-dextrose control diet. This is in contrast to the results obtained in Experiment 1. All casein-dextrose diets, regardless of supplementation, had decreased lactobacilli populations ($P < 0.004$) at all age periods when compared to the corn-SBM diet. The increase in lactobacilli populations in the corn-SBM diet as compared to the casein-dextrose diets is probably a result of the soybean oligosaccharides, raffinose and stachyose. These two compounds represent approximately 4 to 6% of the dry matter of the SBM (Coon et al., 1990), and have been shown to positively influence microbial populations (Hayakawa et al., 1990). Thus, the increased and high level of lactobacilli in the ceca of birds fed the corn-SBM diet may indicate that the diet is less sensitive than the casein-dextrose diet for detecting the effects of dietary prebiotic compounds in cecal

lactobacilli populations. At 7 d, *E. coli* was decreased ($P < 0.03$) in all supplemented dietary treatments and in the corn-SBM diet when compared to the casein-dextrose control diet.

From these two experiments, we can generally conclude that Grobiotic, Dairylac-80, and lactose had no consistent effects on growth performance across age periods when given to chicks for either 3, 7, or 21 days, whether it be in a corn-SBM diet or casein-dextrose diet. However, when these compounds were included in a casein-dextrose diet, weight gain at 21 d was increased ($P < 0.04$) for all supplemented treatments when compared to the unsupplemented casein-dextrose control, which suggests that this type of diet may be more sensitive than a corn-SBM diet for detecting these effects. This can be observed in the results obtained for cecal lactobacilli and *E. coli* populations as well. When GB, Dairylac-80, and lactose were supplemented to a casein-dextrose diet, lactobacilli was increased ($P < 0.03$) at 3 and 21 d, whereas in a corn-SBM diet, there was no effect on lactobacilli for all dietary supplemented treatments at these age periods. At 7 d, *E. coli* was decreased ($P < 0.03$) in all supplemented dietary treatments when compared to the casein-dextrose control diet, but in a corn-SBM diet, *E. coli* was only decreased when 1% lactose was fed.

Experiment 3

Growth performance for chicks infected with *E. acervulina* was significantly reduced ($P < 0.0001$) in comparison with sham-inoculated chicks (Table 4.8). These reductions were largest during the early post-inoculation period (d 0 to 7) and became less severe with increasing days post-inoculation. The depression in chick growth performance was similar to previous studies where coccidiosis was either experimentally induced (Perez et al., 2010; Persia et al., 2006; Willis and Baker, 1981a,b) or naturally occurred in commercial poultry production systems (Haug et al., 2008; Williams, 2005). A significant diet main effect for weight gain was also

observed for the 0 to 7 d post-inoculation period. This effect occurred because both GB and Dairylac-80 increased growth in both the presence and absence of a coccidial infection, but there was no significant interaction between diet and coccidiosis challenge. Persia et al. (2006) reported that the inclusion of 5% GB to the diet of chicks infected with *E. acervulina* completely eliminated the negative growth effects of a coccidiosis challenge in one experiment. The differences between the two studies may be due to the dosage level used (1×10^6 vs. 5×10^5 sporulated oocysts, respectively) and the severity of the coccidial infection between the 2 experiments. In the current study, the magnitude of the growth depression caused by coccidiosis was much greater than in the Persia et al. (2006) study (13 vs. 29%, respectively). Thus, the coccidial infection in the current study may have been too severe for any protective effects of GB to ameliorate the negative response. There was a significant interaction ($P < 0.03$) between diet and *E. acervulina* infection during the 0 to 7 d post-inoculation period for feed intake. This interaction was probably due to the lactose decreasing feed intake in the absence of a coccidial infection, but increasing feed intake in the presence of a coccidial infection. Feed efficiency (Table 4.8) over all time periods was not affected by any dietary treatment.

Acute infection of chicks with *E. acervulina* resulted in large and significant reductions ($P < 0.0001$) in ME_n and AA digestibility at 14 d of age (6 d post-inoculation) when compared to sham-inoculated chicks (Table 4.9). The reductions in ME_n and AA digestibility became less severe by 21 d of age (14 d post-inoculation) when the effect of infection was only significant for Cys, Thr, Val, and ME_n . The 14 d results are in agreement with previous studies that also reported significant reductions in both ME_n and AA digestibility during an *E. acervulina* infection (Persia et al., 2006; Ruff and Wilkins, 1980; Takhar and Farrell, 1979a,b), and is primarily attributed to reduced absorption capabilities due to intestinal tissue damage. The

effects of diet on ME_n and AA digestibility were generally inconsistent across age periods (Table 4.9). ME_n and AA digestibility values at 14 d for non-infected chicks fed diets containing GB, Dairylac-80, and lactose were not statistically different from the control. However, at 21 d, diets which contained any dietary supplement had decreased ($P < 0.007$) ME_n when compared to the corn-SBM control, and diets which contained GB and Dairylac-80 had decreased AA digestibility ($P < 0.05$) for most amino acids. There were no diet x coccidiosis interactions for ME_n or any AA at 14 or 21 d of age, again indicating that the dietary supplements did not ameliorate the coccidial infection.

The *E. acervulina* infection generally had no significant effect on cecal microbial populations, except for increasing cecal *E. coli* ($P < 0.02$) when chicks were fed the corn-SBM diet (Table 4.10). All dietary treatments also generally had no significant effects on any cecal microbial populations, except the 5% GB treatment increased lactobacilli ($P < 0.01$) populations when compared to the corn-SBM control diet. The *E. acervulina* infection reduced ($P < 0.0001$) cecal pH (Table 4.10). Ruff et al. (1973) reported that *E. acervulina* infection increased ($P < 0.05$) cecal pH in inoculated birds 4 to 7 d post-inoculation compared to uninoculated controls; however, at 9 d post-inoculation there was no difference in pH. Cecal pH was also reduced in all chicks fed the GB diet; however, there was no significant interaction between diet and *E. acervulina* infection. This is similar to a previous study conducted by our lab which also showed a reduction ($P < 0.05$) in cecal pH when chicks were fed 5% GB (unpublished data). There was also a small increase in cecal weight during the coccidial challenge.

Similar patterns can be seen for ileal microbial populations (Table 4.11). The *E. acervulina* infection generally had no significant effect on ileal microbial populations, except for decreasing ileal lactobacilli ($P < 0.004$) when chicks were fed the GB or lactose treatments. All

supplemented treatments generally had no significant differences for any microbial populations when compared to the corn-SBM control diet, except chicks fed diets containing Dairylac-80 had a significant increase ($P < 0.04$) in ileal *E. coli* populations when compared to the corn-SBM treatment. McReynolds et al. (2007) also reported supplementation of lactose in the diets of broiler chicks failed to affect the intestinal microbial populations of lactobacilli, *Clostridium* spp., and *E. coli*. The *E. acervulina* infection increased ($P < 0.0001$) small intestine weight in all dietary treatments (Table 4.11). Allen (1984) also reported increased weights of all intestinal segments when birds were inoculated with 1×10^6 sporulated *E. acervulina* oocysts and attributed the increase to swelling and edema within the tissue.

From these three experiments, we can conclude that an *E. acervulina* infection reduced chick growth performance, ME_n, and AA digestibility in Experiment 3, and the addition of GB, Dairylac-80, and lactose was not effective in alleviating the negative effects of a severe coccidiosis infection. In the absence of a coccidiosis infection, the results from this study indicate that the feeding of GB, Dairylac-80, and lactose may yield some beneficial responses in chick growth performance, especially when added to a casein-dextrose diet; however, the responses are not consistent. Also, feeding various potential prebiotics, especially those which contain some lactose, may yield beneficial responses in cecal microbial populations in young chicks, particularly elevated levels of bifidobacteria and lactobacilli and decreased levels of *E. coli*. In addition, these results show that dietary prebiotics can have an influence on cecal and ileal microbial populations even when fed for a short period of only 3 or 7 d. Feeding a highly digestible diet, such as casein-dextrose, improves early growth (0 to 3 d of age) of chicks when compared to feeding a corn-SBM diet, and may be more sensitive than a corn-SBM diet for detecting the effects on cecal microbial populations.

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Tables

Table 4.1. Nutritional composition (%) of control diets used in Experiments 1-3.

Ingredient	Experiment 1 & 3	Experiment 2	
	Corn-SBM ¹	Casein-dextrose	Corn-SBM
Corn	45.69	-	45.69
Soybean meal	39.71	-	38.30
Casein	-	24.20	-
Dextrose	2.90	57.00	5.66
Soybean oil	5.00	3.00	5.00
Limestone	1.20	-	1.40
Dicalcium phosphate	2.02	-	2.00
Salt	0.40	-	0.60
Vitamin mix ²	0.20	-	-
Purified diet vitamin mix ³	-	0.20	0.20
Trace mineral mix ⁴	0.15	-	0.15
Purified diet mineral mix ⁵	-	5.37	-
DL-Methionine	0.19	0.14	0.20
L-Arg	-	0.60	-
Glycine	-	0.90	0.40
MgSO ₄	-	0.20	-
Choline chloride	0.10	0.25	0.10
Solka Flocc ⁶	2.14	7.82	-
DL- α -tocopherol acetate	-	0.002	-
Ethoxyquin	-	0.013	-
Chromic Oxide	0.30	0.30	0.30
Calculated Analysis:			
Crude protein, %	23.2	23.0	23.0
ME _n , kcal/kg	3,065	3,207	3,108
Calcium, %	1.02	1.09	1.09
Nonphytate phosphorus, %	0.50	0.60	0.50

¹ Diets were kept isocaloric within experiment by adjusting the content of dextrose and Solka Flocc within each diet.

² Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μ g; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³ Provided per kilogram of diet: thiamin-HCl, 20 mg; retinyl acetate, 1,789 µg; cholecalciferol, 15 µg; vitamin B₁₂, 0.04 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; niacin, 50 mg; menadione dimethylpyrimidinol bisulfite, 2 mg; pyridoxine- HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; ascorbic acid, 250 mg.

⁴ Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄• 7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

⁵ Provided per kilogram of diet:Ca₃(PO₄)₂, 28g; K₂PO₄, 9g; NaCl, 8.89g; MgSO₄-7H₂O, 3.5 g; ZnCO₃, 0.10g; MnSO₄-H₂O, 0.65 g; FeSO₄-7H₂O, 0.42g; KI, 40 mg; CuSO₄-5H₂O, 20 mg; Na₂MoO₄-2H₂O, 9 mg, H₃BO₃, 9 mg; CoSO₄-7H₂O, 1 mg; NaSeO₃, 0.22 mg.

⁶ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 4.2. Effect of short-term feeding of Grobiotic, Dairylac-80, and lactose in a corn-soybean meal diet on the growth performance of chicks in Experiment 1.

Dietary treatment ^{1, 2}	Weight gain (g/chick)			Feed intake (g/chick)			Gain/feed (g/kg)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Corn-SBM control	14 ^{bc}	47 ^b	350 ^{ab}	47 ^a	136	624	289 ^b	344 ^b	561
5% Grobiotic	16 ^a	47 ^b	341 ^{ab}	38 ^b	123	617	424 ^a	383 ^b	557
1.2% Dairylac-80	15 ^{ab}	53 ^a	368 ^a	39 ^b	119	634	400 ^a	454 ^a	581
1% lactose	13 ^c	46 ^b	329 ^b	36 ^b	127	629	374 ^a	376 ^b	528
Pooled SEM	0.8	2.1	8.9	1.9	6.1	27.2	22.7	23.7	20.0

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² SBM = soybean meal.

Table 4.3. Effect of short-term feeding of Grobiotic, Dairylac-80, and lactose in a corn-soybean meal diet on select cecal microbial populations in Experiment 1.

Dietary treatment ^{1,2}	Bifidobacteria			Lactobacilli			<i>Escherichia coli</i>		
	(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Corn-SBM control	7.10 ^c	6.93 ^b	7.43 ^{ab}	11.08	11.29 ^a	10.13	11.44 ^b	11.20 ^a	11.11 ^{ab}
5% Grobiotic	8.18 ^a	7.86 ^a	7.67 ^a	11.13	10.73 ^b	10.46	12.14 ^a	11.32 ^a	11.42 ^a
1.2% Dairylac-80	8.10 ^{ab}	7.84 ^a	7.29 ^b	10.89	10.80 ^b	10.12	11.73 ^a	11.43 ^a	10.65 ^b
1% lactose	7.85 ^b	7.77 ^a	7.31 ^b	10.78	10.53 ^b	10.28	10.48 ^c	10.00 ^b	9.92 ^c
Pooled SEM	0.12	0.10	0.14	0.14	0.16	0.21	0.21	0.22	0.23

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² SBM = soybean meal.

Table 4.4. Effect of short-term feeding of Grobiotic, Dairylac-80, and lactose in a casein-dextrose diet on growth performance in chicks in Experiment 2.

Dietary treatment ^{1,2}	Weight gain (g/chick)			Feed intake (g/chick)			Gain/feed (g/kg)		
	Days of age			Days of age			Days of age		
	3	7	21 ³	3	7	21	3	7	21
Casein control	20 ^{ab}	36	273 ^b	27	77 ^{bc}	496 ^b	737 ^a	479 ^a	554
5% Grobiotic	21 ^a	40	328 ^a	29	93 ^a	566 ^a	771 ^a	427 ^{ab}	579
1.2% Dairylac-80	19 ^b	38	312 ^{ab}	28	83 ^{abc}	576 ^a	693 ^a	461 ^a	544
1% lactose	19 ^b	35	305 ^{ab}	26	70 ^c	520 ^{ab}	754 ^a	500 ^a	586
Corn-SBM	11 ^c	33	308 ^{ab}	29	89 ^{ab}	538 ^{ab}	386 ^b	373 ^b	571
Pooled SEM	0.7	2.5	13.3	1.6	5.1	22.9	34.9	26.2	20.5

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 15 chicks (3 days), 10 chicks (7 days), or 5 chicks (21 days) each per treatment.

² SBM = soybean meal.

³ Weight gain was increased ($P < 0.04$) for all supplemented dietary treatments when compared to unsupplemented casein control based on a single degree of freedom contrast.

Table 4.5. Effect of diet and age on ME_n (kcal/kg DM) in Experiment 2.

Dietary treatment ^{1,2}	Days of age		Pooled SEM
	7	21	
Casein control	2,872 ^{az}	3,446 ^{aby}	32.7
5% Grobiotic	2,875 ^{az}	3,434 ^{aby}	55.0
1.2% Dairylac-80	2,886 ^{az}	3,396 ^{by}	38.2
1% lactose	2,956 ^{az}	3,453 ^{ay}	26.3
Corn-SBM	2,472 ^{bz}	3,246 ^{cy}	50.3
Pooled SEM	56.2	18.8	

^{a-d} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.05$).

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 10 chicks (7 days) or 5 chicks (21 days) each per treatment.

² SBM = soybean meal.

Table 4.6. Effect of diet and age on selected amino acid digestibility (%) in Experiment 2.

Amino acid and dietary treatment	Days of age		Pooled SEM	Amino acid and dietary treatment	Days of age		Pooled SEM
	7	21			7	21	
Cysteine				Methionine			
Casein control	14 ^y	71 ^z	4.9	Casein control	84 ^y	97 ^z	1.0
5% Grobiotic	15 ^y	67 ^z	9.2	5% Grobiotic	81 ^y	96 ^z	1.9
1.2% Dairylac-80	4 ^y	65 ^{z*}	3.3	1.2% Dairylac-80	85 ^y	97 ^z	1.0
1% lactose	11 ^y	71 ^z	5.9	1% lactose	87 ^y	98 ^z	0.8
Corn-SBM	56 ^{y*}	78 ^{z*}	2.4	Corn-SBM	80 ^y	92 ^{z*}	1.5
Pooled SEM	7.8	1.8		Pooled SEM	1.8	0.4	
Isoleucine				Threonine			
Casein control	74 ^y	93 ^z	1.4	Casein control	66 ^y	91 ^z	1.5
5% Grobiotic	71 ^y	92 ^z	2.3	5% Grobiotic	62 ^y	89 ^z	3.2
1.2% Dairylac-80	74 ^y	93 ^z	1.3	1.2% Dairylac-80	66 ^y	90 ^z	1.5
1% lactose	78 ^y	94 ^z	1.1	1% lactose	71 ^y	92 ^z	1.4
Corn-SBM	73 ^y	87 ^{z*}	1.7	Corn-SBM	64 ^y	81 ^{z*}	1.6
Pooled SEM	2.2	0.6		Pooled SEM	2.7	0.8	
Lysine				Valine			
Casein control	88 ^y	97 ^z	0.9	Casein control	75 ^y	94 ^z	1.4
5% Grobiotic	86 ^y	96 ^z	1.4	5% Grobiotic	71 ^y	94 ^z	2.3
1.2% Dairylac-80	88 ^y	97 ^z	0.7	1.2% Dairylac-80	75 ^y	94 ^z	1.3
1% lactose	90 ^y	97 ^z	0.7	1% lactose	79 ^y	95 ^z	1.2
Corn-SBM	75 ^{y*}	87 ^{z*}	1.5	Corn-SBM	70 ^y	85 ^{z*}	1.8
Pooled SEM	1.5	0.4		Pooled SEM	2.3	0.6	

*Means within a column for each amino acid (diet effect) are significantly ($P < 0.05$) different than the casein control diet.

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 10 chicks (7 days) or 5 chicks (21 days) each per treatment.

² SBM = soybean meal.

Table 4.7. Effect of short-term feeding of Grobiotic, Dairylac-80, and lactose in a casein-dextrose diet on select cecal microbial populations in Experiment 2.

Dietary treatment ^{1,2}	Bifidobacteria			Lactobacilli			<i>Escherichia coli</i>		
	(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Casein control	6.09 ^b	6.15 ^{ab}	6.17 ^b	7.73 ^c	7.53 ^{bc}	8.47 ^b	10.55	10.17 ^a	10.74
5% Grobiotic	6.61 ^a	6.27 ^a	6.25 ^{ab}	9.31 ^b	7.92 ^{ab}	8.91 ^b	11.10	9.43 ^b	11.14
1.2% Dairylac-80	6.59 ^a	6.16 ^{ab}	6.07 ^b	9.82 ^{ab}	7.44 ^c	8.64 ^b	11.02	8.87 ^{bc}	10.36
1% lactose	6.49 ^{ab}	6.41 ^a	6.47 ^{ab}	9.93 ^{ab}	8.06 ^a	10.09 ^a	11.23	9.33 ^b	11.34
Corn-SBM	6.61 ^a	5.70 ^b	6.74 ^a	10.55 ^a	8.29 ^a	9.97 ^a	11.37	8.58 ^c	10.39
Pooled SEM	0.17	0.20	0.20	0.33	0.16	0.31	0.41	0.26	0.40

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 15 chicks (3 days), 10 chicks (7 days), or 5 chicks (21 days) each per treatment.

² SBM = soybean meal.

Table 4.8. Growth performance of chicks fed Grobiotic, Dairylac-80, and lactose and inoculated with or without *Eimeria acervulina* oocysts in Experiment 3^{1,2}.

	Sham				<i>E. acervulina</i>				PSEM	P- value ³		
	C-SBM	GB	DL-80	LAC	C-SBM	GB	DL-80	LAC		Diet	Ch	Inter.
Weight gain ⁴ (g/chick)												
PI d 0 to 7	296	318	310	286	159	189	173	167	9.33	0.02	<0.0001	0.73
PI d 7 to 10	183	174	180	179	139	136	134	132	7.31	0.84	<0.0001	0.94
PI d 0 to 14	656	649	658	641	466	518	478	488	19.35	0.66	<0.0001	0.41
Feed intake ⁴ (g/chick)												
PI d 0 to 7	399	431	417	392	315	343	346	361	10.26	0.03	<0.0001	0.03
PI d 7 to 10	248	255	248	243	213	221	222	220	6.76	0.64	<0.0001	0.74
PI d 0 to 14	911	947	929	900	778	831	829	842	22.22	0.23	<0.0001	0.38
Gain:feed ⁴ (g/kg)												
PI d 0 to 7	740	738	745	730	502	543	501	465	19.30	0.18	<0.0001	0.33
PI d 7 to 10	740	683	727	734	653	606	603	604	25.33	0.24	<0.0001	0.67
PI d 0 to 14	721	686	709	712	598	623	576	581	14.39	0.65	<0.0001	0.06

¹ Data are means of 7 replicates of 6 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age (post-inoculation (PI) day 0).

² C-SBM = corn-soybean meal control; GB = 5% Grobiotic; DL-80 = 1.2% Dairylac-80; LAC = 1% lactose.

³ Effect of diet, Sham vs. *E. acervulina* challenge (Ch), and diet x challenge interaction (Inter.).

⁴ Age periods: PI d 0 to 7 = day 8 to 15 of age; PI d 7 to 10 = day 15 to 18 of age; PI d 0 to 14 = day 8 to 22 of age.

Table 4.9. Effect of diet and *Eimeria acervulina* challenge on selected amino acid (AA) digestibility (%) and ME_n (kcal/kg DM) in Experiment 3¹.

Diet and AA or ME _n	Sham control		<i>E. acervulina</i>	
	Days of age			
	14	21	14	21
Corn-SBM control				
Cysteine	81	82	57	77
Isoleucine	88	85	71	84
Lysine	87	84	72	84
Methionine	92	90	81	89
Threonine	83	82	65	79
Valine	86	84	70	83
ME _n	3,407	3,447	2,493	3,361
5% Grobiotic				
Cysteine	76	74	45	70
Isoleucine	86	83	65	83
Lysine	85	82	66	81
Methionine	91	90	78	89
Threonine	80	78	61	77
Valine	84	82	64	81
ME _n	3,268	3,308	2,103	3,256
1.2% Dairy-lac-80				
Cysteine	78	78	42	73
Isoleucine	87	83	64	83
Lysine	85	82	64	82
Methionine	91	89	76	89
Threonine	80	78	57	76
Valine	85	82	63	81
ME _n	3,290	3,323	1,927	3,280
1% lactose				
Cysteine	78	78	52	73
Isoleucine	87	84	70	82
Lysine	85	83	71	81
Methionine	91	89	80	88
Threonine	80	78	65	76
Valine	85	82	69	81
ME _n	3,283	3,358	2,070	3,267

Table 4.9 con't

Day 14 probability				
	<u>Diet</u>	<u><i>E. acervulina</i></u>	<u>Diet x <i>E. acervulina</i></u>	<u>Pooled SEM</u>
Cysteine	0.02	<0.0001	0.19	2.1
Isoleucine	0.29	<0.0001	0.52	1.8
Lysine	0.18	<0.0001	0.45	1.6
Methionine	0.44	<0.0001	0.56	1.1
Threonine	0.12	<0.0001	0.40	1.7
Valine	0.23	<0.0001	0.53	1.7
ME _n	0.03	<0.0001	0.27	79.3

Day 21 probability				
	<u>Diet</u>	<u><i>E. acervulina</i></u>	<u>Diet x <i>E. acervulina</i></u>	<u>Pooled SEM</u>
Cysteine	< 0.0001	<0.0001	0.89	0.8
Isoleucine	0.03	0.22	0.82	0.5
Lysine	0.008	0.19	0.93	0.5
Methionine	0.21	0.07	0.94	0.4
Threonine	0.0009	0.002	0.78	0.6
Valine	0.02	0.05	0.89	0.5
ME _n	<0.0001	0.0002	0.61	15.1

¹Data are means of 4 replicates of 5 chicks orally gavaged with either a single dose of distilled water (Sham) or 1×10^6 sporulated oocysts of *Eimeria acervulina* on d 8 of age.

Table 4.10. Effect of diet and *Eimeria acervulina* challenge on select cecal microbial populations, cecal weight, and cecal pH in Experiment 3¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Cecal Wt. (% of BW ²)	Cecal pH
	(log ₁₀ CFU/g DM)					
Corn-SBM ² control						
Sham	6.25	8.90	9.80	5.39	1.07	6.13
<i>E. acervulina</i>	6.51	9.77	10.55	5.41	1.11	5.65
5% Grobiotic						
Sham	6.67	9.91	10.40	5.74	1.08	5.64
<i>E. acervulina</i>	6.47	9.74	10.71	5.38	1.23	5.55
1.2% Dairylac-80						
Sham	6.41	9.12	10.52	5.65	1.05	5.89
<i>E. acervulina</i>	6.35	8.99	10.60	5.70	1.14	5.61
1% lactose						
Sham	6.34	9.09	10.39	5.81	1.15	5.94
<i>E. acervulina</i>	6.26	9.01	10.56	5.61	1.15	5.60
Pooled SEM	0.17	0.25	0.20	0.20	0.05	0.10
	Probability					
Diet	0.45	0.01	0.18	0.41	0.40	0.04
<i>E. acervulina</i>	0.87	0.50	0.02	0.40	0.04	<0.0001
Diet x <i>E. acervulina</i>	0.57	0.14	0.34	0.70	0.46	0.27

¹Data are means of 7 replicates of 6 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

²BW = body weight; SBM = soybean meal;

Table 4.11. Effect of diet and *Eimeria acervulina* challenge on select ileal microbial populations and small intestine weight in Experiment 3¹.

Dietary treatment	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>	<i>Clostridium perfringens</i>	Intestine Wt. (% of BW ²)
	(log ₁₀ CFU/g DM)				
Corn-SBM ² control					
Sham	5.30	9.92	9.63	5.64	3.52
<i>E. acervulina</i>	5.61	9.94	9.70	5.63	5.19
5% Grobiotic					
Sham	5.79	10.15	10.19	5.73	3.67
<i>E. acervulina</i>	5.57	9.35	9.54	5.60	4.94
1.2% Dairylac-80					
Sham	5.63	9.49	10.56	5.55	3.59
<i>E. acervulina</i>	5.59	9.85	10.34	5.59	5.43
1% lactose					
Sham	5.65	10.05	10.19	5.68	3.62
<i>E. acervulina</i>	5.29	9.55	9.96	5.24	5.40
Pooled SEM	0.14	0.16	0.27	0.15	0.17
	Probability				
Diet	0.29	0.44	0.04	0.54	0.53
<i>E. acervulina</i>	0.43	0.05	0.18	0.22	<0.0001
Diet x <i>E. acervulina</i>	0.10	0.004	0.63	0.40	0.35

¹Data are means of 7 replicates of 6 chicks orally gavaged with either a single dose of distilled water (Sham) or 1 x 10⁶ sporulated oocysts of *Eimeria acervulina* on d 8 of age.

² BW = body weight; SBM = soybean meal.

Chapter V

The Effects of Short-term Feeding of Several Feed Additives on Growth Performance, Nutrient Digestibility, and Microbial Populations in Young Chicks

Abstract

Six experiments were conducted to evaluate the effects of short-term feeding and the prebiotic potential of several feed additives when fed to young chicks. Experiments 1-3 evaluated the effects of feeding Temulose, partially hydrolyzed Temulose, and Grobiotic in a corn-soybean meal diet on the growth performance, metabolizable energy (ME_n), amino acid digestibility, and select cecal microbial populations of young chicks when fed for 3, 7, or 21 d of age. Experiments 4-6 evaluated the effects of DDGS, Elusieve fiber, and Alternan in a corn-soybean meal or casein-dextrose diet on the growth performance, ME_n , amino acid digestibility, and cecal microbial populations of young chicks when fed for 3, 7, or 21 d. From Experiments 1-3, Temulose and partially hydrolyzed Temulose had no consistent effects on weight gain, feed intake, or feed efficiency. However, crude Temulose did increase ME_n and amino acid digestibility when fed for a period of 21 d. The addition of partially hydrolyzed Temulose to the diet of chicks at 3 d increased populations of bifidobacteria, lactobacilli, and *E. coli* when compared to the original, nonhydrolyzed Temulose. However, both Temulose compounds also decreased ($P < 0.10$) lactobacilli populations at 7 days of age. From Experiments 4-6, diets containing Elusieve fiber and Alternan had no consistent effects on growth performance whether it is in a corn-SBM diet or casein-dextrose diet. Chicks fed all casein-dextrose diets, regardless of supplementation, had increased weight gain and feed efficiency ($P < 0.05$) during the first week when compared to chicks fed the corn-SBM diet, as well as increased ME_n and AA digestibility. Bifidobacteria were decreased ($P < 0.10$) when 2% Alternan was added to a corn-

SBM diet and this same result was observed for the casein-dextrose diet. Also, the overall microbial colony forming units for the diets which were based on corn-SBM were higher than for the diets based on casein-dextrose. Overall, the results from this study indicated that very short-term feeding of various potential prebiotics had no negative effects on chick growth performance, and that dietary ingredients, such as prebiotics, can influence cecal microbial populations even when given to the chick for a period of 3 or 7 d.

Introduction

As poultry production systems become more intensive and competitive, it is important to continually evaluate the current practices in place, as well as explore new opportunities. In light of the current reduction in use and possible future ban of antibiotic growth promoters in the United States, the use of alternative feed ingredients represents what is possibly the greatest opportunity for nutritionists and poultry producers alike. The application of prebiotic-type compounds may provide one such alternative. Many compounds with prebiotic properties such as fructooligosaccharides, mannanoligosaccharides, and galactooligosaccharides have been researched extensively, but there is still interest in the development and evaluation of new prebiotic products. Gibson and Roberfroid (1995) originally classified prebiotics as a “non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the host health”. This definition, however, was based on the use of prebiotics in humans and their use in animals and particularly birds may be more complex.

One such product that may provide beneficial effects to the bird and possibly alter the microbial populations within the gut is Elusieve fiber. This product is produced from DDGS using the Elusieve process. In this process, the combination of sieving and air aspirating

(elutriation) separate fiber from DDGS to produce a lighter, higher fiber fraction (Elusieve fiber) and a heavier, lower fiber fraction (Srinivasan et al., 2005). Fiber has been shown to have positive effects on gut health and welfare in many species, but the effects in poultry are still largely unknown.

Other potential prebiotic-type compounds, Temulose and partially hydrolyzed Temulose, (Temple-Inland, Diboll, TX) are hemicellulose by-products of the fiberboard industry and analysis has shown that they contain a high galactoglucomannan-type oligosaccharide content composed mainly of mannanoligosaccharides (MOS), xylooligosaccharides, and glucooligosaccharides (Faber et al., 2010). Several studies have shown that these compounds exert beneficial effects in the intestinal tract in many species by increasing the production of short chain fatty acids, lowering pH, and altering microbial populations to that of a healthier profile (Djouzi and Andrieux, 1997; Djouzi et al., 1995; Flickinger et al., 2000; Smiricky-Tjardes et al., 2003; Swanson and Fahey, 2002). However, the beneficial effects of the galactoglucomannan-type oligosaccharide substrate in Temulose have not been previously explored as a prebiotic in poultry.

Another potential prebiotic product is alternansucrase-derived oligosaccharides. When grown on sucrose, the bacteria *Leuconostoc mesenteroides* NRRL B-21297 produces the glucansucrase, alternansucrase, which catalyses the production of a polysaccharide (alternan) consisting of alternating α -1,3 and α -1,6 linkages (Cote and Holt, 2007). In the presence of acceptor carbohydrates, alternansucrase presents the same specificity as for alternan synthesis and catalyses the production of oligoalternans (Cote et al., 2008). In pure culture, these products show some bifidogenic properties, in addition to preventing coliform and pathogenic bacteria

growth (Cote and Holt, 2007). However, there are no data available on their effects using mixed bacteria or their effect in poultry.

Also, since microorganisms play an important role in the health of the digestive tract and the health of the host animal, early establishment of beneficial microbial populations in the young chick is crucial in the development of the immune system and in resisting colonization of pathogenic species, since chicks have a virtually sterile gastrointestinal tract upon hatch (Dibner and Richards, 2005). Diet plays a very important role in determining the composition of the indigenous gut microbiota and their effect on the host animal (Swanson and Fahey, 2002). Incorporating prebiotics into the diet may beneficially alter microbial populations in the gut (Gibson and Roberfroid, 1995), thereby preventing pathogenic organism establishment, and possibly improving growth performance. In addition, previous studies conducted with prebiotics have typically involved feeding them for 21 or more days post-hatch, but it may be possible that feeding potential prebiotics for shorter time periods of only 3 to 7 d post-hatch may improve microbial populations in the gastrointestinal tract, which could lead to improvement in the bird's growth and health. Feeding prebiotics for shorter time periods also would be less expensive.

Therefore, the objective of the following experiments was to evaluate the prebiotic potential of Elusieve fiber, Temulose products, and alternansucrase-derived oligosaccharides (alternan) and examine the effects of these products on growth performance, metabolizable energy, and amino acid digestibility at several ages, as well as the effect on cecal microbial populations of bifidobacteria, lactobacilli, and *E. coli*.

Materials and Methods

Animals and Husbandry

The University of Illinois Institutional Animal Care and Use Committee approved all procedures. All experiments were conducted with New Hampshire x Columbian male crossbred chicks. Chicks were housed in thermostatically controlled starter batteries with raised wire floors in an environmentally controlled room with light provided continuously. The wire floors of the batteries in all experiments were left soiled from a previous experiment in an attempt to increase the chicks' exposure to a bacterial challenge. Both feed and water were provided ad libitum. Diets were formulated based on NRC (1994) feeding requirements for young chicks and were adequate in all nutrients unless otherwise noted. At hatch, chicks were weighed, wing-banded, and allotted to pens and dietary treatments so that the initial mean weight was similar among treatment groups.

Metabolizable Energy and Amino Acid Analysis

In assays where metabolizable energy (ME_n) and amino acid (AA) digestibility were evaluated, the following procedures were used. Chromic oxide was included in all diets at 0.3% as an indigestible marker. Excreta from each replicate pen was collected over a 24-hour period on day 7 and 21 post-hatch and freeze-dried. Feed and excreta samples from d 7 and 21 were analyzed for gross energy using an adiabatic bomb calorimeter. Amino acid concentrations [method 982.30E (a,b,c); (AOAC International, 2006)] and chromium [method 990.08; (AOAC International, 2006)] in the feed and excreta samples and nitrogen analyses [method 990.03; (AOAC International, 2006)] were determined at the University of Missouri-Columbia Experiment Station Chemical Laboratory. The ME_n of the diets was calculated using the

equation described by Hill and Anderson (1958). Apparent digestibility of amino acids was calculated using the equation described by Adedokun et al. (2007).

Microbial Populations and pH

At the end of the feeding period, chicks were euthanized via CO₂ inhalation and cecal contents were extracted from all birds. The contents of one cecum from each bird were used to determine percent dry matter of cecal contents and pH, while the contents of the other cecum was utilized for DNA extraction followed by quantitative PCR (qPCR) techniques described by Middelbos et al. (2007) with minor adaptations. Cecal DNA was extracted from freshly collected samples that had been stored at -20°C until analysis using a QIAmp DNA Stool Mini-kit (Qiagen Inc., Valencia, CA) according to manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed using specific primers for *Bifidobacterium* spp., *Lactobacillus* spp., and *E. coli*. Briefly, a 10 µl final volume contained 5 µl 2X SYBER Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted cecal DNA. Standard curves were obtained by previous harvesting of pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution. Bacterial DNA was extracted from each dilution and amplified with the cecal DNA to create triplicate standard curves. Colony forming units (CFU) in each dilution were determined by plating on specific agars: *Lactobacillus* genus on Difco Lactobacilli MRS broth (Becton Dickinson Company, Sparks, MD), *Bifidobacterium* genus on Difco Reinforced Clostridial Medium (Becton Dickinson Company) and *E. coli* on Luria-Bertani Medium [10 g/l tryptose, 5 g/l yeast extract, 5 g/l NaCl (pH = 7)]. The calculated log CFU/mL of

each serial dilution was plotted against the cycle threshold to create a linear equation to calculate CFU/g of dry cecal contents.

Experiment 1

The objective of this preliminary experiment was to determine the effects of feeding graded levels of raw Temulose on growth performance, ME_n, and amino acid digestibility of young chicks when fed from 0 to 21 days of age. Five pens of 5 chicks were allowed access to 1 of 4 diets: 1) corn-soybean meal (SBM) control diet (Table 5.1); 2) 0.5% Temulose; 3) 1% Temulose; or 4) bacitracin methylene disalicylate (25 mg/kg). All diets were formulated to contain 23% crude protein and 3,088 ME_n / kg. Temulose and bacitracin were added to the control diet (Table 5.1) in place of Solka Floc. It was assumed that Temulose would pass through the digestive system relatively undigested, so it was assumed to contain no ME_n. Chick weight gain and feed intake were recorded at the beginning and the end of the experiment and feed efficiency was calculated for each pen replicate.

Experiment 2

The objective of this experiment was to evaluate the effects of short-term feeding of Grobiotic (previously described) and raw Temulose in a corn-soybean meal diet on the growth performance and cecal microbial populations of young chicks. Four pens of 10 chicks were allowed access to 1 of 6 diets: 1) corn-soybean meal (SBM) control diet fed for 7 days (Table 5.1); 2) 5% Grobiotic fed for 3 days post-hatch; 3 and 4) 0.5% Temulose fed for 3 or 7 days post-hatch; or 5 and 6) 5% Grobiotic + 0.5% Temulose fed for 3 or 7 days post-hatch. All diets were formulated to contain 23% crude protein and 3,050 ME_n / kg. To keep the diets isocaloric, Grobiotic and Temulose were added to the control diet (Table 5.1) in place of dextrose and Solka Floc. It was assumed that the ME_n of Grobiotic was 2000 kcal/kg. The ME_n value for Grobiotic is

an estimated value based on the gross energy assuming that the dairy fraction would be poorly digested and that the yeast and dried fermentation extracts would be moderately to well digested. At the end of the 3 d feeding periods for Grobiotic and Temulose, the chicks were fed the Treatment 1 corn-SBM control diet for the remainder of the 7 d experiment. Chick weight gain and feed intake were recorded at the beginning and the end of the experiment and feed efficiency was calculated for each pen replicate.

Experiment 3

The objective of this experiment was to evaluate the effects of short-term feeding of raw Temulose and partially hydrolyzed Temulose (purified form of Temulose where the galactoglucomannan-type oligosaccharides are separated from arabinoxylan) on the growth performance and cecal microbial populations of young chicks. Four pens of 8 chicks were allotted to 1 of 10 dietary treatments: 1, 2, and 3) corn-soybean meal (SBM) control diet fed for either 3, 7, or 21 days (Table 5.1); 4, 5, and 6) 0.5% Temulose fed for 3, 7, or 21 days post-hatch; 7, 8, and 9) 0.5% partially hydrolyzed Temulose fed for 3, 7, or 21 days post-hatch; and 10 and 11) 0.5% partially hydrolyzed Temulose fed for either 3 or 7 days followed by corn-SBM until 21 days post-hatch. The diets were formulated to contain 23% crude protein and 3,050 kcal of ME_n / kg and were kept isocaloric by adjusting the content of dextrose and Solka Floc within each diet (Table 5.1). As stated earlier for Temulose, it was assumed that partially hydrolyzed Temulose would pass through the digestive system relatively undigested, so it was given a ME_n value of zero. When the end of a feeding period for Temulose or partially hydrolyzed Temulose was reached, the treatments were ended at that time. Chick weight gain and feed intake were recorded at the beginning and the end of the experimental feeding periods and feed efficiency

was calculated for these time periods. The effect of diet on cecal microbial populations was also determined for each time period.

Experiment 4

The objective of this experiment was to evaluate the effects of DDGS and Elusieve fiber fed for short-time periods on the growth performance and cecal microbial populations of young chicks. Four pens of 8 chicks were allotted to each of 7 dietary treatments: 1 and 2 were corn-soybean meal (SBM) control diet fed for either 3 or 21 days (Table 5.1); 3 and 4 were 10% DDGS fed for 3 or 21 days post-hatch; 5 and 6 were 5% Elusieve fiber fed for 3 or 21 days post-hatch; and 7 was bacitracin methylene disalicylate (25 mg/kg) fed for 21 days. The diets were formulated to contain 23% crude protein and 3,100 kcal of ME_n / kg and were kept isocaloric by adjusting the content of corn, soybean meal, dextrose, and Solka Floc within each diet. A ME_n value of 2,705 kcal/kg was used for the Elusieve fiber; this value was the TME_n value estimated in a precision-fed rooster assay conducted by Kim et al. (2010). When the end of a feeding period for each treatment was reached, the treatment was ended at that time. Chick weight gain and feed intake were recorded at the beginning and the end of the experimental feeding periods and feed efficiency was calculated for these time periods.

Experiment 5

The objective of this experiment was to evaluate the effects of short-term feeding of 1 or 2% Alternan in a corn-soybean meal (SBM) diet on the growth performance, ME_n, AA digestibility, and cecal microbial populations of young chicks. Four pens of 8 chicks were allowed access to 1 of 9 diets for either 3, 7, or 21 days post-hatch, respectively: 1, 2, and 3 were corn-soybean meal control diet (Table 5.1); 4, 5, and 6 were 1% Alternan; and 7, 8, and 9 were 2% Alternan. The diets were formulated to contain 23% crude protein and 3,078 kcal of ME_n /

kg and were kept isocaloric by adjusting the content of dextrose and Solka Floc within each diet (Table 5.1). It was assumed that the Alternan would pass through the digestive system relatively undigested, so it was given a ME_n value of zero. When the end of a feeding period for Alternan was reached, the treatments were ended at that time. Chick weight gain and feed intake were recorded at the beginning and the end of the experimental feeding periods and feed efficiency was calculated for these time periods. The effect of diet on cecal microbial populations was also determined for each time period.

Experiment 6

The objective of this experiment was to evaluate the effects of short-term feeding of Alternan or Elusieve DDGS fiber in a casein-dextrose diet on the growth performance, ME_n, AA digestibility, and cecal microbial populations of young chicks. This diet was used because it contains little or no indigestible oligosaccharides whereas a corn-soybean meal diet contains large amounts of indigestible oligosaccharides which may diminish and confound the effects of the potential prebiotic ingredients. Thus, it is anticipated that the casein-dextrose diet will yield larger effects of the dietary prebiotics on cecal microbial populations and will be a more sensitive model for evaluating prebiotic ingredients. In addition, the inclusion of highly digestible ingredients, such as dextrose and casein, might increase feed intake, nutrient digestibility, and growth performance early in life (Longo et al., 2007). Four pens of 5, 10, or 15 chicks (depending on the age of collection) were allowed access to 1 of 12 diets for either 3, 7, or 21 days post-hatch, respectively: 1, 2, and 3 were casein-dextrose control diet (Table 5.2); 4, 5, and 6 were 5% Elusieve DDGS fiber; 7, 8, and 9 were 2% Alternan; and 10, 11, and 12 were corn-SBM control diet (Table 5.2). When the end of a feeding period for each diet was reached, the treatments were ended at that time. Chick weight gain and feed intake were recorded at the

beginning and the end of the experimental feeding periods and feed efficiency was calculated for these time periods. The effect of diet on cecal microbial populations was also determined for each time period.

Statistical Analysis

All data were analyzed by the general linear models (GLM) procedure of SAS (SAS Institute Inc., 1990) appropriate for completely randomized designs. If the F-test for treatment effect was significant in the ANOVA, differences among treatment means were determined using the least significant difference test (Carmer and Walker, 1985). Differences in growth performance, ME_n, and AA digestibility were considered significant when the $P < 0.05$, while differences in cecal microbial populations were considered significant when the $P < 0.10$.

Results and Discussion

Experiment 1

Weight gain, feed intake, and feed efficiency (Table 5.3) were not significantly affected by the inclusion of Temulose when compared to the corn-SBM control diet. The ME_n and the digestibility of most amino acids (Table 5.4) increased significantly ($P < 0.05$) when the 1% level of Temulose was fed when compared to the corn-SBM control diet. The addition of bacitracin also increased the digestibility of all amino acids and increased ME_n ($P < 0.05$) when compared to the corn-SBM control diet.

Experiment 2

Weight gain (Table 5.5) was not significantly affected by the inclusion of Grobiotic or Temulose when compared to the corn-SBM control diet. This is in agreement with Experiment 1 when two levels of Temulose were fed. Feed intake (Table 5.5) was increased ($P < 0.05$) when the diets were supplemented with 5% Grobiotic alone or the combination of 5% Grobiotic and

0.5% Temulose for 3 d. This is in agreement with previous studies conducted in our lab where low concentrations of Grobiotic (3 or 5%) resulted in an increase in feed intake when fed for up to 3 d (unpublished data), so the increase in feed intake observed in the 0.5% Temulose and 5% Grobiotic diet is probably due to the addition of the Grobiotic rather than the Temulose. Feed efficiency for the chicks supplemented with 5% Grobiotic, 0.5% Temulose, or the combination thereof was decreased ($P < 0.05$) when fed for only 3 d, but was similar among the remaining treatments. Previous studies in our lab have also shown that chicks fed 5% Grobiotic for either 2 or 3 d had lower ($P < 0.05$) feed efficiency than chicks fed the control diet (unpublished data).

Bacterial enumeration by qPCR did not detect any significant differences in bifidobacteria, lactobacilli, or *E. coli* populations when Grobiotic, Temulose, or combinations thereof were included in the diet for 3 or 7 d (Table 5.6). The lack of a response is in agreement with Faber et al. (2010) who reported that graded levels of Temulose from 0 to 8% in dog diets did not increase fecal microbial concentrations of *E. coli* or lactobacilli. Swanson et al. (2002) and Middelbos et al. (2007) also observed no changes in fecal microbial populations when dogs were fed 0.5 to 1% yeast cell wall, another source of MOS. Strickling et al. (2000) also noted no significant changes in bifidobacteria, lactobacilli, and *E. coli* when dogs were fed 0.5% yeast cell wall and xylooligosaccharides. The results of these earlier studies and the experiments presented herein indicate that an inclusion level of 0.5% is insufficient to elicit any positive responses in microbial populations.

Experiment 3

Weight gain (Table 5.7) was not significantly affected by the supplementation of Temulose or partially hydrolyzed Temulose throughout the experiment when compared to the corn-SBM control diet. This is in agreement with Experiments 1 and 2. Feed intake (Table 5.7)

at 3 and 7 d was increased ($P < 0.05$) for the 0.5% Temulose treatment or two of the partially hydrolyzed Temulose treatments were fed. There were no differences among treatments in feed intake for the 0 to 21 d feeding period when compared to the control. For feed efficiency, none of the prebiotic treatments were significantly different from the corn-SBM control treatment during the 0 to 3 d period. Over the 0 to 7 d period, as well as over the entire experiment (0 to 21 d), however, the feed efficiency for the 0.5% Temulose diet was significantly lower ($P < 0.05$) than the corn-SBM control diet. This was similar to the results in Experiment 2. Feed efficiency for all of the 0.5% partially hydrolyzed Temulose treatments was not significantly different from the control.

The effects of diet on cecal microbial populations are presented in Table 5.8. At 3 d of age, there was an increase ($P < 0.10$) in bifidobacteria and *E. coli* populations in chicks being fed a diet supplemented with 0.5% partially hydrolyzed Temulose when compared to chicks fed the corn-SBM control diet. At 7 d of age, chicks receiving both the 0.5% Temulose and partially hydrolyzed Temulose diet had a decrease in lactobacilli ($P < 0.10$) populations. At 21 d of age, there were no significant differences in any microbial populations among the dietary treatments. Since Temulose and partially hydrolyzed Temulose contain high concentrations of mannose (52 and 73%, respectively; Temple-Inland, unpublished data), the main component of MOS, these results were unexpected as several experiments conducted with broiler chickens have reported that supplemental MOS increases lactobacilli and bifidobacteria populations in the ceca compared to diets containing no antibiotic growth promoters (Baurhoo et al., 2007; Fernandez et al., 2002).

From these three experiments, we can conclude that raw or partially hydrolyzed Temulose have no negative effects on weight gain and no consistent effects on feed intake or

feed efficiency when fed to chicks for either 3, 7, or 21 days. Although several previous studies have reported positive effects of MOS (one of the main components of Temulose) on growth performance, no such effects were observed in this study. Hooge et al. (2004a,b) conducted a meta-analysis of 44 broiler trials and 27 turkey trials and reported that MOS supplementation improved body weight in 79.5 and 70.4% of cases, respectively. In contrast, additional studies by Stanczuk et al. (2005) and Waldroup et al. (2003) reported no significant changes in body weight gain or feed conversion when MOS was added to the diet of either turkeys, or broilers, respectively. Thus, a positive effect of MOS on growth does not occur in all studies including the current study. The addition of partially hydrolyzed Temulose to the diet of chicks at 3 d resulted in significantly higher populations of bifidobacteria, lactobacilli, and *E. coli* when compared to the original, nonhydrolyzed Temulose. However, both Temulose compounds also decreased ($P < 0.10$) lactobacilli populations at 7 days of age, with no other consistent effects seen in the other microbial populations at the three different ages. These results were unexpected as several studies have shown that the compounds contained within Temulose (mannanoligosaccharides, xylooligosaccharides, and glucooligosaccharides) exert beneficial effects on growth performance and increase microbial populations of lactobacilli and bifidoabacteria (Djouzi and Andrieux, 1997; Flickinger et al., 2000; Hooge, 2004 a,b; Smiricky-Tjardes et al., 2003; Swanson and Fahey, 2002). However, the results of the current study do indicate that dietary ingredients such as Temulose can influence cecal microbial populations even when given to the chick for a period of 3 or 7 d.

Experiment 4

Weight gain, feed intake, and feed efficiency (Table 5.9) from 0 to 3 d or 0 to 21d were generally not significantly affected by the supplementation of DDGS or Elusieve fiber. Loar et

al. (2010), Lumpkins et al. (2004), and Wang et al. (2007) also showed that inclusion of 0 to 12% DDGS had no effect on growth performance when fed during the starter phase. However, it was expected that the addition of Elusieve fiber may have an effect on growth performance because of the higher fiber content when compared to DDGS (51.09 vs 36.88% NDF; Kim et al., 2010). For example, Gonzalez-Alvarado et al. (2007) and Jimenez-Moreno et al. (2009; 2010) demonstrated that the inclusion of fiber in a low-fiber diet (3% oat hulls, soy hulls, or sugar beet pulp) improved performance of broilers from 1 to 21 d of age and nutrient digestibility at 18 d of age. These sources contain 67 to 44 % NDF, which is similar to that in Elusieve fiber. The differences in results between the current study and previous ones may be due to the level of ingredient fed, the type and source of fiber fed (soluble vs. insoluble), and the level of fiber in the diet to which the high fiber source is being added.

The effects of diet on cecal microbial populations are presented in Table 5.10. At 3 d of age, there was a decrease ($P < 0.10$) in lactobacilli and *E. coli* populations in chicks receiving bacitracin when compared to the control and all other treatments. Chicks fed a diet supplemented with 10% DDGS also showed a decrease in *E. coli* at 3 d when compared to the corn-SBM control diet. Although there is little or no research concerning the effects of DDGS inclusion has on the colonization of *E. coli* in poultry, Jacob et al. (2008) reported a positive association between the use of DDGS in the diet of cattle and reduction of *E. coli* in the feces. A similar relationship seems to be present in this experiment as well. However, at 21 d of age, there were no significant differences in any microbial populations for any of the dietary treatments when compared to the control. These results suggest that 10% DDGS and 5% Elusieve fiber have no consistent effect on the growth performance or cecal microbial populations of young chicks from 0 to 21 d.

Experiment 5

Weight gain, feed intake, and feed efficiency (Table 5.11) were not significantly affected by the supplementation of 1 or 2% Alternan for any of the three time periods. The ME_n of all diets increased significantly ($P < 0.05$) from 7 to 21 d of age; however, there was no significant effect of dietary treatment within each age period (Table 5.12). As was observed with the ME_n of the diets, the AA digestibility increased for all amino acids at 21 d when compared to values at 7 d (Table 5.13). These results are similar to previous results reported by our lab, which indicated that the ability of the chick to utilize energy and AA increases until the chick is approximately two weeks of age, then plateaus, suggesting that digestibility has reached its maximum at this time (Batal and Parsons, 2002a). There were no differences in AA digestibility among treatments at 7 d; however, at 21 d, the addition of 1 or 2% Alternan increased the digestibility of all amino acids when compared to the corn-SBM control diet, except for cysteine digestibility which was increased only by the 1% level of Alternan.

At 3 d of age, there were no significant differences in any microbial populations for any of the dietary treatments when compared to the corn-SBM control diet (Table 5.14). At 7 d, bifidobacteria were decreased ($P < 0.10$) when 2% Alternan was added to the diet, but no other differences were seen in any of the other microbial populations. At 21 d, *E. coli* were increased ($P < 0.10$) when 1 or 2% Alternan was added to the diet, but no other differences were seen in any of the other microbial populations. When using pure colonic bacteria *in vitro*, Holt et al. (2005) determined that alternansucrase-derived oligosaccharides (Alternan) increased the growth of *Bifidobacterium* spp., while coliforms or pathogenic bacteria displayed little or no growth; however, the opposite effect was observed in this experiment. Further studies involving mixed

cultures and the influence of the complex intestinal microbiota of the animal will need to be conducted in order to determine a clear relationship *in vivo*.

These results suggest that of 1 or 2 % Alternan can be added to young chick diets with no adverse effects on growth performance and may increase amino acid digestibility at 21 d. However, the addition of Alternan did not have any positive effects on any selected microbial populations across all age periods and actually decreased bifidobacteria and increased *E. coli* populations in some cases.

Experiment 6

Weight gain (Table 5.15) was increased ($P < 0.05$) during the 0 to 3 and 0 to 21 d time periods for all chicks fed the diet containing 5% Elusieve fiber when compared to the control casein-dextrose diet, while the diet containing 2% Alternan was not different from the control. Jimenez-Moreno et al. (2010) also showed that the inclusion of different fiber sources in a very low fiber diet improved weight gain from 1 to 21 d of age, suggesting that young broilers require a minimal amount of fiber in the diet when the objective is to maximize growth. All casein-dextrose diets, regardless of supplementation, had increased weight gain ($P < 0.05$) during the first week when compared to the corn-SBM diet. Batal and Parsons (2002b) also reported that chicks fed a casein-dextrose diet immediately after hatching had significantly ($P < 0.05$) higher weight gains at 48 h and 1 wk post-hatching when compared to chicks fed a corn-SBM diet; however, at 3 wk of age, there were no differences in weight gain between these two treatments in the current study. Feed intake over all time periods was not affected by any dietary treatment when compared to the casein control. Feed efficiency for the chicks fed the corn-SBM diet was reduced ($P < 0.05$) compared to the casein diet treatments during the first 3 days, but this result did not persist throughout the remainder of the experiment. This result is in agreement with Batal

and Parsons (2002b) who also showed that chicks fed a casein-dextrose diet had significantly ($P < 0.05$) better feed efficiency than those fed a corn-SBM diet during the first week post-hatch.

The ME_n of all diets increased significantly ($P < 0.05$) from 7 to 21 d of age (Table 5.16). At 7 days of age, all diets containing casein-dextrose yielded significantly higher ME_n values ($P < 0.05$) when compared to the corn-SBM diet. This in agreement with Batal and Parsons (2002b) who also showed increased ME_n values for a casein-dextrose diet when compared to a corn-SBM diet. In addition, these differences (332 - 405 kcal/kg) were much greater than the 99 kcal/kg difference based on calculated values (Table 5.2). The very high ME_n of the casein-dextrose diet indicates that glucose and casein are very well utilized by the young chick. At 21 days, however, only the diet containing 5% Elusieve fiber still continued to show a significantly increased ($P < 0.05$) ME_n value when compared either the casein-dextrose control or corn-SBM diet.

As was observed with the ME_n , amino acid digestibility increased for all amino acids at 21 d when compared to values at 7 d (Table 5.17). There were generally no differences in AA digestibility among the casein-dextrose treatments at 7 and 21 d. When compared to the casein-dextrose control diet, AA digestibility was decreased for the corn-SBM diet. Again, this is in agreement with Batal and Parsons (2002b) who showed increased AA digestibility values for a casein-dextrose diet compared to a corn-SBM diet, indicating that chicks have a high ability to absorb AA and to digest proteins even at a young age.

At 3 d of age, there was a significant decrease ($P < 0.10$) in bifidobacteria for all treatments when compared to the casein-dextrose control diet (Table 5.18). This was unexpected as Holt et al. (2005) determined that Alternan increases the growth of *Bifidobacterium* spp. *in vitro*. At 21 d, bifidobacteria and *E. coli* were decreased ($P < 0.10$) when 5% Elusieve fiber was

added to the diet, while the addition of 2% Alternan resulted in a decrease ($P < 0.10$) in lactobacilli populations.

From these experiments, we can generally conclude that Elusieve fiber and Alternan had no consistent effects on growth performance when given to chicks for either 3, 7, or 21 days, whether it be in a corn-SBM diet or casein-dextrose diet. For example, in Experiment 4, 5% Elusieve fiber increased feed intake from 0 to 3 d, but had no effects on weight gain or feed efficiency. In Experiment 6, when 5% Elusieve fiber was included in a casein-dextrose diet, weight gain was increased at 3 and 21 d, but there were no significant effects on feed intake or feed efficiency. In addition, all casein-dextrose diets, regardless of supplementation, had increased weight gain and feed efficiency ($P < 0.05$) during the first week when compared to the corn-SBM diet, as well as increased ME_n and AA digestibility. This suggests that any improvement in growth performance was due to the highly digestible ingredients in the casein-dextrose diet rather than the Elusieve fiber or Alternan.

Also, in an attempt to yield more positive and consistent effects of Elusieve fiber and Alternan on cecal microbes as compared to the corn-SBM diets in the previous experiments, a casein-dextrose diet which contains little or no indigestible oligosaccharides was used in Experiment 6. It was hypothesized that the lack of significant effects when the prebiotic-type compounds were added to a corn-SBM diet may be due to the high oligosaccharide content of the SBM. The soybean oligosaccharides, raffinose and stachyose, represent approximately 4 to 6% of the dry matter of the SBM (Coon et al., 1990), and these two compounds have been shown to influence microbial populations in the human colon (Hayakawa et al., 1990). However, the results obtained using this diet were similar to those obtained using a corn-SBM diet. For example, bifidobacteria were decreased ($P < 0.10$) when 2% Alternan was added to a corn-SBM

diet and this same result was observed in the casein-dextrose diet. Also, the overall colony forming units in the diets which were based on corn-SBM were higher than diets based on casein-dextrose.

Overall, the results from this study indicate that the feeding of various potential prebiotics may yield some beneficial responses in chick growth performance and in select cecal microbial populations; however, the responses are not consistent. In addition, these results, especially those observed for cecal microflora, show that dietary prebiotics can have an influence even when fed for a short period of only three days. However, more extensive research needs to be conducted to develop a clear picture of the nutritional properties, dosage level, and management environment needed to yield more consistent responses to potential prebiotic ingredients.

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Tables

Table 5.1. Nutritional composition (%) of control diets used in Experiments 1-5.

Ingredient	Experiment 1 ¹	Experiment 2 & 3 ¹	Experiment 4	Experiment 5 ¹
Corn	49.00	45.69	49.00	50.00
Soybean meal	38.15	39.71	38.60	38.44
Soybean oil	5.00	5.00	5.00	5.00
Limestone	1.20	1.20	1.31	1.20
Dicalcium phosphate	2.05	2.02	1.75	2.02
Salt	0.40	0.40	0.40	0.40
Vitamin mix ²	0.20	0.20	0.20	0.20
Mineral mix ³	0.15	0.15	0.15	0.15
DL-Methionine	0.22	0.19	0.19	0.19
Choline chloride	0.10	0.10	0.10	0.10
Dextrose	-	2.54	1.55	-
Solka Floc ⁴	1.53	2.50	1.45	2.00
Chromic Oxide		0.30	0.30	0.30
Celite	2.00	-	-	-
Calculated Analysis:				
Crude protein, %	23.0	23.0	23.0	23.0
ME _n , kcal/kg	3,088	3,050	3,100	3,078
Calcium, %	1.00	1.00	1.00	1.00
Nonphytate P, %	0.50	0.45	0.45	0.45

¹ Diets were kept isocaloric within an experiment by adjusting the content of dextrose and Solka Floc within each diet.

² Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 4.41 mg; D-Ca-pantothenate, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

³ Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄•7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

⁴ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 5.2. Nutritional composition (%) of chick diets used in Experiment 6.

Ingredient	Casein-dextrose	Corn-SBM
Corn	-	45.69
Soybean meal	-	38.30
Casein	24.20	-
Dextrose	57.00	5.66
Soybean oil	3.00	5.00
Limestone	-	1.40
Dicalcium phosphate	-	2.00
Salt	-	0.60
Vitamin mix ¹	0.20	0.20
Trace mineral mix ²	-	0.15
Purified diet mineral mix ³	5.37	-
DL-Methionine	0.14	0.20
L-Arg	0.60	-
Glycine	0.90	0.40
MgSO ₄	0.20	-
Choline chloride	0.25	0.10
Solka Floc ⁴	7.82	-
DL- α -tocopherol acetate	0.002	-
Ethoxyquin	0.013	-
Chromic Oxide	0.30	0.30
Calculated Analysis:		
Crude protein, %	23.0	23.0
ME _n , kcal/kg	3,207	3,108
Calcium, %	1.09	1.09
Nonphytate phosphorus, %	0.60	0.50

¹ Provided per kilogram of diet: thiamin-HCl, 20 mg; retinyl acetate, 1,789 µg; cholecalciferol, 15 µg; vitamin B₁₂, 0.04 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; niacin, 50 mg; menadione dimethylpyrimidinol bisulfite, 2 mg; pyridoxine- HCl, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; ascorbic acid, 250 mg.

² Provided as milligrams per kilogram of diet: Mn, 75 from MnO; Fe, 75 from FeSO₄• 7H₂O; Zn, 75 from ZnO; Cu, 5 from CuSO₄•5H₂O; I, 0.75 from ethylene diamine dihydroiodide; Se, 0.1 from Na₂SeO₃.

³ Provided per kilogram of diet: Ca₃(PO₄)₂, 28g; K₂PO₄, 9g; NaCl, 8.89g; MgSO₄-7H₂O, 3.5 g; ZnCO₃, 0.10g; MnSO₄-H₂O, 0.65 g; FeSO₄-7H₂O, 0.42g; KI, 40 mg; CuSO₄-5H₂O, 20 mg; Na₂MoO₄-2H₂O, 9 mg; H₃BO₃, 9 mg; CoSO₄-7H₂O, 1 mg; NaSeO₃, 0.22 mg.

⁴ Purified cellulose, International Fiber Corp., North Tonawanda, NY.

Table 5.3. Effect of Temulose on growth performance of chicks from 0 to 21 d in Experiment 1.

Dietary treatment ¹	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)
Corn-soybean meal	799	1127	708
0.5% Temulose	805	1141	705
1% Temulose	764	1082	706
Bacitracin	773	1075	719
Pooled SEM	22.1	28.0	9.3

¹ Means represent 5 replicates of 5 chicks each per treatment.

Table 5.4. Effect of diet on ME_n values (kcal/kg DM) and select amino acid (AA) digestibility (%) at 21 d in Experiment 1.

AA or ME _n ¹	Corn-SBM ²	0.5% Temulose	1% Temulose	Bacitracin	Pooled SEM
ME _n	3,179 ^c	3,218 ^{bc}	3,280 ^{ab}	3,348 ^a	0.02
Arginine	91 ^b	92 ^{ab}	93 ^a	93 ^a	0.3
Cysteine	77 ^b	78 ^b	79 ^{ab}	82 ^a	0.9
Isoleucine	86 ^c	87 ^{bc}	88 ^{ab}	89 ^a	0.5
Lysine	87 ^b	87 ^b	88 ^{ab}	89 ^a	0.5
Methionine	92 ^c	94 ^b	93 ^{bc}	95 ^a	0.3
Threonine	81 ^c	83 ^{bc}	84 ^{ab}	85 ^a	0.6
Valine	85 ^c	86 ^{bc}	87 ^{ab}	89 ^a	0.5

^{a-b} Means within a row with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 5 replicates of 5 chicks each per treatment.

² SBM = soybean meal.

Table 5.5. Effect of Grobiotic and Temulose on growth performance of chicks from 0 to 7 d in Experiment 2.

Dietary treatment ¹	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)
Corn-SBM ² control	56 ^{ab}	88 ^{cd}	630 ^a
5% Grobiotic (GB) fed for 3 d	57 ^a	105 ^{ab}	544 ^c
0.5% Temulose (TL) fed for 3 d	52 ^b	95 ^{bc}	552 ^{bc}
0.5% TL fed for 7 d	54 ^{ab}	84 ^d	640 ^a
5% GB + 0.5% TL fed for 3 d	54 ^{ab}	110 ^a	497 ^c
5% GB + 0.5% TL fed for 7 d	55 ^{ab}	90 ^{cd}	611 ^{ab}
Pooled SEM	1.5	3.7	21.3

^{a-d} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 10 chicks each per treatment.

² SBM = soybean meal.

Table 5.6. Effect of short-term feeding of Grobiotic and Temulose on select cecal microbial populations in 7-day-old chicks in Experiment 2.

Dietary treatment ¹	Bifidobacteria	Lactobacilli	<i>Escherichia coli</i>
	(log ₁₀ CFU/g DM)		
Corn-SBM ² control	8.64	10.49	9.99
5% Grobiotic (GB) for 3 d	9.85	10.68	10.15
0.5% Temulose (TL) for 3 d	9.48	10.83	10.24
0.5% Temulose for 7 d	9.87	10.59	10.24
5% GB + 0.5% TL for 3 d	9.20	10.73	9.85
5% GB + 0.5% TL for 7 d	8.86	10.67	9.81
Pooled SEM	0.52	0.15	0.30

¹ Means represent 4 replicates of 10 chicks each per treatment.

² SBM = soybean meal.

Table 5.7. Effect of short-term feeding of Temulose and partially hydrolyzed Temulose on growth performance of chicks in Experiment 3.

Dietary treatment ^{1,2}	Weight gain (g/chick)			Feed intake (g/chick)			Gain/feed (g/kg)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Corn-SBM control	12	38	299	28 ^c	79 ^c	461	430 ^{ab}	490 ^b	650 ^a
0.5% Temulose	12	35	286	31 ^{ab}	89 ^{ab}	500	381 ^b	399 ^c	573 ^b
0.5% HTL	13	41	298	29 ^{bc}	83 ^{bc}	484	456 ^a	500 ^{ab}	615 ^{ab}
0.5% HTL for 3d, C-SBM until 21 d	13	41	299	31 ^{ab}	70 ^d	503	415 ^{ab}	578 ^a	594 ^{ab}
0.5% HTL for 7 d, C-SBM until 21 d	12	40	296	33 ^a	93 ^a	501	378 ^b	435 ^{bc}	595 ^{ab}
Pooled SEM	0.5	2.4	10.9	0.9	2.4	14.7	19.6	30.0	24.7

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² C-SBM = corn soybean meal diet; HTL = hydrolyzed Temulose;.

Table 5.8. Effect of short-term feeding of Temulose and partially hydrolyzed Temulose on select cecal microbial populations in chicks in Experiment 3.

Dietary treatment ^{1,2}	Bifidobacteria			Lactobacilli			<i>Escherichia coli</i>		
	(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)		
	Days of age	Days of age	Days of age	Days of age	Days of age	Days of age	Days of age	Days of age	Days of age
	3	7	21	3	7	21	3	7	21
Corn-SBM control	6.77 ^b	6.66	6.45	11.44 ^{ab}	11.11 ^a	10.08	11.65 ^b	10.61	10.17
0.5% Temulose	7.05 ^b	6.87	6.38	11.16 ^b	10.59 ^b	10.21	11.39 ^b	10.24	8.81
0.5% HTL	7.36 ^a	6.90	6.41	11.53 ^a	10.64 ^b	9.74	12.13 ^a	10.47	9.71
0.5% HTL for 3 d, C-SBM until 21 d	-	-	6.62	-	-	9.78	-	-	9.97
0.5 % HTL for 7 d, C-SBM until 21 d	-	-	6.61	-	-	10.12	-	-	10.25
Pooled SEM	0.12	0.21	0.09	0.12	0.16	0.23	0.18	0.32	0.53

^{a-b} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² C-SBM = corn soybean meal diet; HTL = hydrolyzed Temulose.

Table 5.9. Effect of short-term feeding of distillers dried grains with solubles and Elusieve fiber on growth performance of chicks in Experiment 4.

Dietary treatment ^{1,2}	Weight gain (g/chick)		Feed intake (g/chick)		Gain/feed (g/kg)	
	— Days of age —		— Days of age —		— Days of age —	
	3	21	3	21	3	21
Corn-SBM control	13 ^{ab}	406	26 ^b	661	497	614
10% DDGS	12 ^b	405	23 ^c	634	530	640
5% Elusieve Fiber	15 ^a	418	29 ^a	648	504	647
Bacitracin	14 ^a	406	28 ^{ab}	663	510	615
Pooled SEM	0.5	9.1	0.9	18.0	19.0	12.9

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² SBM = soybean meal; DDGS = distillers dried grains with solubles.

Table 5.10. Effect of short-term feeding of distillers dried grains with solubles and Elusieve fiber on cecal microbial populations in chicks in Experiment 1.

Dietary treatment ^{1,2}	Bifidobacteria		Lactobacilli		<i>Escherichia coli</i>	
	— Days of age —		(log ₁₀ CFU/g DM) — Days of age —		— Days of age —	
	3	21	3	21	3	21
Corn-SBM control	8.12	7.70 ^{ab}	10.87	9.11 ^{ab}	11.51 ^a	8.39
10% DDGS	7.06	8.80 ^a	10.55	9.05 ^{ab}	10.14 ^{bc}	8.49
5% Elusieve fiber	7.51	7.63 ^{ab}	10.52	9.32 ^a	10.81 ^{ab}	8.74
Bacitracin ³	-	7.17 ^b	-	8.67 ^b	-	9.13
Pooled SEM	0.58	0.57	0.19	0.22	0.35	0.28

^{a-c} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² SBM = soybean meal; DDGS = distillers dried grains with solubles.

³ This diet was only fed from 0 to 21 d.

Table 5.11. Effect of short-term feeding of Alternan in a corn-soybean meal diet on the growth performance of chicks in Experiment 5.

Dietary treatment ¹	Weight gain (g/chick)			Feed intake (g/chick)			Gain/feed (g/kg)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Corn-SBM ² control	14	43	334	31	86	519	451	509	644
1% Alternan	14	43	358	30	80	535	478	534	669
2% Alternan	14	44	334	30	78	535	491	564	626
Pooled SEM	0.5	2.2	9.9	1.1	2.7	11.7	19.1	24.1	20.6

¹Means represent 4 replicates of 8 chicks each per treatment.

²SBM = soybean meal.

Table 5.12. Effect of age and diet on ME_n values (kcal/kg DM) in Experiment 5.

Dietary treatment ¹	Days of age		Pooled SEM
	7	21	
Corn-SBM ² control	2,719 ^z	3,382 ^y	35.9
1% Alternan	2,716 ^z	3,353 ^y	66.5
2% Alternan	2,677 ^z	3,414 ^y	69.3
Pooled SEM	85.1	23.4	

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$). There was no significant effect of dietary treatment within age period.

¹Means represent 4 replicates of 8 chicks each per treatment.

²SBM = soybean meal.

Table 5.13. Effect of age and diet on select amino acid digestibility (%) in Experiment 5.

Amino acid and dietary treatment ^{1,2}	Days of age		Pooled SEM	Amino acid and dietary treatment	Days of age		Pooled SEM
	7	21			7	21	
Arginine				Methionine			
Corn-SBM	85 ^z	92 ^y	1.0	Corn-SBM	83 ^z	93 ^y	0.9
1% Alternan	86 ^z	93 ^{y*}	1.2	1% Alternan	83 ^z	94 ^{y*}	1.4
2% Alternan	84 ^z	93 ^{y*}	1.5	2% Alternan	80 ^z	94 ^{y*}	1.7
Pooled SEM	1.6	0.2		Pooled SEM	1.9	0.2	
Cysteine				Threonine			
Corn-SBM	69 ^z	81 ^y	1.7	Corn-SBM	70 ^z	84 ^y	1.4
1% Alternan	73 ^z	83 ^{y*}	1.9	1% Alternan	70 ^z	86 ^{y*}	1.8
2% Alternan	64 ^z	82 ^y	2.8	2% Alternan	67 ^z	86 ^{y*}	2.4
Pooled SEM	2.9	0.7		Pooled SEM	2.5	0.4	
Isoleucine				Valine			
Corn-SBM	75 ^z	89 ^y	1.5	Corn-SBM	72 ^z	87 ^y	1.5
1% Alternan	78 ^z	91 ^{y*}	1.7	1% Alternan	73 ^z	89 ^{y*}	1.6
2% Alternan	74 ^z	91 ^{y*}	2.1	2% Alternan	69 ^y	88 ^{y*}	2.4
Pooled SEM	2.3	0.3		Pooled SEM	2.5	0.2	
Lysine							
Corn-SBM	78 ^z	90 ^y	1.3				
1% Alternan	79 ^z	91 ^{y*}	1.6				
2% Alternan	76 ^z	91 ^{y*}	1.8				
Pooled SEM	2.1	0.2					

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

* Means within a column for each amino acid (diet effect) are significantly ($P < 0.05$) different than the corn-SBM control diet.

¹ Means represent 4 replicates of 8 chicks each per treatment.

²SBM = soybean meal.

Table 5.14. Effect of short-term feeding of Alternan in a corn-soybean meal diet on select cecal microbial populations in chicks in Experiment 5.

Dietary treatment ^{1,2}	Bifidobacteria			Lactobacilli			<i>Escherichia coli</i>		
	(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Corn-SBM control	7.48	9.70 ^a	8.28	11.57	11.06	9.97	11.82	11.34	10.38 ^b
1% Alternan	8.29	9.83 ^a	7.80	11.49	11.07	10.18	12.23	11.89	11.00 ^a
2% Alternan	7.59	8.67 ^b	8.63	11.57	10.93	10.11	12.48	11.37	11.09 ^a
Pooled SEM	0.39	0.37	0.37	0.09	0.12	0.10	0.25	0.29	0.22

^{a-c} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.10$).

^{x-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 8 chicks each per treatment.

² SBM = soybean meal.

Table 5.15. Effect of short-term feeding of Elusieve DDGS fiber and Alternan in a casein-dextrose diet on growth performance in chicks in Experiment 6.

Dietary treatment ¹	Weight gain (g/chick)			Feed intake (g/chick)			Gain/feed (g/kg)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Casein control	20 ^b	50 ^a	324 ^b	27 ^{ab}	83	541	782 ^a	609 ^{ab}	600 ^b
5% Elusieve fiber	25 ^a	55 ^a	373 ^a	30 ^a	88	554	836 ^a	630 ^a	675 ^a
2% Alternan	22 ^b	50 ^a	340 ^b	27 ^{ab}	85	541	826 ^a	592 ^{ab}	629 ^{ab}
Corn-soybean meal	13 ^c	41 ^b	346 ^{ab}	24 ^b	84	551	586 ^b	515 ^b	629 ^{ab}
Pooled SEM	1.2	3.6	9.4	3.2	7.9	12.7	69.8	50.2	15.2

^{a-c} Means within a column with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 15 chicks (3 days of age), 10 chicks (7 days of age), and 5 chicks (21 days of age) each per treatment.

Table 5.16. Effect of age and diet on ME_n (kcal/kg DM) in Experiment 6.

Dietary treatment ¹	Days of age		Pooled SEM
	7	21	
Casein control	3,123 ^{az}	3,391 ^{bcy}	26.5
5% Elusieve fiber	3,138 ^{az}	3,461 ^{ay}	28.0
2% Alternan	3,196 ^{az}	3,424 ^{aby}	26.9
Corn-soybean meal	2,791 ^{bz}	3,246 ^{cy}	63.1
Pooled SEM	53.1	16.8	

^{a-c} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.05$).

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

¹ Means represent 4 replicates of 10 chicks (7 days of age) and 5 chicks (21 days of age) each per treatment.

Table 5.17. Effect of age and diet on select amino acid digestibility (%) in Experiment 6.

Amino acid and dietary treatment ^{1,2}	Days of age		Pooled SEM	Amino acid and dietary treatment	Days of age		Pooled SEM
	7	21			7	21	
Cysteine				Methionine			
Casein control	48 ^z	76 ^y	2.1	Casein control	92 ^z	97 ^y	0.5
5% Elusieve	60 ^{z*}	79 ^y	1.8	5% Elusieve	94 ^z	97 ^y	0.3
2% Alternan	55 ^{z*}	76 ^y	2.1	2% Alternan	92 ^z	97 ^y	0.5
Corn-SBM	71 ^{z*}	83 ^{y*}	1.7	Corn-SBM	85 ^{z*}	93 ^{y*}	1.0
Pooled SEM	2.3	1.4		Pooled SEM	0.8	0.3	
Isoleucine				Threonine			
Casein control	84 ^z	94 ^y	0.6	Casein control	79 ^z	92 ^y	0.8
5% Elusieve	87 ^z	90 ^{y*}	0.7	5% Elusieve	82 ^z	91 ^y	0.9
2% Alternan	84 ^z	93 ^y	0.7	2% Alternan	78 ^z	91 ^y	1.1
Corn-SBM	80 ^{z*}	90 ^{y*}	1.6	Corn-SBM	72 ^{z*}	85 ^{y*}	1.9
Pooled SEM	1.3	0.6		Pooled SEM	1.7	0.6	
Lysine				Valine			
Casein control	93 ^z	97 ^y	0.5	Casein control	85 ^z	95 ^y	0.5
5% Elusieve	94 ^z	96 ^y	0.2	5% Grobiotic	87 ^z	93 ^y	0.8
2% Alternan	93 ^z	97 ^y	0.3	1.2% Dairy-lac-80	85 ^z	94 ^y	0.6
Corn-SBM	82 ^{z*}	90 ^{y*}	1.5	Corn-SBM	78 ^{z*}	89 ^{y*}	1.6
Pooled SEM	1.1	0.3		Pooled SEM	1.3	0.5	

^{y-z} Means within a row (age effect) with no common superscript differ significantly ($P < 0.05$).

*Means within a column for each amino acid (diet effect) are significantly ($P < 0.05$) different than the casein-dextrose control diet.

¹ Means represent 4 replicates of 10 chicks (7 days) and 5 chicks (21 days of age) each per treatment.

² SBM = soybean meal.

Table 5.18. Effect of short-term feeding of Alternan and Elusieve DDGS fiber in a casein-dextrose diet on select cecal microbial populations in chicks in Experiment 6.

Dietary treatment ^{1,2}	Bifidobacteria			Lactobacilli			<i>Escherichia coli</i>		
	(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)			(log ₁₀ CFU/g DM)		
	Days of age			Days of age			Days of age		
	3	7	21	3	7	21	3	7	21
Casein control	6.62 ^a	6.10 ^{ab}	6.21 ^a	9.47 ^{ab}	8.45 ^{ab}	9.96 ^a	10.40	9.68 ^{ab}	10.59 ^a
5% Elusieve fiber	6.07 ^b	6.00 ^{ab}	5.72 ^b	9.61 ^{ab}	8.49 ^{ab}	9.49 ^{ab}	10.55	9.18 ^b	9.68 ^b
2% Alternan	6.02 ^b	6.45 ^a	6.27 ^a	8.97 ^b	7.68 ^b	8.98 ^b	10.37	9.38 ^{ab}	10.21 ^{ab}
Corn-SBM	6.19 ^b	5.92 ^b	6.20 ^{ab}	9.99 ^a	9.42 ^a	9.07 ^b	11.07	10.55 ^a	10.28 ^a
Pooled SEM	0.16	0.20	0.19	0.33	0.43	0.22	0.34	0.52	0.22

^{a-b} Means within a column (diet effect) with no common superscript differ significantly ($P < 0.10$).

¹ Means represent 4 replicates of 15 chicks (3 days), 10 chicks (7 days), and 5 chicks (21 days of age) each per treatment.

² SBM = soybean meal.

General Summary

The gastrointestinal tract of animals is known to harbor extensive and diverse microorganisms and communities. The host animal, through hundreds of years of adaptation, has formed a complex symbiotic relationship with these communities and ultimately depends on them for much of its survival. Depending on which species are present, these microorganisms can either have a beneficial or harmful effect on the intestinal and overall health of the host. Early establishment of beneficial microbial populations is crucial in the development of the immune system and in resistance to colonization by pathogenic organisms. The control of pathogenic bacteria, and thus, the maintenance of a healthy gut, has historically been accomplished with the addition of antibiotic growth promoters (AGPs) to the diets of livestock. Recently, this practice has been the cause of increased concern among consumers and politicians because it is believed to promote antibiotic resistance among microorganisms, especially those that may be considered human pathogens. Consequently, producers and researchers are trying to find new dietary strategies or alternative feed additives that may improve the growth and maintain the intestinal health of poultry when AGPs cannot be included in the diet. Feeding prebiotic-type compounds and whole or large particle grains may be possible alternatives. In order for these compounds to be viable alternatives, however, they need to maintain intestinal health by supporting the growth of beneficial microbial populations, while having no negative effects on growth performance or nutrient digestibility. In addition, if these additives can elicit a positive response even when being fed for short-time periods or when the bird has a challenge to its immune system, such as during an acute coccidiosis infection, they may give producers an economically viable way to maintain the health of their flocks.

The objectives of the first study were to evaluate large particle corn, whole sorghum, and Grobiotic (GB), a prebiotic-type product that provides yeast cell wall and yeast soluble components, lactose, and other fermentable carbohydrates in a corn-soybean meal (SBM) diet with or without the addition of distillers dried grains with solubles (DDGS). Generally, weight gain and feed efficiency ($P < 0.05$) were decreased during the first week for chicks fed diets containing large ground corn or whole sorghum in a corn-SBM diet (no DDGS), but these results did not persist throughout the remainder of the experiment. When DDGS was included in the diet, this depression was not seen. Diets containing 20% whole sorghum or 5% GB showed an increase ($P < 0.05$) in ME_n when compared to all other treatments. The effects of diet on amino acid digestibility were generally small and inconsistent in both experiments. The data from this study showed a consistent increase in relative gizzard weight (% of body weight) in all experiments when large ground corn, whole sorghum, and DDGS were fed. There was no consistent effect of dietary treatment on cecal bifidobacteria, lactobacilli, or *E. coli* populations or gizzard pH. These results indicate that larger particle sizes from large ground corn or whole sorghum, as well as the addition of GB, can be supplemented to a corn-SBM or corn-SBM DDGS diet with no adverse effect on weight gain when fed from 0 to 21 d of age. Also, relative gizzard weights can be significantly increased when large ground corn or sorghum are included in the diet of very young chicks, which may have beneficial effects on intestinal health.

The next study was conducted to evaluate the effects of fine and large ground corn, whole sorghum, and whole wheat in the presence and absence of an acute *Eimeria acervulina* coccidiosis infection. In the first experiment, the coccidial challenge was generally not effective in significantly reducing growth performance, so a second experiment was performed. In the second experiment, the *E. acervulina* infection reduced chick performance, ME_n , and AA

digestibility ($P < 0.05$), and the addition of large ground corn, whole sorghum, or whole wheat was not effective in alleviating the negative effects of a coccidiosis infection. In the absence of a coccidiosis infection, the addition of whole or large particle grains had no consistent effects on weight gain, feed intake, or feed efficiency. In both experiments, relative gizzard weights were increased ($P < 0.05$) and cecal *E. coli* populations were decreased when large ground corn or whole grains were included in the diet, which may result in a healthier intestinal environment. Moreover, the increases in gizzard weights with the large ground corn and whole wheat or sorghum diets were greater in the presence than in the absence of a coccidiosis infection, indicating some particularly potential intestinal health benefits of feeding larger particle or whole grains in chicks infected or exposed to a coccidial challenge.

The next study evaluated the effects of short-term feeding of GB, Dairy-lac-80, and lactose in both a corn-soybean meal diet and a casein-dextrose diet on growth performance, nutrient digestibility, and microbial populations of bifidobacteria, lactobacilli, and *E. coli* in two experiments. In addition, the effects of these compounds in the presence and absence of an *E. acervulina* infection were also determined in a third experiment. In the first two experiments, diets containing GB, Dairy-lac-80, and lactose generally had no consistent effects on growth performance whether they were in a corn-SBM diet or casein-dextrose diet. However, chicks fed all casein-dextrose diets had increased weight gain and feed efficiency ($P < 0.05$) during the first week when compared to chicks fed the corn-SBM diet, as well as increased ME_n and AA digestibility. The addition of GB, Dairy-lac-80, and lactose to both types of diets resulted in a significant increase ($P < 0.05$) in cecal bifidobacteria populations, while cecal lactobacilli increased ($P < 0.03$) and *E. coli* decreased ($P < 0.03$) when casein-dextrose diets were supplemented with GB, Dairy-lac-80, and lactose. The *E. acervulina* infection in the third

experiment significantly reduced chick growth performance, ME_n, and AA digestibility ($P < 0.05$). However, the addition of GB, Dairylac-80, and lactose to the diet had no consistent effects on growth performance and did not ameliorate the negative effects of the coccidiosis infection. Overall, the results from this study indicated that very short-term feeding of various potential prebiotics, especially those which contain some lactose, had no negative effects on chick growth performance, and that dietary prebiotic ingredients can positively influence cecal microbial populations even when given to the chick for a period of only 3 or 7 d.

The final study evaluated the prebiotic potential of several feed additives and examined the effects of these products on growth performance, nutrient digestibility, and select cecal microbial populations at several different ages in young chicks. The first three experiments evaluated the effects of feeding Temulose, partially hydrolyzed Temulose, and GB in a corn-soybean meal diet, while the last three experiments evaluated the effects of DDGS, Elusieve fiber, and Alternan in a corn-soybean meal or casein-dextrose diet. Both Temulose compounds had no negative effects on weight gain and no consistent effects on feed intake or feed efficiency when fed to chicks for either 3, 7, or 21 days. The addition of partially hydrolyzed Temulose to the diet of chicks at 3 d resulted in significantly higher ($P < 0.10$) populations of bifidobacteria, lactobacilli, and *E. coli* when compared to the original, nonhydrolyzed Temulose. However, both Temulose compounds also decreased ($P < 0.10$) lactobacilli populations at 7 days of age, with no other consistent effects seen in the other microbial populations. In the last three experiments, diets containing Elusieve fiber and Alternan had no consistent effects on growth performance whether it was in a corn-SBM diet or casein-dextrose diet. However, chicks fed all casein-dextrose diets, regardless of supplementation, had increased weight gain and feed efficiency ($P < 0.05$) during the first week when compared to chicks fed the corn-SBM diet, as well as increased

ME_n and AA digestibility. Bifidobacteria were decreased ($P < 0.10$) when 2% Alternan was added to a corn-SBM diet and this same result was observed for the casein-dextrose diet.

Overall, the results from this study indicated that short-term feeding of various potential prebiotics had no negative effects on chick growth performance. In addition, these results show that several potential prebiotic feed ingredients or additives had beneficial effects on cecal microflora, particularly those containing lactose, even when fed to the chick for a period of only 3 or 7 d.

Appendix A

Denaturing Gradient Gel Electrophoresis (DGGE)

This appendix includes information about the DDGE procedures used in select experiments presented in this thesis, as well as the results obtained from the DGGE process including dendrograms and Dice coefficients of similarity. DGGE procedures were performed according the techniques of Lubbs et al. (2008) and Vester et al. (2009).

PCR Amplification of 16S rDNA

Briefly, template DNA was amplified using PCR involving primers (25 pmol of each per reaction mixture) targeting the conserved regions flanking the variable region 3 (V3) of the 16S rDNA gene as described by Muyzer et al. (1996) and Simpson et al. (1999). ‘Eubacterial’ primer 341F (5′-CCTACGGGAGGCAGCAG-3′) and the ‘universal’ primer 543R (5′-ATTACCGCGGCTGCTGG-3′), the number corresponding to *E. coli* 16S rDNA (Muyzer et al., 1996), were obtained from Operon Biotechnologies, Inc. (Huntsville, AL). To provide a more stable melting behavior of fragments in DGGE, a GC-rich sequence (GC-clamp) was attached to the 5′ end of 341F (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGGCACGGGGGG-3′). Primers 341F and 543R amplify PCR products containing approximately 200 base pairs. Parameters for PCR amplification were done according to Lubbs et al. (2008).

Removal of Single-stranded DNA from PCR products

Single-stranded DNA (ssDNA) may remain in some of the samples following the PCR process and can present problems for image analysis. Thus, mung bean nuclease (Stratagene, LA Jolla, CA) treatment was performed on PCR products to eliminate ssDNA as described by Simpson et al. (1999). The reaction mixture contained 10 µl PCR product, 1.5 µl 10X Tris-acetate EDTA (TAE) buffer, 1.0 µl mung bean nuclease (diluted 1:20 in sterile deionized water),

and 2.5 µl sterilized deionized water. This mixture was then incubated at 37°C for 10 min. 15 µl of 2X DDGE loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol, sterile deionized water) was then added to stop the reaction.

Denaturing Gradient Gel Electrophoresis

Mung bean nuclease-treated samples were separated on polyacrylamide gels [non-deionized 40% acrylamide/bis stock solution 37.5:1 (Bio-Rad laboratories, Hercules, CA)] on a 29 to 48% gradient. A 100% denaturing solution contains 40% (vol/vol) formamide and 7 M urea. The gradient was formed using a Bio-Rad Gradient Former Model 475 (Bio-Rad Laboratories) and gels were polymerized onto a gel support film (FMC, Rockland, ME). Aliquots of 12 µl of mung bean nuclease-treated samples and 3 µl of 2X DGGE loading dye were mixed and loaded into sample wells. Electrophoresis was performed using a D-Code Universal Mutation Detection System (Bio-Rad Laboratories) using 0.5X TAE run buffer at 60 °C for 10 min at 50 V and then at 60 °C for 3 h at 150 V. Bacterial standard ladders (3 lanes per gel) were included on all DDGE gels prepared in each experiment. Inclusion of the ladder permits mapping of band migration within the standard ladder on each gel. Ladder band migration is then used to standardize all banding patterns on each gel, allowing analysis across gels. Bands were stained in the gel using SYBR Green Stain I (Invitrogen, Eugene, OR) using a 4 µl 10,000X stain/20 ml 1X TAE buffer dilution and developed for 20 min. Gels were read using a Gel Doc XR scanner (Bio-Rad Laboratories) under a UV light with a SYBR Green emissions filter and analyzed using QuantityOne (Bio-Rad Laboratories) and Bionumerics 5.0 (Applied Maths Inc., Austin, TX) software. Lanes and bands were automatically detected by the program. Phylogenetic trees for each age period were formed using the unweighted pair group

method using Arithmetic Averages (UPGMA) clustering based on Dice coefficient similarity values to create a dendrogram.

Grobiotic, Dairylac-80, and Lactose in a Corn-Soybean Meal Diet (Chapter IV - Experiment 1)

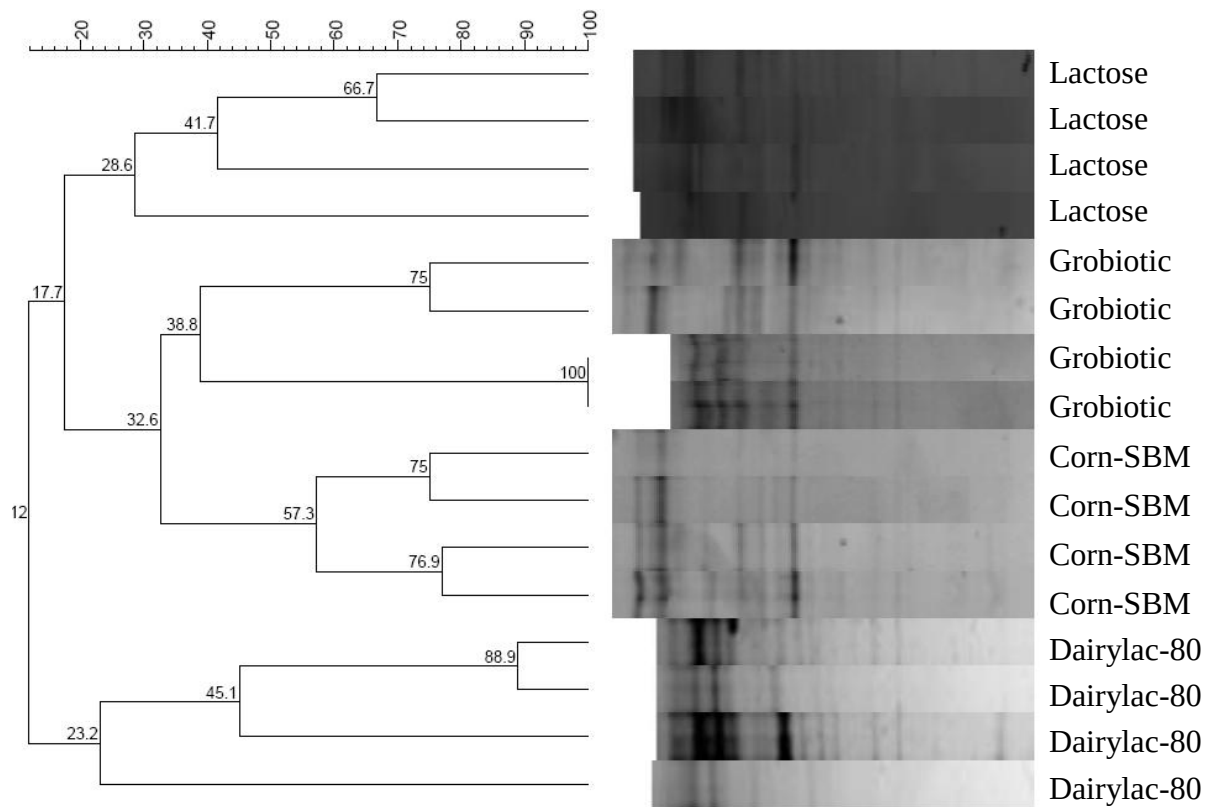


Figure A.1. DGGE Gel and Dendrogram of 3 D Old Chicks Fed Corn-SBM, 5% Grobiotic, 1.2% Dairylac-80, and 1% Lactose. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

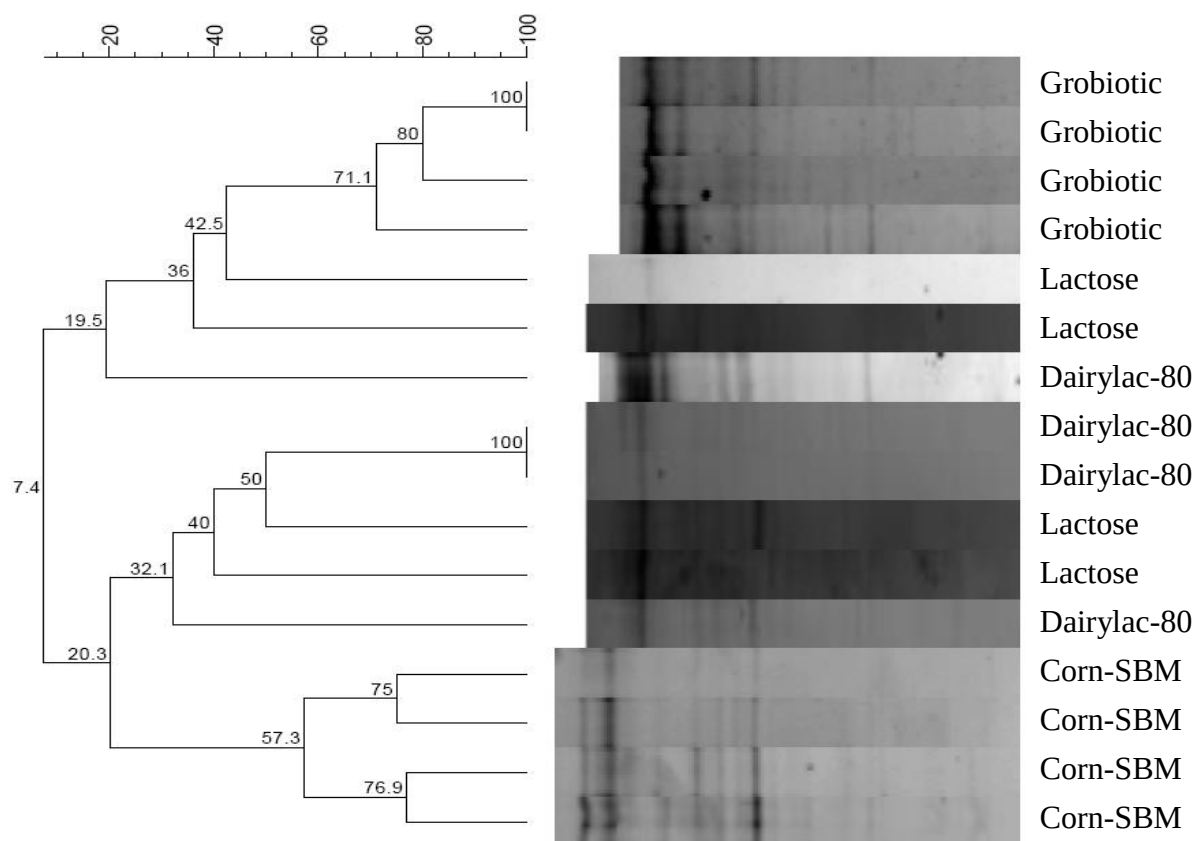


Figure A.2. DGGE Gel and Dendrogram of 7 D Old Chicks Fed Corn-SBM, 5% Grobiotic, 1.2% Dairylac-80, and 1% Lactose. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

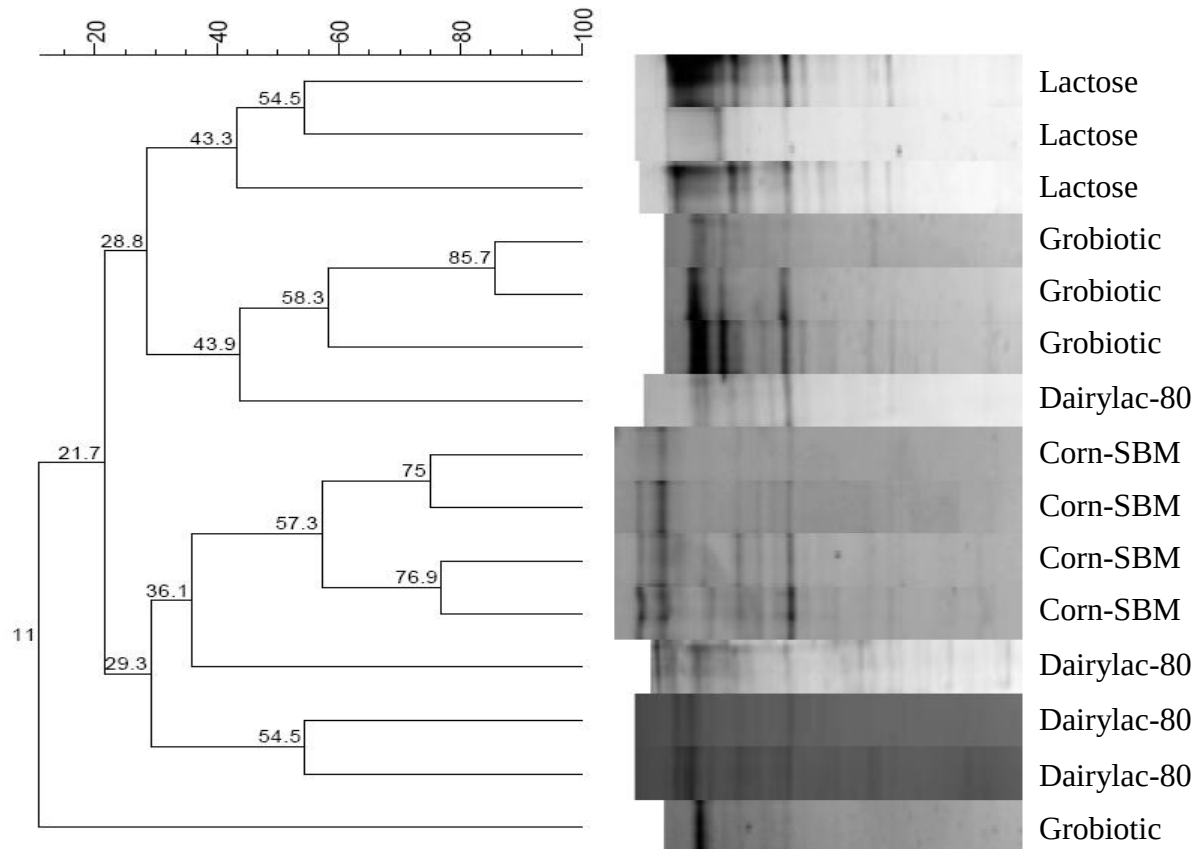


Figure A.3. DGGE Gel and Dendrogram of 21 D Old Chicks Fed Corn-SBM, 5% Grobiotic, 1.2% Dairylac-80, and 1% Lactose. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

Fingerprint analysis of DDGE banding patterns at 3 d (Figure A.1) shows a distinct separation due to diet. Using separation by Dice coefficients, the DGGE fingerprints determined 4 major clusters sharing 12% similarity. Each cluster represents each specific diet that was fed for the 3 d period. Fingerprint analysis at 7 d (Figure A.2) again shows a separation due to diet; however, only 3 major clusters sharing 7.4% similarity emerged compared to 4 previously. The first cluster primarily contained replicates of chicks fed the Grobiotic diet, while the second cluster contained replicates of chicks fed both Dairylac-80 and lactose. The third cluster contained replicates of chicks exclusively fed the corn-SBM control diet. DGGE banding patterns at 21 d (Figure A.3) once again showed a general trend toward separation of replicates

by diet, with 3 major clusters emerging. One cluster containing the lactose replicates, one containing mainly Grobiotic replicates, and one containing a combination of Dairylac-80 and corn-SBM replicates were formed.

Alternan in a Corn-Soybean Meal Diet (Chapter V- Experiment 5)

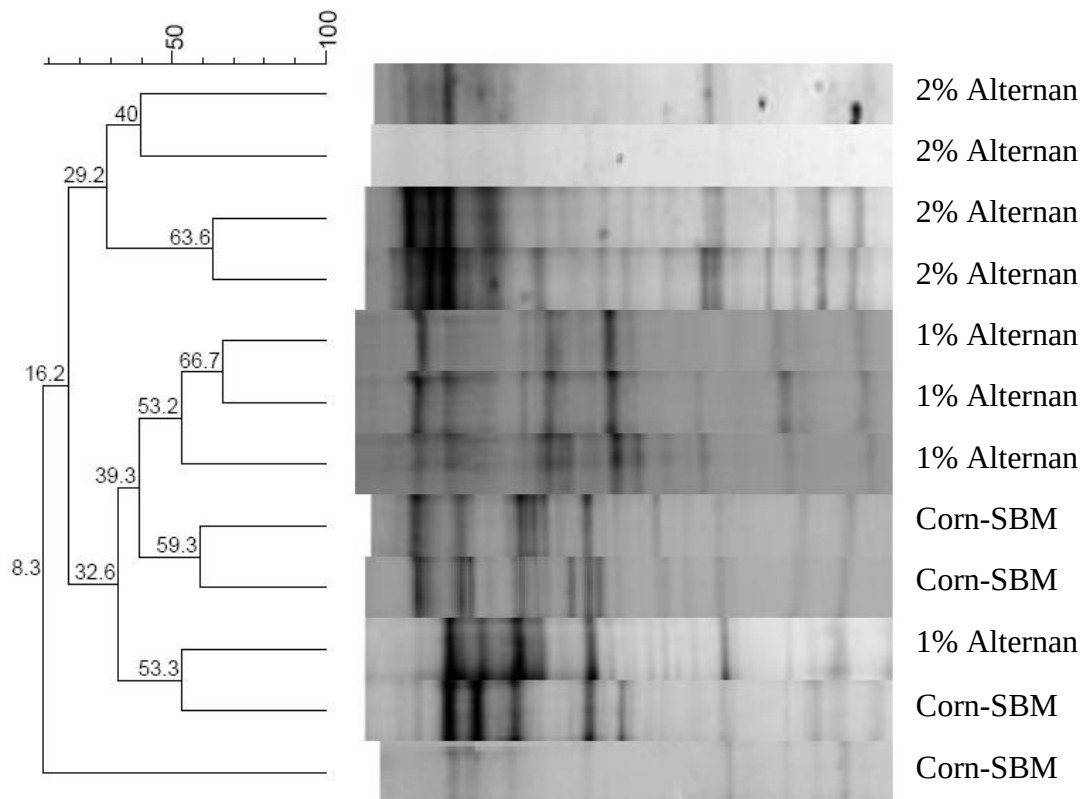


Figure A.4. DGGE Gel and Dendrogram of 3 D Old Chicks Fed Corn-SBM and 1 or 2% Alternan. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

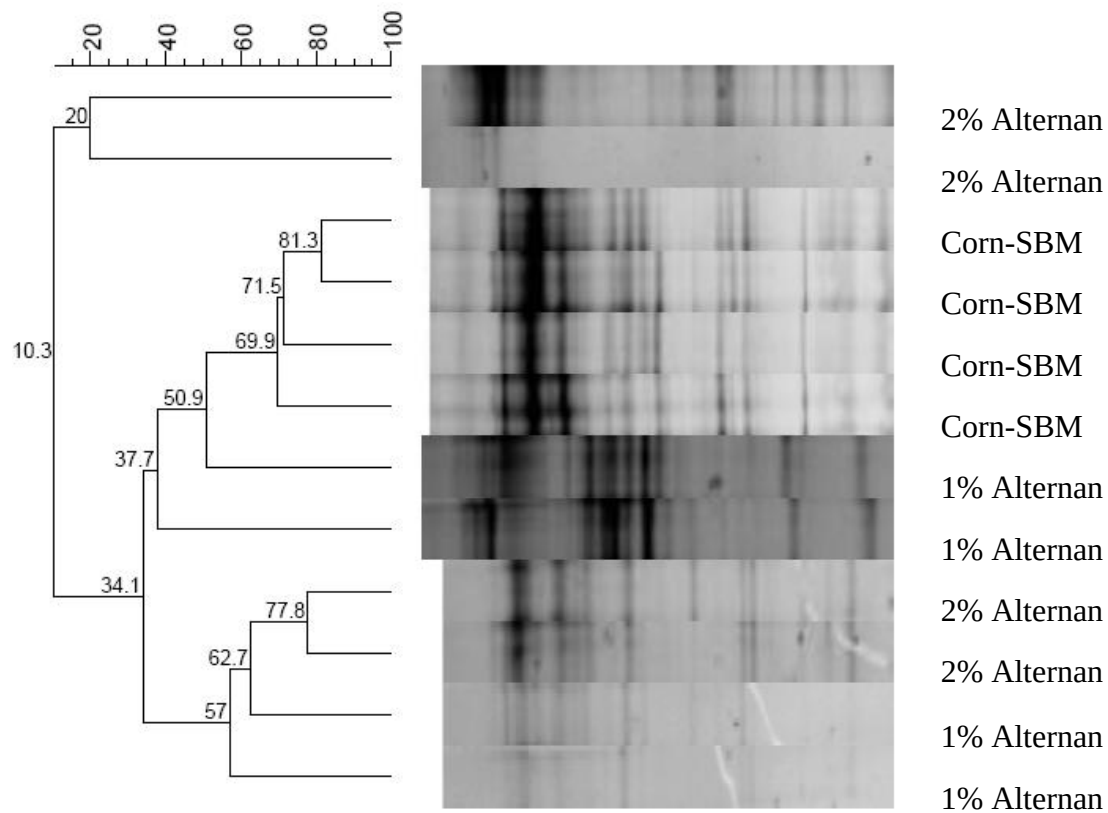


Figure A.5. DGGE Gel and Dendrogram of 7 D Old Chicks Fed Corn-SBM and 1 or 2% Alternan. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

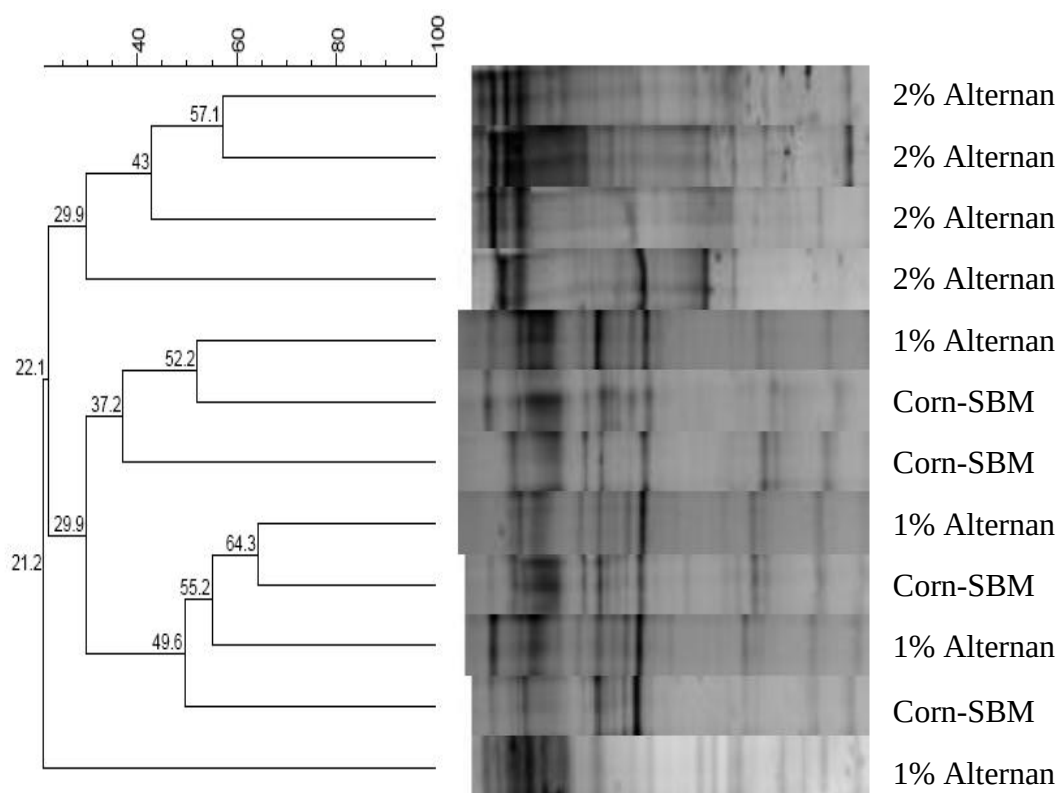


Figure A.6. DGGE Gel and Dendrogram of 21 D Old Chicks Fed Corn-SBM and 1 or 2% Alternan. Relatedness was calculated using Dice coefficients of similarity and are reported as percent similarity. SBM = Soybean meal.

Fingerprint analysis of DDGE banding patterns at 3 d (Figure A.4) shows a general trend toward separation due to diet. Using separation by Dice coefficients, the DGGE fingerprints determined 2 major clusters sharing 16.2% similarity. The first cluster contained replicates of chicks exclusively fed the 2% Alternan diet, while the second cluster contained replicates of chicks fed the 1% Alternan and corn-SBM control treatments. DGGE fingerprint analysis at 7 d (Figure A.5) determined 3 major clusters. The first cluster contained 2 replicates of chicks fed the 2% Alternan diet, while the second cluster contained primarily replicates of chicks fed the corn-SBM control diet. The third cluster contained replicates of chicks fed both the 1 and 2% Alternan treatments. DGGE banding patterns at 21 d (Figure A.6) once again showed the 2%

Alternan replicates formed one distinct cluster, while the other clusters were a combination of both the 1% Alternan and corn-SBM control replicates.

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Curriculum Vitae

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Education

- **Doctor of Philosophy, Animal Sciences** December 2010
University of Illinois, Urbana, IL
Advisor: Dr. Carl Parsons; Interest: Poultry Nutrition
- **Master of Science, Animal Sciences** December 2007
University of Illinois, Urbana, IL
Advisor: Dr. Carl Parsons
- **Midwest Poultry Consortium Center of Excellence Program** Summer 2004-2005
University of Wisconsin, Madison, WI
- **Bachelor of Science, Animal Sciences** May 2005
University of Illinois, Urbana, IL

Work Experience

Graduate Research Assistant

August 2005 to Present

Nutritional expertise includes:

- Diet formulation and mixing of both practical and purified experimental diets
- Design and setup of experimental feeding trials for laying hens and broiler chicks
- Design and application of coccidiosis challenge studies utilizing broiler chicks
- Surgical procedures for cecectomy on roosters,
 - Dosing and administration of anesthesia, surgery, and suture techniques
- Microbial culture analysis including serial dilution and plating, quantitative PCR, and DGGE
- Assisting in precision-fed bioassays with adult roosters and chicks
- Collection of excreta and various tissue samples such as blood, tibia, intestinal tissue, meat components, and internal organs
- Evaluation of egg components
 - Grading, candling, specific gravity, Haugh units, and quality
- Hatchery and grower management
 - Egg transfer and incubator setup
 - Experience in vaccinating (aerosol and injection), dubbing, toe-clipping, beak trimming, dewatting, and Pullorum testing

Laboratory Skills

- Nutritional analysis of feed ingredients including dry matter, ADF, NDF, and ADL analysis, bomb calorimetry, and Kjeldahl procedures
- PCR techniques including isolation of DNA from blood and digesta, amplification, and gel electrophoresis
- Proficient in SAS (statistical analysis program), Microsoft Office, and Windows

- As lead TA, delivered presentations, graded term papers, and assisted undergraduate students and fellow TAs in the World Animal Resources course, which is an intensive writing class
- Taught labs in the introductory undergraduate Animal Sciences courses including Domestic Animals in Their Environment, Introduction to Animal Sciences, and Poultry Production
- Completed Writing Across the Curriculum Graduate Teaching Assistant Training Seminar, Spring 2009

Publications and Presentations

Peer-reviewed journal articles

- Mejia, L., **C. M. Jacobs**, P. L. Utterback, C. M. Parsons, D. Rice, C. Sanders, B. Smith, C. Iiams, and T. Sauber. 2010. Evaluation of nutritional equivalency of soybean meal with the genetically modified trait DP-3Ø5423-1 when fed to laying hens. *Poult. Sci.* (Submitted).
- Perez, V. G., **C. M. Jacobs**, J. Barnes, M. C. Jenkins, M. S. Kuhlenschmidt, G. C. Fahey, Jr., C. M. Parsons, and J. E. Pettigrew. 2010. Effect of corn distillers dried grains with solubles and *Eimeria acervulina* infection on growth performance and the intestinal microbiota of young chicks. *Poult. Sci.* (Submitted).
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- Kim, E. J., **C. M. Jacobs**, P. L. Utterback, and C. M. Parsons. Amino acid digestibility of various feedstuffs of plant and animal origin using three different methods. *Poult. Sci.* 89 (Suppl. 1): 89.
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Presentations/Posters

- **Jacobs, C. M.**, P. L. Utterback, and C. M. Parsons. 2010. Cecal microbial populations of young chicks fed several prebiotic-type compounds as determined by DGGE and quantitative PCR. *Poult. Sci.* 89 (Suppl. 1): 283.
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- **Jacobs, C. M.**, P. L. Utterback, and C. M. Parsons. 2007. Effects of corn particle size in a corn-soybean meal diet on growth performance and nutrient digestibility. *Poult. Sci.* 86 (Suppl. 1):220.
- **Jacobs, C. M.**, P. L. Utterback, C. M. Parsons, B. Smith, M. Hinds, D. Rice, M. Liebergesell, and T. Sauber. 2007. Feeding performance in laying hens fed diets containing DAS-59122-7 maize grain compared with diets containing non-transgenic maize grain. *Poult. Sci.* 86 (Suppl. 1): 519.

Awards/Activities

- Mitchell Fellowship in Animal Nutrition, Department of Animal Sciences (August 2009-2010)
- 2008 International Ingredient Corporation Pinnacle Award
- National Dean's List and Vice-President's List
- Co-founder and President of The Illini Poultry Club, 2005-2010
- Member of Gamma Sigma Delta, Poultry Science Association, American Society of Animal Science, World Poultry Science Association
- PSA Hatchery Student Champion, 2006-current