EFFECTS OF DISTILLERS DRIED GRAINS WITH SOLUBLES AND DIETARY FIBER ON THE INTESTINAL HEALTH OF YOUNG PIGS AND CHICKS

BY

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DISSERTATION

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ABSTRACT

The objective of this research was to determine the value of dietary fiber (DF) from distillers dried grains with solubles (DDGS) on the promotion of intestinal health in young pigs and chicks. Furthermore, purified sources of DF were used in pigs to elucidate whether the soluble or insoluble fraction of DF, or its absence from the diet, is beneficial in the prevention and recovery from post-weaning colibacillosis (PWC). The experimental approach consisted of developing a disease challenge model of PWC in pigs and coccidiosis in chicks. In pigs, a strain of β-hemolytic F18 *Escherichia coli* (Ecoli) was selected based on its capability to colonize and cause mild diarrhea. The inoculation consisted of 3 consecutive daily doses of either distilled water (Sham) or 10^{10} cfu of Ecoli/dose. Inoculation began on d 3 or 5 after weaning, considered post-inoculation (PI) d 0. In chicks, a dose of *Eimeria acervulina* (EA) was selected to reduce growth by about 20%. The inoculation consisted of a single dose of either distilled water (Sham) or 10^6 sporulated EA oocysts. Inoculation occurred on d 10 of age. Variables of response included signs of disease such as growth depression and diarrhea; changes in fecal coliforms by culturing; changes in bacterial populations of intestinal mucosa by molecular methods, targeting the V3 region of the bacterial 16S ribosome; and intestinal morphology. In pigs, the inoculation with Ecoli changed the populations of fecal coliforms over time. The pathogenic coliforms gradually replaced the commensal coliforms until PI d 6; then, the commensal coliforms gradually recovered and replaced the pathogenic coliforms (time quadratic, $P < 0.001$). This pattern of response shows the course of disease from infection to recovery. The inclusion of 10 or 20% DDGS in the diet delayed the drop in commensal coliforms during the infection and hastened the drop in pathogenic coliforms during recovery (diet × challenge × time, $P < 0.01$). This effect was observed in 2 consecutive experiments. During recovery from PWC, increasing concentrations of dietary DDGS decreased diarrhea (diet linear, $P < 0.01$) and increased villus height in jejunum of Ecoli pigs (diet linear × challenge, $P < 0.001$). Also in recovery, pigs fed either 5 or 10% DDGS had a larger bacterial diversity in cecum ($P = 0.02$) and colon ($P = 0.08$), than those fed 0 or 20% DDGS. When purified sources of DF were used, pigs fed a diet with insoluble dietary fiber recovered faster ($P < 0.05$) from PWC diarrhea. In chicks, the inclusion of up to 20% DDGS in the diet did not ameliorate the reduction in performance caused by EA. However, the cecal bacterial diversity and homogeneity within treatments were increased by feeding 10% DDGS (diet quadratic, $P < 0.001$). Those changes in cecal microbiota can be

interpret as beneficial for the intestinal health. In conclusion, DF did not prevent PWC in pigs or coccidiosis in chicks. However, the inclusion of insoluble DF from either DDGS or a purified source in pig diets hastened the recovery from PWC.

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To Úrsula, for the inspiration

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CHAPTER I

INTRODUCTION

The fiber fraction in diets for pigs and chicks mainly represents the cell wall of plants. Dietary fibers are indigestible carbohydrates because they cannot be hydrolyzed by endogenous enzymes (Bach Knudsen and Canibe, 2000) in the small intestine. Thus, the inclusion of fiber in diets for non-ruminants can dilute energy and nutrient concentration, which in turn may reduce growth performance (NRC, 1998). Therefore, dietary fiber has typically been an undesirable component of the diet. However, 2 main factors recently have driven the interest in dietary fiber inclusion in diets for pigs and chicks. First, the need for using non-traditional ingredients opens the opportunity to include feedstuffs with larger concentrations of fiber; for example, corn distillers dried grains with solubles (DDGS) is a by-product from the ethanol production. The DDGS has a large concentration of dietary fiber and is a suitable dietary ingredient for the pig (Shurson et al., 2000; Stein et al., 2006) and chicken (Martinez-Amezcua et al., 2007; Pahm et al., 2009). The second factor is a growing public concern regarding the use of in-feed antimicrobials for livestock production. The concern is because of the potential transmission of antibiotic resistance from animals to humans (McDermott et al., 2002; Witte et al., 2002). Consequently, in-feed antimicrobials as growth promoters have been banned in the European Union (Stein, 2002). Therefore, the interest in dietary alternatives to promote or maintain health in pigs and chicks has grown.

Dietary fiber can be classified as soluble or insoluble in water and weak alkali solutions. Each type of fiber has a different effect on the digestive physiology of non-ruminants (Bach Knudsen, 2001). Moreover, soluble fiber is rapidly fermentable by intestinal microbes, whereas insoluble fiber is slowly fermented (Grieshop et al., 2001). Dietary fiber serves as the main source of energy for the intestinal microbiota through fermentation (Williams et al., 2001). The composition of carbohydrates in dietary fiber can change the microbial populations and the products of microbial fermentation (Högberg and Lindberg, 2006; Pieper et al., 2008). Those changes can have an important effect in promoting intestinal health of non-ruminants through the diet (Montagne et al., 2003; Richards et al., 2005; Bauer et al., 2006). However, the effect of dietary fiber on health of non-ruminants is controversial. Some authors suggest that soluble fiber protects against enteric diseases (Thomsen et al., 2007; Wellock et al., 2008). In contrast, others have observed that soluble fiber is detrimental for pigs' health (Pluske et al., 1996; McDonald et

al., 1999). In addition, insoluble fiber prevents post-weaning colibacillosis in pigs (Mateos et al., 2006; Kim et al., 2008), whereas low-fiber diets based on rice reduced the susceptibility to enteric diseases (Montagne et al., 2004; Pluske et al., 2003).

It has been suggested that insoluble dietary fiber from DDGS may help to protect pigs from intestinal infections. However, a series of disease challenge experiments using *Lawsonia intracellularis*, the agent of porcine proliferative enteropathy, did not show consistent benefits of feeding DDGS (Whitney et al., 2006a,b,c). Nevertheless, the notion that DDGS ameliorates enteric infections in both pigs and chicks persists in the industry.

The objective of this research was to determine the value of dietary fiber from DDGS on the promotion of intestinal health in young pigs and chicks. Furthermore, purified sources of dietary fiber were used in pigs to elucidate whether the soluble or insoluble fraction of dietary fiber, or its absence from the diet, is beneficial in the prevention and recovery from enteric disease. The experimental approach consisted of developing a disease challenge model of colibacillosis in pigs and coccidiosis in chicks to assess the dietary effects on health. Preliminary experiments were conducted in pigs to select a strain of β-hemolytic F18 *Escherichia coli* capable of colonizing the gastrointestinal tract and causing mild diarrhea. In chicks, a dose of *Eimeria acervulina* was selected to reduce growth rate by about 20%.

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CHAPTER II

LITERATURE REVIEW

Definition of Dietary Fiber

The major source of energy in diets for pigs and chicks is carbohydrate, which can be categorized roughly as starch and dietary fiber (DF). Starch is the polysaccharide that plants use to store energy in the form of amylose (linear polymer of glucose units with α(1→4) linkages) and amylopectin (branched polymer of glucose units with α(1→4) and α(1→6) linkages). The DF mainly represents the plant cell wall polysaccharides and lignin. Lignin is a non-digestible phenolic polymer that anchors the cell wall polysaccharides. The major polysaccharides in plant cell wall are cellulose, hemicelluloses, and pectins. Cellulose is a linear polymer of glucose units with β(1→4) linkages. Hemicelluloses are a complex matrix of polysaccharides that include arabinose, xylose, galactose, mannose, glucuronic and galacturonic acids, and β-glucans. The pectins contain α(1→4) linkages and galacturonic acid residues (Bach Knudsen, 1997, 2001; Grieshop et al., 2001; Montagne et al., 2003). The DF can be classified as soluble or insoluble based on its solubility in water or weak alkali. In cereals, the polysaccharides that constitute insoluble DF include lignin, cellulose, and hemicelluloses. The polysaccharides that constitute soluble DF include mostly pectins, gums, some hemicelluloses, and may include a variable amount of fructan. The inclusion or not of some polysaccharides in the determination of soluble and insoluble DF may vary depending on the methodology to measure DF (Marlett et al., 1989; Bach Knudsen, 1997, 2001; Campbell et al., 1997; Montagne et al., 2003; NRC, 2007).

Dietary fiber has been defined differently throughout the years (DeVries, 2004). The general agreement among all definitions is the reference to non-digestible carbohydrates. The disagreement, however, resides in the concepts that are included or excluded in the definition. For example, some definitions of dietary fiber specifically refer to carbohydrates from plan material, which exclude carbohydrates from animal material, yeast, and endogenous losses. Other definitions refer to carbohydrates that escape enzymatic digestion specifically in humans, or that have a specific physiological effect. Dietary fiber also can be defined by analytical method. In general, there are 4 methods to measure DF as described below:

1) The crude fiber method. This method is part of the proximate analysis system (AOAC, 1980). It consists of a gravimetric determination. This method is the oldest one and underestimates the concentration of DF (Bach Knudsen, 2001).

2) The detergent fiber or Van Soest method. It is an enzymatic-gravimetric method to determine neutral detergent fiber (cellulose, hemicelluloses, and lignin), acid detergent fiber (hemicelluloses and lignin), and acid detergent lignin (Van Soest, 1963; Van Soest and Wine, 1967; Van Soest et al., 1991). The main limitation of this method is that it does not account for soluble DF (Marlett, 1989; Campbell et al., 1997).

3) The Prosky total DF method or the Association of Official Analytical Chemists method. This method is an enzymatic-gravimetric determination of soluble and insoluble DF (Prosky et al., 1985). The differentiation between soluble and insoluble DF offers a functional advantage, as the solubility is related to the ability of the fiber to be fermented by nonruminats. Soluble DF is rapidly fermented, whereas insoluble DF is fermented much slower than the soluble DF (Bach Knudsen, 2001).

4) The Uppsala total dietary fiber or enzymatic-chemical Uppsala method measures the DF as monomeric sugar components by chromatography or colorimetry (Theander et al., 1994).

In the present study, the definition and analysis of DF corresponds to the Prosky total DF method, following the procedure 991.43 as described by the Association of Official Analytical Chemists International (AOAC, 2007).

Colibacillosis in Pigs

Economic losses due to post-weaning colibacillosis (PWC) remain major problems in the swine industry through increased mortality, increased medication costs, and growth depression. Current strategies to prevent PWC include biosecurity measures, vaccination, sanitation, prophylactic antibiotics, and feed additives such as probiotics, prebiotics, and zinc. Despite these strategies, cases of severe *E. coli*-associated diarrhea outbreaks continue to occur (Pittman, 2010), perhaps because of the emergence of more virulent *E. coli* strains and development of antibiotic resistance (Fairbrother et al., 2005). The PWC often is caused by β-hemolytic F18 *E. coli* strains because the receptors for the F18 fimbria are expressed in pigs after weaning (Bertschinger and Fairbrother, 1999).

Coccidiosis in Chicks

Coccidiosis is a pervasive problem in poultry production (Williams, 2005). In broilers, the disease ranges from light infections, with no apparent reduction in chick health or performance to severe infections that result in clinically sick birds and mortality. However, the most common presentation of coccidiosis in the poultry industry is a subclinical infection that

does not produce clinical signs of disease but reduces growth performance, causing economically important losses (Williams, 1999; Haug et al., 2008). Among the *Eimeria* species that cause subclinical coccidiosis in chicks, *Eimeria acervulina* (EA) appears to be one of the most prevalent (Williams, 2005; Haug et al., 2008; Martynova-VanKley et al., 2008). Vaccines against *Eimeria* (Williams, 2002) and in-feed anticoccidial drugs (Chapman, 2001) have been used successfully to reduce the impact of the disease.

Dietary Fiber and Intestinal Health

Dietary fiber is the major source of energy to support microbial populations in the gastrointestinal tract (Bach Knudsen et al., 1991; Bach Knudsen and Hansen, 1991; Jensen and Jørgensen, 1994; Jørgensen et al., 1996). The chemical properties of DF, as well as its fermentation in the intestine, have an important role in keeping the balance between communities of commensals and pathogens (Bach Knudsen, 2001; Montagne et al, 2003). Also, the intestinal microbiota has the capacity to modulate the host's immune response (Bauer et al., 2006; Magalhaes et al., 2007). Thus, the source and concentration of DF in the diets of pigs and chicks represent an opportunity to manipulate the susceptibility and capacity to recover from enteric diseases.

The overall effects that DF has on intestinal health can be exerted, directly and indirectly, through the microbial fermentation and the physiochemical characteristics of DF. In nonruminants, some microbial fermentation occurs in the small intestine, but a greater microbial fermentation occurs in the large intestine, particularly in pigs and chicks (Bach Knudsen et al., 1993; Jensen and Jørgensen, 1994; Glitsø et al., 1998; Wenk, 2001). The soluble DF is usually fermented more proximal in the gastrointestinal tract, because it is rapidly fermented (Bach Knudsen et al, 1993; Glitsø et al., 1998; Bach Knudsen and Canibe, 2000). In contrast, insoluble DF is more resistant to fermentation because of its large concentration of lignin (Bach Knudsen, 1997; Glitsø et al., 1999). Fermentation of DF promotes the development of microbial communities (Bach Knudsen et al., 1991; Jensen and Jørgensen, 1994), which can inhibit the growth of pathogens (Wenk, 2001; Williams et al., 2001; Lesser and Mølbak, 2009). This concept is known as *colonization resistance* or *competitive exclusion* (Snel et al., 2002; Montagne et al., 2003; Lallès et al., 2007). In addition, the microbial fermentation of DF can promote the development of specific bacterial populations that are considered to be beneficial (Pieper et al., 2008), mostly because of their production of short-chain fatty acids (SCFA) in both

the small and large intestine (Williams et al., 2001; Bikker et al., 2006; Högberg and Lindberg, 2006; Reilly et al., 2010).

The microbial fermentation of DF produces SCFA, mainly acetate, propionate, and butyrate (Le Goff et al., 2003; Montagne et al., 2003). The SCFA are the major contributors of energy from fermentation to the host (Dierick et al., 1989; Yen et al., 1991; Jørgensen et al., 1996). The SCFA are rapidly consumed by the enterocyte (Bach Knudsen and Hansen, 1991; Bach Knudsen, 2001); particularly, butyrate is rapidly consumed by the colonocyte (Ruppin et al., 1980; Roediger, 1982; Rérat et al., 1987). The concentration of SCFA in the digesta may reduce the pH. A more acidic pH may inhibit the proliferation of some pathogens such as *Escherichia coli* (Montagne et al., 2003), *Clostridium difficile* (May et al., 1994), and *Salmonella spp*. (Cummings, 1983). Digesta pH has been used as an indirect estimation of SCFA concentration and microbial fermentation (Franklin et al., 2002; Högberg and Lindberg, 2004). However, digesta pH does not always reflect the concentration of SCFA (Pluske et al., 2003; Nyachoti et al., 2006). The discrepancy between digesta pH and the measured concentration of SCFA has been attributed to the rapid absorption of SCFA in the enterocyte, which is even faster under low pH and at greater concentrations of SCFA (Von Engelhardt et al., 1989). Also, it has been suggested that the eventual lack of correlation between pH and SCFA in digesta can be due to other factors that alter digesta pH, such as the pK_a and proportion of specific SCFA present in the digesta, or the buffering capacity of some nutrients as protein (Pluske et al., 1998, 2003; Nyachoti et al., 2006). In addition, the SCFA have a trophic effect in the intestine, increasing the enterocyte turnover rate (Roediger, 1982; Sakata, 1987; Tappenden and McBurney, 1998). All of these described effects of SCFA in digestive physiology help in maintaining the integrity of the intestinal mucosa, which is a barrier to protect against pathogenic bacteria (Magalhaes et al., 2007). Furthermore, SCFA may ameliorate diarrhea and prevent dehydration by promoting resorption of water and sodium in the large intestine (Roediger and Moore, 1981).

Two of the most important physicochemical characteristic of DF are their viscosity and capacity to retain water (Bach Knudsen, 2001; Wellock et al., 2007). Soluble DF is more viscous than insoluble DF (Dikeman and Fahey, 2006; Dikeman et al., 2006). The soluble DF increases the viscosity of digesta and reduces the rate of passage (Rainbird and Low, 1986a,b; Glitsø et al., 1999); these effects can reduce the absorption of nutrients (Rainbird et al., 1984) and increase satiety (Bach Knudsen, 2001). In contrast, insoluble DF reduces the viscosity of digesta and

increases the rate of passage (Stanogias and Pearce, 1985; Potkins et al., 1991), which has been suggested to stimulate early feed intake in newly weaned pigs (Reis de Souza et al., 2005b). The capacity of DF to retain water is call *water holding capacity* (Bach Knudsen, 2001). This characteristic of DF increases the bulk of digesta and rate of passage (Bach Knudsen and Hansen, 1991; Mcrorie et al., 2000; Molist et al., 2009). The bulk of digesta promotes intestinal development in both pigs and chicks (Wyatt et al., 1988; Jørgensen et al., 1996; McDonald et al., 1999, 2001; Reis de Souza et al., 2005a) which, in turn, may increase motility (Mateos et al., 2006).

The Controversy

Conflicting results have been published regarding the effects of DF on intestinal health (Wellock et al., 2007). This controversy is based on 2 major concepts. First, the effects of DF in the organism depend on the physicochemical properties of different types of DF. Secondly, DF can promote or suppress health depending on what is considered to be beneficial. For example, some humans considered to be beneficial the increase in viscosity of digesta that soluble DF promotes. As described above, a more viscous digesta reduces the absorption of nutrients and increases satiety. These effects are beneficial to control energy intake in obese humans and to reduce cholesterol concentration. In limit-fed gestating sows, reducing absorption of nutrients and increasing satiety is also beneficial (Robert et al., 1993, 1997; Meunier-Salaün et al., 2001). However, the same effects are detrimental in growing pigs and broilers because of the reduction in growth performance (NRC, 1994, 1998). Similarly, microbial fermentation is considered to be beneficial when it contributes energy to the host; it happens when the energy is not readily available for enzymatic digestion (Bach Knudsen and Hansen, 1991; Jørgensen et al., 1996; Renteria-Flores et al., 2008). However, when dietary energy is in the form of carbohydrates that are easy to digest and easily absorbed by the host, the microbial populations compete with the host for energy (Maramatsu et al., 1994; Jørgensen et al., 1996; Flint et al., 2007).

The promotion of microbial growth by fermentation of DF is desirable because of the competitive exclusion of pathogens. However, fermentation of viscous soluble DF has been shown to promote PWC (McDonald et al., 1999, 2001) and other enteric diseases (Pluske et al., 1996, 1998; Hopwood et al., 2002). In addition, some products of microbial fermentation, such as SCFA, are beneficial for intestinal health. However, under excess of protein or amino acids imbalances in the diet, the products of microbial fermentation are detrimental for the intestinal

health (Bikker et al., 2006; Jeaurond et al, 2008; Heo et al., 2009; Hermes et al., 2009; Opapeju et al., 2009).

The effect of DF in the protection against enteric diseases in pigs and chicks has been investigated, but the results are controversial. The information available on this issue can be separated into the following 4 concepts:

1. Soluble DF are protective against PWC and swine dysentery.
2. Soluble DF promote PWC and swine dysentery.
3. Low concentrations of DF in rice-based diets are protective against PWC diarrhea.
4. Insoluble DF in diets for pigs are protective against PWC diarrhea.

The conditions under which these results were observed are reviewed below.

Soluble fibers are protective against PWC and swine dysentery

The beneficial effects of soluble DF in the protection against PWC (Wellock et al., 2008) and swine dysentery (Thomsen et al., 2007) have been documented. Both of those authors recognized that increasing the viscosity of digesta by using a source of soluble DF in the diet for pigs may promote PWC diarrhea. However, they suggested that the viscosity, and not the fermentation of soluble DF, can be the detrimental factor. Therefore, they used inulin as the source of soluble, non-viscous DF.

Inulin has a prebiotic effect (Flickinger et al., 2003; Waltz et al., 2005). Therefore, the protective effect observed in those cases is more likely to happen because of the prebiotic effect of inulin rather than the fermentation of soluble DF *per se*.

Soluble fibers promote PWC and swine dysentery

Soluble DF promote PWC (McDonald et al., 1999; 2001; Hopwood et al., 2002, 2004; Montagne et al., 2004), swine dysentery (Pluske et al., 1996, 1998), and colibacillosis in the rat (Wyatt et al., 1988) and mice (Swidsinski et al., 2009). The detrimental effect has been clearly associated with the fermentation capacity and viscosity of the soluble dietary fiber. The detrimental effects also include damage to the intestinal mucosa.

Low concentrations of DF in rice-based diets are protective against PWC diarrhea

Feeding pigs with low-fiber diets based on rice ameliorates PWC diarrhea (McDonald et al., 1999; 2001; Hopwood et al., 2002; Pluske et al., 2003, 2007; Montagne et al., 2004; Mateos et al., 2007; Vicente et al., 2008), as well as the number of sick pigs in commercial production (Perez et al., 2006). Diets based on cooked rice have not only low in DF concentrations, but also

have a large concentration of starch that is rapidly digested and absorbed. It is possible that the beneficial effects observed in pigs fed rice-base diets are not because of the low concentration of fiber, but to the rice *per se*, because rice has a direct effect in reducing diarrhea (Macleod et al., 1995) through a compound that has been called *the rice factor* (Mathews et al., 1999).

Insoluble DF in diets for pigs are protective against PWC diarrhea

The inclusion of insoluble DF reduces PWC diarrhea (Mateos et al., 2006; Kim et al., 2008). Insoluble DF may promote health, directly and indirectly, by maintaining the integrity of the intestinal mucosal barrier and promoting the excretion of pathogens through a washout effect.

The insoluble DF may exert a physical effect on the excretion of pathogens by reducing viscosity of digesta and increasing rate of passage and motility. In addition, insoluble DF promotes the enterocyte turnover rate (Jin et al., 1994) and growth of villous length (Hedemann et al., 2006). Thus, more cells slough off to the intestinal lumen, contributing to the physical excretion of pathogens. Although fermentation of insoluble DF is less than that of soluble DF, insoluble DF also promotes the production of SCFA (Högberg and Lindberg, 2006; Reilly et al., 2010). As previously described, the SCFA promote the integrity of intestinal tissues and the resorption of water and sodium. Moreover, insoluble DF may also promote the secretion of saliva, gastric juice, pancreatic juice, and bile (Zebrowska et al., 1983; Low, 1989; Dongowski et al., 2002; Lallès et al., 2007; Piel et al., 2007; Wilfart et al., 2007), which contain bactericidal enzymes and antibacterial peptides (Montage et al., 2003).

DDGS and Health

Corn distillers dried grains with solubles (DDGS) is a feedstuff with a large concentration of insoluble fiber (Martinez-Amezcua et al. 2007; Pahm et al., 2009) and is suitable for pig (Shurson et al., 2000; Stein et al.,2006) and poultry (Parsons and Baker, 1983) diets. Therefore, it has been proposed that insoluble dietary fiber from DDGS may help protect pigs and chicks against enteric disorders.

In this regard, Whitney et al. (2006a,b,c) conducted a series of studies using *Lawsonia intracellularis*, the agent of porcine proliferative enteropathy (ileitis). However, no consistent protective benefits were observed as the incidence and severity of intestinal lesions typical of ileitis were reduced in only 1 of 3 experiments. No clinical benefits were observed as well. In another study with finisher pigs, pig mortality in a commercial environment decreased as more DDGS was included in the diet (Cook et al., 2005). Despite the lack of more data, the notion that

DDGS helps to ameliorate enteric infections in pigs persists in the industry. We hypothesized that the inclusion of insoluble dietary fiber from either DDGS or a purified source can be protective against enteric diseases in young pigs and chicks.

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CHAPTER III

INCLUSION OF DISTILLERS DRIED GRAINS WITH SOLUBLES IN PIG DIETS HASTENS RECOVERY FROM POST-WEANING COLIBACILLOSIS: CLINICAL SIGNS AND HISTOLOGY

Abstract

Three experiments were conducted to determine whether dietary distillers dried grains with solubles (DDGS) can prevent post-weaning colibacillosis (PWC) and promote recovery in pigs. A pathogenic F18 *E. coli* strain (SLT2) was selected based on its capability to colonize the gastrointestinal tract (Exp. 1). Experiment 2 was a factorial arrangement of 2 diets (inclusion of 0 vs. 20 % DDGS) × 4 challenge treatments: 1) distilled water (Sham); 2) single dose of SLT2 to provide 10^{10} cfu (EcAL); 3) single dose of SLT2 to provide 10^{11} cfu (EcAH); and 4) 3 consecutive doses of SLT2 to provide 10^{10} cfu/dose (EcCR). Experiment 3 had a factorial arrangement of 4 diets (inclusion of 0, 5, 10, or 20% DDGS) × 2 challenge treatments (Sham vs. EcCR). Each treatment was replicated with 6 pigs (21 d old, 6.23 ± 0.34 kg BW in Exp. 2, and 5.96 ± 0.48 kg BW in Exp. 3). Inoculation began on d 3 post-weaning, considered post-inoculation (PI) d 0. Measurements included ADG, diarrhea score (1= normal feces through 5= watery diarrhea), fecal coliforms culture score (0= no growth through 10= very heavy growth), and intestinal histology on PI d 11. Data were analyzed using the MIXED procedure of SAS; culture score data were analyzed as repeated measures. Pigs in EcAH or EcCR treatments grew less ($P < 0.05$) from PI d 0 to 7 than those in Sham or EcAL, regardless of diet. No differences among treatments in ADG were detected from PI d 7 to 11. Diarrhea score from PI d 8 to 11 decreased as dietary DDGS increased: 2.15, 1.98, 1.81, and 1.29 (SEM= 0.22; diet linear, $P < 0.01$) for diets with 0, 5, 10, and 20% DDGS, respectively. Fecal coliforms shifted from commensal to pathogenic colonies until PI d 6, then shifted again showing a gradual recovery of commensals and excretion of pathogenic coliforms (time quadratic, $P < 0.001$). The commensal coliforms in pigs fed 20% DDGS recovered faster than those in pigs fed 0% DDGS (time × diet, $P < 0.01$). Villus height in the jejunum of EcCR pigs increased as more DDGS was included in the diet (diet linear × challenge, $P < 0.001$). The inclusion of up to 20% DDGS in diets for pigs did not prevent PWC; however, signs of a faster recovery were observed when DDGS was included in the diet.

Key words: colibacillosis, distillers dried grains with solubles, fecal coliforms, pig

Introduction

Economic losses due to post-weaning colibacillosis (PWC) remain major problems in the swine industry through increased mortality, increased medication costs, and growth depression. Current strategies to prevent PWC include biosecurity measures, vaccination, sanitation, prophylactic antibiotics, and feed additives such as probiotics, prebiotics, and zinc. Despite these strategies, cases of severe *E. coli*-associated diarrhea outbreaks continue to occur (Pittman, 2010), perhaps because of the emergence of more virulent *E. coli* strains and development of antibiotic resistance (Fairbrother et al., 2005). The PWC is often caused by β-hemolytic F18 *E. coli* strains, because the receptors for the F18 fimbria are expressed in pigs after weaning (Bertschinger and Fairbrother, 1999).

An effective strategy to maintain pig health should include diet modifications as a preventive measure (Pettigrew, 2006). The inclusion of dietary fiber in diets of young pigs has been proposed to be beneficial in promoting intestinal health (Wellock et al., 2007). Insoluble dietary fiber in particular, has been shown to prevent post-weaning diarrhea in pigs (Mateos et al., 2006; Kim et al., 2008). Corn distillers dried grains with solubles (DDGS) is a feedstuff with a large concentration of insoluble fiber (Martinez-Amezcua et al. 2007) and is suitable for pig diets (Shurson et al., 2000). Therefore, it has been proposed that insoluble dietary fiber from DDGS may help to protect pigs from intestinal infections. In this regard, Whitney et al. (2006a,b,c) conducted a series of studies using *Lawsonia intracellularis*, the agent of porcine proliferative enteropathy (ileitis), and found no consistent protective benefits from DDGS. Nevertheless, the notion that DDGS helps to ameliorate enteric infections in pigs persists in the industry. Thus, it is possible that the inclusion of DDGS, as a source of insoluble dietary fiber, in diets of newly weaned pigs can ameliorate PWC. The objective of this research was to determine whether the inclusion of up to 20% DDGS in the diet of pigs may prevent PWC infection and promote recovery.

Materials and Methods

A series of 3 disease challenge experiments was conducted to evaluate the effect of dietary DDGS in the prevention of, and recovery from, PWC. First, a pathogenic *E. coli* strain was selected based on its capability to colonize the intestine (Exp. 1). Then, the effects of dietary DDGS were evaluated under different dose regimens of pathogenic *E. coli* (Exp. 2), and by using increasing doses of DDGS in the diet (Exp. 3). Partial results of Exp. 3 on intestinal

microbiology were reported separately (Chapter IV). All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental Design

Experiment 1 was a completely randomized design with 5 treatments to evaluate the capability of 2 pathogenic *E. coli* strains, given at either of 2 doses, to colonize the gastrointestinal tract. The first strain expressed the heat-stable toxins a and b (STAB); the second strain expressed the Shiga-like toxin II (SLT2). The 2 dose concentrations were 10^8 cfu (Low) and 10^{10} cfu (High) per dose. Treatments were as follow: 1) inoculation with distilled water (Sham); 2) inoculation with STAB at Low dose; 3) inoculation with STAB at High dose; 4) inoculation with SLT2 at Low dose; and 5) inoculation with SLT2 at High dose.

Experiment 2 was a completely randomized design with a factorial arrangement of 2 diets (inclusion of 0 vs. 20 % DDGS) × 4 challenge treatments: 1) inoculation with a single dose of distilled water (Sham); 2) inoculation with a single dose of SLT2 *E. coli* to provide 10^{10} cfu (EcAL); 3) inoculation with a single dose of SLT2 *E. coli* to provide 10^{11} cfu (EcAH); and 4) inoculation with 3 consecutive daily doses of SLT2 *E. coli* to provide 10^{10} cfu per dose (EcCR).

Experiment 3 was a completely randomized design with a factorial arrangement of 4 diets (inclusion of 0, 5, 10, or 20% DDGS) × 2 challenge treatments: inoculation with 3 consecutive daily doses of distilled water (Sham), or SLT2 *E. coli* to provide 10^{10} cfu per dose (EcCR).

The pig was the experimental unit for all measurements; each treatment was replicated with 3 pigs in Exp. 1, or with 6 pigs in Exp. 2 and 3. Litter of origin and sex were balanced among treatments.

Animals, Housing, and Diets

All pigs were weaned at 21 d of age (BW was 7.43 ± 0.31 kg in Exp. 1, 6.23 ± 0.34 kg in Exp. 2, and 5.96 ± 0.48 kg in Exp. 3) and housed in disease containment chambers. Each chamber housed 3 pigs to encourage proliferation of *E. coli* within the chamber. The ventilation system kept a negative air pressure within chamber to reduce the chances of cross-contamination; animal biosafety level 2 protocols were followed after the first inoculation. Each chamber had 4.65 m^2 of floor space and was equipped with a plastic-coated expanded metal floor, a nipple drinker, and a stainless-steel 4-hole feeder. Temperature was set to remain constant at 28^{o}C; light period was set to 12 h per day and ventilation was automatically controlled. Feed and water were offered to allow ad libitum intake. Pigs were a crossbred of sire

line 337 to dam line C-22 (Pig Improvement Company, Hendersonville, TN). All pigs received an intramuscular dose of 300,000 units of penicillin G procaine (Bimeda, Oakbrook, IL) on d 3 of age; no more antibiotics were used before and during the experiment. Pigs were selected from litters with no β-hemolytic coliforms detected in feces of pigs and their dams 3 d before weaning. No antibiotics were used during the experiment.

Diets were formulated to meet or exceed the nutrient requirements estimated by NRC (1998) and to provide similar concentration of nutrients among treatments (Table 3.1). Amino acid supply was calculated on a standardized ileal digestibility basis (Stein et al., 2007) to meet the ideal amino acid profile estimated by Chung and Baker (1992). The chemical composition of feed ingredients and their percentages of amino acid digestibility were taken from NRC (1998), except for soybean meal and DDGS. Chemical composition of soybean meal was taken from the Soy in Animal Nutrition Database (Kusina et al., 2008); chemical composition and percentages of amino acid digestibility in DDGS were those reported by Stein et al. (2006). The same batch of DDGS (Lincolnland Agri-Energy LLC, Palestine, IL) was used in Exp. 2 and 3. All diets, including the diet for lactating sows, were free of antibiotics and antimicrobial compounds. No creep fed was allowed. Experiment 1 had no dietary treatments and only used the diet described as 0% DDGS in Table 3.1. Experimental diets were introduced on the day of weaning.

Inoculation Procedures

All experiments used a pathogenic β-hemolytic *E. coli* serotype F18. Experiment 1 used 2 strains (STAB and SLT2) to select the one with the better capacity to colonize the pig intestine; based on those results, Exp. 2 and 3 only used the SLT2 *E. coli* strain. Both strains were isolated from field outbreaks by the Veterinary Diagnostic Laboratory, University of Illinois College of Veterinary Medicine. The pathogenic *E. coli* was grown in sheep blood agar plates (Thermo Fisher Scientific, Lenexa, KS) and incubated overnight at 37ºC. Then, the colonies were collected and diluted as needed in distilled water to reach a concentration of either 10^8 or 10^{10} cfu per dose of 3 mL. All doses were prepared within 3 h before use. The inoculum was delivered orally using a 3-mL syringe with a 1-inch plastic tube attached at the end. Inoculation occurred (Exp. 1) or began (Exp. 2 and 3) on d 3 after weaning (BW was 7.79 ± 0.44 kg in Exp. 1, 6.55 ± 0.37 kg in Exp. 2, and 6.58 ± 0.51 kg in Exp. 3), and it was considered post-inoculation (PI) d 0.

Measurements and Sample Collection

Pigs were weighed at weaning, and then on PI d 0, 7, and 11. The exception was Exp. 2, in which the last BW was recorded on PI d 10. The severity of diarrhea was assessed daily by 2 persons trained to visually score feces consistency using the following scale: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea. Fecal samples were collected on PI d 0, 1, 2, 4, 7, and 10 (Exp. 1), or PI d 0, 2, 4, 6, 8, and 10 (Exp. 2), for the detection of β-hemolytic and non-hemolytic coliforms. Fecal samples were harvested directly from the rectum using a fecal loop, or a cotton culture swab in case of severe diarrhea, and placed in plastic tubes. The samples were transported on ice to the laboratory and plated within 2 h after collection.

Pigs were anesthetized by intramuscular injection of Telazol:Ketamine:Xylazine (1:0.5:0.5 mg/10 kg of BW), and then euthanized by intra-cardiac injection of sodium pentobarbital (70 mg/kg of BW) on PI d 11 (Exp. 1 and 3), or PI d 10 (Exp. 2). In Exp. 3, a 6-cm tissue sample was harvested from both jejunum (2 m before ileal-cecal junction) and middle colon. Half of each sample was cut longitudinally, so that one end was flat and the other end kept its round shape; samples were stored in 10% neutral buffered formalin to fix the tissue structure within 5 min post-euthanasia. Histological measurements were villus height, crypt depth, villus height to crypt depth ratio (VCR), and the number of lymphocytes infiltrated in the mucosa layer of the jejunum. In Exp. 3, the small and large intestines were emptied, washed with water, dried with paper towels, and weighed.

Laboratory Analyses

The experimental diets were analyzed for soluble and insoluble dietary fiber (method 991.43; AOAC International, 2007).

Fecal samples were cultured on sheep blood agar plates and incubated overnight at 37ºC. Non-hemolytic and β-hemolytic colonies were scored separately on a scale from 0 = no growth through 10 = very heavy growth. Those colonies were further re-plated in differential agar media and incubated overnight at 37ºC to verify that they were coliforms, and that the non-hemolytic colonies were non-pathogenic; the differential agar media used were MacConkey, Triple Sugar Iron, Lysine Iron Agar, Simmon's Citrate, and Motility Indole Ornithine (Thermo Fisher Scientific, Lenexa, KS). Thus, non-hemolytic colonies were interpreted to be commensal coliforms and β-hemolytic colonies were interpreted to be pathogenic coliforms.

Tissue samples were embedded in paraffin and 2 cross-sections were obtained, one from the flat side and another one from the round side of the sample. Tissues were stained with hematoxylin and eosin stain. Histological analyses were performed using Carl Zeiss equipment (Carl Zeiss Inc., Thornwood, NY) of light microscope (Zeiss Axiovert 200M), digital camera (AxioCam MRc5), and image analysis software (AxioVision, release 4.7). An average of 21 villi and crypts were measured per sample, and the lymphocytes infiltrated in mucosal layer of jejunum were counted in 4 villi per sample.

Statistical Analysis

Each experiment was analyzed as a completely randomized design. Normal distribution of the data and homogeneity of variance were determined by analysis of the residuals using the UNIVARIATE procedure (SAS Inst., Inc., Cary, NC). All data were analyzed using the MIXED procedure (Littell et al., 1996). Treatment means were calculated by the LSMEANS procedure.

In Exp. 1, the effect of inoculation with *E. coli* was tested by contrast analysis of Sham vs. the other treatments. The 4 treatments that used *E. coli* as inoculum were used in a set of orthogonal contrasts to evaluate the effects of *E. coli* strain (STAB vs. SLT2), dose concentration (Low vs. High), and the strain by dose interaction.

The Exp. 2 and 3 were analyzed using a 2 × 4 factorial arrangement of treatments. When no interaction was detected, only the main effects of diet and challenge treatment were presented. In Exp. 2, treatment means were separated by pairwise comparisons using the PDIFF procedure. In Exp. 3, the 3 degrees of freedom sum of squares from both main effect of DDGS and DDGS × challenge treatment interaction were separated into single-degree-of-freedom sums of squares by contrast analysis for the linear and quadratic effects.

The culture score of fecal coliforms data were analyzed as repeated measures in time (Littell et al., 1998). The First-Order Autoregressive covariance model was used to fit the structure of the data; the selection of covariance model was based on graphic visualization of the data structure, number of parameters estimated, values from fit statistics, and normal distribution of residuals. The linear and quadratic effects of time were analyzed by contrast.

Results

Experiment 1

Pigs inoculated with *E. coli* had both a lower ($P < 0.05$) culture score of non-hemolytic fecal coliforms and a greater ($P < 0.001$) score of β-hemolytic coliforms compared to Sham pigs

(Table 3.2); the effect on β-hemolytic culture score also interacted with time (P = 0.003). Between *E. coli* strains, only pigs inoculated with SLT2 gradually decreased their non-hemolytic culture score, which reached the lowest score between PI d 2 and 4, and then increased (time quadratic effect, $P < 0.001$). Simultaneously, the β-hemolytic culture score gradually increased only in pigs inoculated with SLT2 strain, reaching the greatest score between PI d 2 and 4, and then decreased (time quadratic effect, $P < 0.001$). The effect of High dose was greater than Low dose for both non-hemolytic (time × dose, $P = 0.05$) and β-hemolytic (overall, $P < 0.05$) culture scores. No differences were detected in ADG (data not shown).

Experiment 2

Inoculation with either EcAH or EcCR decreased ($P < 0.05$) ADG from PI d 0 to 7 compared to Sham and EcAL pigs, regardless of dietary treatment. No differences among treatments in ADG were detected from PI d 7 to 10; diet and challenge treatment did not interact (Table 3.3). Pigs did not develop diarrhea throughout the experiment; the average diarrhea score from PI d 1 through 7 was 1.50 ± 0.31 and from PI d 8 through 10 was 1.59 ± 0.41. Three pigs from EcAH challenge treatment were euthanized on PI d 2, as recommended by the resident veterinarian; those pigs presented coughing and abdominal breathing. Post-mortem examination showed severe lung damage, but no enteric damage was detected. No further analyses were performed because the focus of this study was on enteric diseases.

Pigs fed either diet gradually dropped their non-hemolytic culture score until PI d 6, and then it gradually increased (time quadratic effect, $P < 0.001$) (Table 3.4). However, pigs fed 20% DDGS increased their non-hemolytic score faster than those fed 0% DDGS (time × diet, $P < 0.01$); pigs fed 0% DDGS also had a lower score than the one they initially had (time linear effect, $P < 0.05$). Only pigs challenged with any *E. coli* treatment gradually increased their β-hemolytic culture score (time × challenge, $P < 0.001$), and then it decreased (time quadratic effect, $P < 0.001$). Simultaneously, their non-hemolytic culture score gradually decreased (time × challenge, $P < 0.001$), and then it increased (time quadratic effect, $P < 0.001$). Pigs in the Sham challenge treatment gradually decreased their non-hemolytic culture score over time (time linear effect, $P < 0.05$). Diet and challenge treatment did not interact on any fecal culture score.

Experiment 3

During the first 7 d PI, pigs in EcCR challenge treatment had a lower ADG ($P = 0.06$) and greater diarrhea score ($P = 0.05$) than those in the Sham treatment (Table 3.5). No diet

effect or diet by challenge interaction was detected. From PI d 7 to 11, no differences in ADG were detected among treatments. The average diarrhea score from PI d 8 to 11 decreased as the inclusion of DDGS increased in the diet (diet linear effect, $P < 0.01$). Diet and challenge treatment did not interact on these variables.

The large intestine of EcCR pigs weighed less ($P = 0.003$) and their crypt depth in both colon and jejunum was lower ($P < 0.05$) than those in Sham pigs, regardless of diet (Table 3.6). Villus height in jejunum of EcCR pigs increased as more DDGS was included in diet (diet linear effect × challenge, $P < 0.001$). The number of lymphocytes per villus infiltrated in jejunal mucosa layer of Sham pigs was larger than that in EcCR pigs (36.2 vs. 31.1 lymphocytes/villus; SEM = 1.35; $P = 0.01$); also, pigs fed 5% DDGS had more lymphocytes infiltrated than those in pigs fed either 0 or 10% DDGS: 31.6, 38.5, 29.2, and 35.2 lymphocytes/villus (SEM = 1.91; $P < 0.01$) for pigs fed diets with 0, 5, 10, and 20% DDGS, respectively.

Discussion

The inclusion of DDGS in the diet did not prevent infection with *E. coli*; however, dietary DDGS hastened pig recovery from infection. The pathogenic coliforms gradually colonized the intestine and replaced commensal coliforms, regardless of dietar-y treatment; then, the population of commensal coliforms gradually recovered and replaced the pathogenic coliforms. This shift in fecal coliforms suggests a competition for colonization between the 2 groups, as well as an infection and a recovery process. The pathogenic *E. coli* used in these experiments competes against commensal coliforms for sites to colonize, because it needs to be attached to intestinal epithelial cells to release its toxins (Bertschinger and Fairbrother, 1999). The observed changes in growth rate also supported the infection and recovery process, as the ADG in Exp. 2 and 3 was reduced in pigs infected with *E. coli* during the first 7 d PI; then, those pigs recovered from infection and grew as much as the Sham pigs from PI d 7 to euthanasia.

In Exp. 3, the inoculation with *E. coli* induced diarrhea from PI d 1 to 7 regardless of dietary treatment. However, in the recovery phase, the diarrhea was reduced as more DDGS was included in the diet. This observation was associated with a reduction in fecal pathogenic coliforms in pigs fed DDGS (Chapter IV). In the present study, the recovery of fecal commensal coliforms in Exp. 2 was hastened in pigs fed 20% DDGS. Perhaps this enhanced recovery explains the observation by Cook et al. (2005) that pig mortality in a commercial environment decreased as more DDGS was included in the diet. Similarly, in a series of pig experiments using

a *Lawsonia intracellularis* challenge, Whitney et al. (2006a,b,c) found that dietary DDGS did not prevent infection but reduced lesion development in the intestine. Corn DDGS has a large concentration of insoluble dietary fiber (Shurson et al., 2000; Martinez-Amezcua et al., 2007). The inclusion of insoluble dietary fiber in pig diets has been helpful to reduce post-weaning diarrhea (Mateos et al., 2006; Kim et al., 2008). Insoluble dietary fiber reduces digesta viscosity and increases its passage rate (Dikeman et al., 2006), which may help to prevent the adhesion of pathogenic bacteria. Also, about half of the insoluble fiber in corn DDGS can be rapidly fermented (Urriola and Stein, 2010). Fermentation of dietary fiber may have promoted the repopulation of commensal bacteria and thus hastened the recovery process. Insoluble fiber also promotes a larger population of lactic acid bacteria, which can benefit the intestinal health (Högberg and Lindberg, 2006).

The histological measurements were done on PI d 11, when signs of advanced recovery were observed in EcCR pigs. The villus height in jejunum of EcCR pigs increased proportionally to the concentration of dietary DDGS. Similarly, Hedemann et al. (2006) observed that feeding pigs a diet with large concentration of insoluble fiber improved their intestinal morphology by increasing villus height. Insoluble dietary fiber has been shown to increase enterocyte turnover rate (Jin et al., 1994). Thus, insoluble dietary fiber in DDGS may have stimulated the recovery of villi after *E. coli* infection. As dietary DDGS did not change crypt dept, but the EcCR challenge reduce it, the largest VCR were observed in EcCR pigs fed either 10 or 20% DDGS. A larger VCR is considered a sign of intestinal health (Pluske et al., 1997). Aggressions to intestinal epithelium induce inflammation of adjacent tissue layers and a cell-mediated immune response, in which lymphocytes migrate to the mucosa layer. The cell proliferation in crypts and rate of cell migration toward the villus top are also increased. Those processes are very energy-dependent (Lalles et al., 2004) and that may explain the reduction in crypt depth observed in EcCR challenge pigs. The EcCR pigs also had less lymphocytes infiltrated in jejunal mucosa layer, as compared with Sham pigs. It is possible that the increased rates of cell proliferation and migration toward the villus top caused a dilution of lymphocytes infiltrated per villus; this effect can be interpreted as part of the recovery process.

In summary, an *E. coli* SLT2 strain was selected to induce mild PWC in pigs based on its capability to colonize the intestine and cause mild infection. The inclusion of up to 20% DDGS in pig diets did not prevent PWC infection. However, pigs fed a diet with 20% DDGS excreted

the pathogenic coliforms and recovered their commensal coliforms faster than did pigs fed diets without DDGS. During the recovery phase, pigs showed less diarrhea when more DDGS was added to the diet; similarly, the villus height in the small intestine of infected pigs increased proportionally to the concentration of dietary DDGS. We interpret these results as indicating dietary DDGS is beneficial to recovery from PWC.

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Tables

Table 3.1. Experimental diets (as-fed)

Ingredient, % of diet	Distillers dried grains with solubles (DDGS)			
	0%	5%	10%	20%
DDGS	0.00	5.00	10.00	20.00
Corn	39.30	36.15	31.92	23.45
Soybean meal, 47.5% CP	10.00	10.00	10.00	10.00
Whey, spray-dried	20.00	20.00	20.00	20.00
Lactose	7.00	7.00	7.00	7.00
Fish, Menhaden select[1]	9.44	6.43	5.28	2.98
Animal plasma, spray-dried[2]	5.00	5.00	5.00	5.00
Soy protein concentrate[3]	5.00	5.00	5.00	5.00
Soybean oil	2.99	3.34	3.47	3.73
Limestone, ground	0.36	0.57	0.69	0.92
Calcium phosphate, 21% P	0.16	0.53	0.62	0.80
Salt	0.40	0.40	0.40	0.40
Mineral and vitamin premix[4]	0.30	0.30	0.30	0.30
L-Lysine·HCl	0.000	0.154	0.200	0.294
DL-Methionine	0.038	0.076	0.072	0.077
L-Threonine	0.015	0.056	0.055	0.054
Chemical composition[5]				
ME, Mcal/kg	3.48	3.48	3.48	3.48
Lactose, %	21.00	21.00	21.00	21.00
CP, %	23.66	23.08	23.42	24.12
SID Lys, %[6]	1.45	1.45	1.45	1.45
Soluble dietary fiber, %[7]	1.10	0.60	0.60	1.80
Insoluble dietary fiber, %[7]	5.00	6.90	7.30	9.60

[1] Menhaden fish meal (Special Select Menhaden Fish Meal, Omega Protein Inc., Hammond, LA)
[2] Spray-dried animal plasma (AP-920, APC Co., Ankeny, IA).
[3] Soy protein concentrate (Soycomil K, ADM, Decatur, IL).

Table 3.1 (cont.)

[4] Supplied per kilogram of complete diet: Ca, 0.9%; available P, 0.55%; Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); Zn, 100 mg (ZnO); Fe, 90 mg ($FeSO_4 \cdot 5H_2O$); Mn, 20 mg (MnO); I, 0.35 mg (CaI_2); Se, 0.3 mg (Na_2SeO_3); NaCl, 3 g; retinyl acetate, 2,273 μg; cholecalciferol, 17 μg; DL-α-tocopheryl acetate, 88 mg; menadione sodium bisulfite complex, 4 mg; niacin, 33 mg; d-Ca-pantothenate, 24 mg; riboflavin, 9 mg; vitamin B12, 35 μg; choline chloride, 324 mg.
[5] Calculated analysis unless something different is reported.
[6] Standardized ileal digestible.
[7] Analyzed value.

Table 3.2. Culture score of fecal coliforms in young pigs challenged or not with β-hemolytic *E. coli* (Exp. 1)[1]

		STAB		SLT2			
Response[2]	Sham	Low	High	Low	High	Effect[3]	*P*-value
Non-hemolytic							
PI d 0	8.3	9.0	6.0	8.7[4]	7.7[4]	T	0.001
PI d 1	6.7	8.3	7.7	6.7	2.7	T × *E. coli*	0.21
PI d 2	6.7	8.0	7.3	3.3	0.0	T × Strain	0.001
PI d 4	7.0	7.3	7.3	0.0	2.7	T × Dose	0.05
PI d 7	7.3	6.7	6.3	1.3	5.6	T × S × D	0.12
PI d 11	6.7	7.3	6.0	6.0	6.7		
Overall[5]	7.1	7.8	6.8	4.3	4.2		
β-hemolytic							
PI d 0	0.0	0.0	0.0	0.0[4]	0.0[4]	T	0.001
PI d 1	1.3	1.7	5.0	3.3	5.0	T × *E. coli*	0.01
PI d 2	1.3	0.0	2.7	7.7	8.0	T × Strain	0.001
PI d 4	0.0	1.0	3.3	8.0	7.0	T × Dose	0.21
PI d 7	0.0	0.0	2.7	7.3	6.7	T × S × D	0.57
PI d 11	0.0	1.0	2.3	0.7	4.5		
Overall[6]	0.4	0.6	2.7	4.5	5.2		

[1] Least square means of 3 pigs per treatment. Pigs were challenged with a single dose of distilled water (Sham), or either of 2 β-hemolytic *E. coli* strains that expressed the STa and STb toxins (STAB), or the Shiga-like toxin II (SLT2); both strains were used at a dose concentration of either 10^8 cfu (Low), or 10^{10} cfu (High).

[2] Bacterial growth in blood agar was scored from 0 (no growth) through 10 (very heavy growth). Inoculation occurred on d 3 after weaning, considered post-inoculation (PI) d 0.

[3] Analysis of repeated measures in time (T) to test the effects of *E. coli* inoculation (Sham vs. the other treatments), strain (STAB vs. SLT2), dose (Low vs. High), strain × dose interaction (S × D). The linear and quadratic effects of time were tested within treatment. Pooled SEM over time was 1.29 in non-hemolytic, and 1.53 in β-hemolytic.

[4] Time quadratic effect ($P < 0.001$).

[5] Overall effect of *E. coli* inoculation ($P < 0.05$) and strain ($P < 0.001$). Pooled SEM = 0.51.

[6] Overall effect of *E. coli* inoculation ($P < 0.001$), strain ($P < 0.001$), and dose ($P = 0.05$). Pooled SEM = 0.62.

Table 3.3. Growth rate of young pigs fed a diet with 0 or 20% distillers dried grains with solubles (DDGS) and challenged with different doses of β-hemolytic *E. coli* (Exp. 2)[1]

	DDGS in diet[2]			Challenge[3]					*P*-value[4]		
Response[5]	0%	20%	SEM	Sham	EcAL	EcAH	EcCR	SEM	Diet	Challenge	D × C
ADG, g/d											
PI d 0 to 7	381	375	25	441[a]	433[a]	320[b]	318[b]	39	0.87	0.02	0.35
PI d 7 to 10	398	450	30	389	428	412	467	47	0.22	0.58	0.66

[ab] Within a row, means without a common superscript differ ($P < 0.05$).
[1] Least square means of main effects.
[2] Pigs were fed a diet with 0 or 20% DDGS; n = 24 pigs/diet.
[3] Challenge treatments were a single dose of distilled water (Sham), a single dose of *E. coli* at 10^{10} cfu (EcAL), a single dose of *E. coli* at 10^{11} cfu (EcAH), or 3 consecutive daily doses of *E. coli* at 10^{10} cfu per dose (EcCR); n = 12 pigs/challenge treatment.
[4] Effect of 0 vs. 20% DDGS in the diet (Diet), effect of challenge dose regimen (Challenge), and Diet × Challenge interaction (D × C).
[5] Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.

Table 3.4. Culture score of fecal coliforms in young pigs fed a diet with 0 or 20% distillers dried grains with solubles (DDGS) and challenged with different doses of β-hemolytic *E. coli* (Exp. 2)[1]

	DDGS in diet[2]		Challenge[3]					
Response[4]	0%	20%	Sham	EcAL	EcAH	EcCR	Effect[5]	*P*-value
Non-hemolytic								
PI d 0	7.8[6,7]	7.1[7]	7.4[6]	7.7[7]	7.8[7]	6.8[7]	T	0.001
PI d 2	5.8	4.0	7.9	4.4	4.8	2.6	T × Diet	0.01
PI d 4	3.1	3.7	6.7	2.9	1.5	2.3	T × Challenge	0.001
PI d 6	2.7	3.7	6.3	3.0	1.3	2.3	T × D × C	0.15
PI d 8	4.5	6.2	6.1	4.3	5.3	5.7		
PI d 10	5.1	6.0	5.9	5.8	5.3	5.2		
Overall[8]	4.8	5.1	6.7	4.7	4.3	4.1		
β-hemolytic								
PI d 0	1.5[7]	0.4[7]	1.2	0.7[7]	1.2[7]	0.8[7]	T	0.001
PI d 2	5.2	5.3	0.3	7.3	6.1	7.1	T × Diet	0.53
PI d 4	5.4	5.1	0.5	6.3	6.8	7.4	T × Challenge	0.001
PI d 6	5.4	5.4	0.5	6.8	8.1	6.3	T × D × C	0.14
PI d 8	3.4	2.8	0.3	4.1	4.3	3.6		
PI d 10	2.2	1.6	0.3	2.6	2.3	2.5		
Overall[9]	3.9	3.4	0.5	4.6	4.8	4.6		

[1] Least square means of main effects.
[2] Pigs were fed a diet with 0 or 20% DDGS; n = 24 pigs/diet. Pooled SEM over time was 0.50 in non-hemolytic and 0.42 in β-hemolytic.
[3] Challenge treatments were a single dose of distilled water (Sham), a single dose of *E. coli* at 10^{10} cfu (EcAL), a single dose of *E. coli* at 10^{11} cfu (EcAH), or 3 consecutive daily doses of *E. coli* at 10^{10} cfu per dose (EcCR); n = 12 pigs/challenge treatment. Pooled SEM over time was 0.80 in non-hemolytic and 0.67 in β-hemolytic.
[4] Bacterial growth in blood agar was scored from 0 (no growth) through 10 (very heavy growth). Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.
[5] Analysis of repeated measures in time (T) to test the effects of dietary DDGS (Diet), challenge treatment (Challenge), and Diet × Challenge interaction (D × C). The linear and quadratic effects of time were tested within treatments.
[6] Time linear effect ($P < 0.05$).
[7] Time quadratic effect ($P < 0.001$).
[8] Overall effect of Challenge ($P < 0.001$). Pooled SEM was 0.25 in Diet and 0.38 in Challenge.
[9] Overall effect of Challenge ($P < 0.001$). Pooled SEM was 0.21 in Diet and 0.32 in Challenge.

Table 3.5. Growth rate and diarrhea score of young pigs fed increasing concentrations of distillers dried grains with solubles (DDGS) and challenged or not with β-hemolytic *E. coli* (Exp. 3)[1]

	DDGS in diet[2]					Challenge[3]			*P*-value[4]		
Response[5]	0%	5%	10%	20%	SEM	Sham	EcCR	SEM	Diet	Challenge	D × C
ADG, g/d											
PI d 0 to 7	379	289	295	329	30	352	294	21	0.14	0.06	0.19
PI d 7 to 11	531	585	556	592	46	527	605	32	0.78	0.10	0.81
Diarrhea score[6]											
PI d 1 to 7	1.49	1.35	1.55	1.20	0.12	1.27	1.52	0.09	0.19	0.05	0.13
PI d 8 to 11[7]	2.15	1.98	1.81	1.29	0.22	1.74	1.88	0.15	0.04	0.53	0.20

[1] Least square means of main effects.
[2] Pigs were fed a diet with 0, 5, 10, or 20% DDGS; n = 12 pigs/diet.
[3] Pigs were challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EcCR); n = 24 pigs/challenge treatment.
[4] Effects of DDGS inclusion in diet (Diet), Sham vs. EcCR challenge (Challenge), and Diet × Challenge interaction (D × C). The linear and quadratic effects of Diet were tested.
[5] Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.
[6] Diarrhea was scored daily as follows: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea.
[7] Diet linear effect ($P < 0.01$).

Table 3.6. Histological changes in the intestine of young pigs fed increasing concentrations of distillers dried grains with solubles (DDGS) and challenged or not with β-hemolytic *E. coli* (Exp. 3)[1]

	Sham[2]				EcCR[2]							
	DDGS in diet[2]									*P*-value[3]		
Response[4]	0%	5%	10%	20%	0%	5%	10%	20%	SEM	Diet	Challenge	D×C
Intestine wt, g												
Small	494	523	473	485	420	463	454	509	32	0.49	0.16	0.40
Large	187	171	162	195	143	134	134	160	16	0.29	0.003	0.96
Jejunum												
Villus height, µm[5]	561	661	538	531	528	540	556	660	28	0.09	0.93	0.001
Crypt depth, µm	282	284	252	264	235	260	241	247	15	0.42	0.03	0.67
VCR[6]	1.99	2.34	2.17	2.02	2.27	2.13	2.35	2.70	0.130	0.37	0.02	0.01
Lymphocytes[7]	36.2	43.1	28.9	36.4	27.0	33.9	29.4	33.9	2.70	0.01	0.01	0.20
Colon												
Crypt depth, µm	634	697	612	477	388	393	353	490	68	0.77	0.001	0.10

[1] Least square means of 6 pigs per treatment.
[2] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EcCR).
[3] Effects of DDGS inclusion in diet (Diet), Sham vs. EcCR challenge (Challenge), and Diet × Challenge interaction (D × C). The linear and quadratic effects of Diet were tested.
[4] Inoculation began on d 3 after weaning and samples were harvested on d 11 after first inoculation.
[5] Diet linear × Challenge interaction ($P < 0.001$).
[6] Villus height to crypt depth ratio. Diet quadratic × Challenge interaction ($P < 0.05$).
[7] Average lymphocytes per villus infiltrated in the mucosa layer. Diet cubic effect ($P < 0.001$).

CHAPTER IV

INCLUSION OF DISTILLERS DRIED GRAINS WITH SOLUBLES IN PIG DIETS HASTENS RECOVERY FROM POST-WEANING COLIBACILLOSIS: INTESTINAL MICROBIOTA AND DIGESTA CHARACTERISTICS

Abstract

The experiment assessed changes in intestinal bacterial populations and digesta characteristics by adding corn distillers dried grains with solubles (DDGS) to a diet for weaned pigs (21 d of age and 5.96 ± 0.48 kg of BW). It was a completely randomized design with a factorial arrangement of 4 diets (inclusion of 0, 5, 10, or 20% DDGS) × 2 challenge treatments: inoculation with 3 consecutive daily doses of either distilled water (Sham) or pathogenic *E. coli* (EC). Each treatment was replicated with 6 pigs. Inoculation began on d 3 post-weaning, considered post-inoculation (PI) d 0. Fecal samples were harvested every 2 d PI and cultured in blood agar; non-hemolytic (commensal) and β-hemolytic (pathogenic) coliforms were scored from 0 (no growth) to 10 (very heavy growth); these data were analyzed by repeated measures in time. Digesta and intestinal mucosa samples were harvested on PI d 11. Microbial populations were analyzed in mucosa samples by denaturing gradient gel electrophoresis, using the V3 region of bacterial 16S ribosome. Fecal coliforms in EC pigs shifted from commensal to pathogenic ones. However, the addition of more DDGS to the diet (diet linear × challenge, $P < 0.001$) delayed both the drop in commensal and the shedding of pathogenic coliforms, as well as hastened the reduction of pathogenic coliforms in feces of EC pigs. Pigs fed either 5 or 10% DDGS had a larger bacterial diversity in cecum ($P = 0.02$) and colon ($P = 0.08$), than those fed 0 or 20% DDGS. The bacterial population in cecum of EC pigs became less homogeneous among pigs within treatments as more DDGS was added to the diet (diet linear × challenge, $P < 0.01$). As more DDGS was added to the diet, the concentrations of short-chain fatty acids in ileal digesta increased in Sham pigs but it decreased in EC pigs (diet linear × challenge, $P = 0.02$): 40.4, 47.5, 52.4, 59.3, 69.3, 54.1, 45.8, 45.7 mmol×kg^{-1} digesta DM for 0, 5, 10, and 20% DDGS in Sham and EC pigs, respectively. Overall, the observed effects suggest a repopulation process of intestinal bacteria going on as part of the recovery from *E. coli* infection, which seems to be promoted by increasing concentrations of DDGS in the diet.

Key words: colibacillosis, distillers dried grains with solubles, fecal coliforms, microbial populations, pig

Introduction

The fiber component of the diet is an important substrate for microbial fermentation (Pieper et al., 2008); therefore, it can change the intestinal microbial populations and its environment. Dietary fiber may contribute to a balance between commensal microbiota and pathogens (Montagne et al., 2003). It has been suggested that dietary fiber may promote growth of commensal bacteria (Bikker et al., 2006), and that may inhibit the growth of pathogenic bacteria (Wenk, 2001; Williams et al., 2001); this phenomenon is known as colonization resistance. Dietary fiber also may exert potentially beneficial effects on the digestive physiology (Jin et al., 1994) and microbial activity (Varel and Yen, 1997). The effect of dietary fiber on preventing colibacillosis diarrhea has been documented, but its mechanism of action is poorly understood and controversial (Wellock et al., 2007). Changes in intestinal microbial populations may have a potentially important effect on the susceptibility to enteric diseases, as well as recovery.

The ethanol co-product, corn distillers dried grains with solubles (DDGS), has a large concentration of fiber, which is mainly insoluble fiber (Martinez-Amezcua et al., 2007). Insoluble fiber may promote a healthier intestine and intestinal environment (Hedemann et al., 2006; Högberg and Lindberg, 2006). The objective of this study was to assess the effect of increasing concentrations of dietary DDGS on the intestinal bacterial populations of young pigs infected with a pathogenic *Escherichia coli*.

Materials and Methods

A disease challenge experiment was conducted to evaluate the effects of increasing concentrations of dietary DDGS (Lincolnland Agri-Energy LLC, Palestine, IL), on fecal and intestinal microbial populations and some characteristics of the digesta. Partial results of this experiment on clinical and histological observations were reported separately (Chapter III). All procedures with animals were approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental Design

The experiment had a completely randomized design with a factorial arrangement of 4 diets (inclusion of 0, 5, 10, or 20% DDGS) × 2 challenge treatments: inoculation with 3 consecutive daily doses of either distilled water (Sham), or pathogenic *E. coli* (EC) to deliver

10^{10} cfu per dose. Inoculation began on d 3 post-weaning. Each treatment was replicated with 6 pigs; pig was the experimental unit. Litter of origin and sex were balanced across treatments.

Animals, Housing, and Diets

All pigs were weaned at 21 d of age (5.96 ± 0.48 kg of BW) and housed in disease containment chambers. Each chamber housed 3 pigs to encourage proliferation of *E. coli* within chamber. The ventilation system kept a negative air pressure within chamber to reduce the chances of cross-contamination; animal biosafety level 2 protocols were followed after first inoculation. Each chamber had 4.65 m^2 of floor space and was equipped with a plastic coated expanded metal floor, a nipple drinker, and a stainless-steel 4-hole feeder. Temperature was set to remain constant at 28^oC; light period was set to 12 h per day and ventilation was automatically controlled. Feed and water were offered to allow ad libitum intake. Pigs were a crossbred of sire line 337 to dam line C-22 (Pig Improvement Company, Hendersonville, TN). All pigs received an intramuscular dose of 300,000 units of penicillin G procaine (Bimeda, Oakbrook, IL) on d 3 of age; no more antibiotics were used before and during the experiment. Pigs were selected from litters with no β-hemolytic coliforms detected in feces of pigs and their dams 3 d before weaning. No in-feed nor parenteral antibiotics were used during the experiment.

Diets were formulated to meet or exceed the nutrient requirements estimated by NRC (1998) and to provide similar concentration of nutrients among treatments (Table 4.1). Amino acids supply was calculated on the standardized ileal digestibility basis (Stein et al., 2007), to meet the ideal amino acid profile estimated by Chung and Baker (1992). The chemical composition of feed ingredients and their percentages of amino acids digestibility were taken from NRC (1998), except for soybean meal and DDGS. Chemical composition of soybean meal was taken from the Soy in Animal Nutrition Database (Kusina et al., 2008); chemical composition and percentages of amino acids digestibility in DDGS were those reported by Stein et al. (2006). All diets, including the diet for lactating sows, were free of antibiotics and antimicrobial compounds. No creep fed was allowed. Experimental diets were offered on weaning day.

Inoculation Procedures

The pathogenic *E. coli* used for inoculation was a β-hemolytic strain, serotype F18, that expressed the Shiga-like toxin II. The selection of *E. coli* strain and inoculation dose was done based on the results of 2 preliminary experiments, which assessed its capacity to colonize and

cause mild infection in newly weaned pigs (Chapter III). The strain was isolated from a field outbreak by the Veterinary Diagnostic Laboratory, University of Illinois College of Veterinary Medicine. The pathogenic *E. coli* was grown in sheep blood agar plates (Thermo Fisher Scientific, Lenexa, KS) and incubated overnight at 37ºC. Then, colonies were collected and diluted in distilled water to reach a concentration of 10^{10} cfu per dose of 3 mL. All doses were prepared within 3 h before use. The inoculum was delivered in the back of the mouth ,using a 3-mL syringe with a 1-inch plastic tube attached at the end. Pigs received 3 doses delivered in 3 consecutive days. Inoculation began on d 3 after weaning (6.58 ± 0.51 kg of BW), considered post-inoculation (PI) d 0.

Measurements and Sample Collection

Fecal samples were collected on PI d 0, 1, 2, 4, 7, and 11 for the detection of β-hemolytic and non-hemolytic coliforms. Fecal samples were harvested directly from the rectum using a fecal loop, or a cotton culture swab in case of severe diarrhea, and placed in plastic tubes. The samples were transported on ice to the laboratory and plated within 2 h after collection.

Pigs were anesthetized by intramuscular injection of Telazol:Ketamine:Xylazine (1:0.5:0.5 mg/10 kg of BW), and then euthanized by intra-cardiac injection of sodium pentobarbital (70 mg/kg of BW) on PI d 11. Digesta and intestinal mucosa samples were harvested within 10 min post-euthanasia. Dry matter content and pH were measured in digesta samples from stomach, jejunum, ileum, cecum, and colon. Digesta pH was measured immediately after collection of samples. Digesta samples to measure dry matter content were kept in ice after collection and stored at -20^{o}C until analysis. The concentration of short-chain fatty acids (SCFA) was measured in digesta samples from ileum, cecum, and colon; those samples were diluted in meta-phosphoric acid (1:1 sample to acid ratio) immediately after collection and stored at -20^{o}C until analysis. Intestinal mucosa samples were collected from jejunum (2 m before ileal-cecal junction), ileum (20 cm before ileal-cecal junction), middle cecum, and middle colon for the molecular analysis of intestinal microbiota. About 10 cm of tissue from each site was taken, longitudinally opened, and gently rinsed with distilled water to wash out the digesta content; then, the mucosa sample was taken by scraping the intestinal wall. These samples were frozen in liquid nitrogen immediately after collection and stored at -80°C until analysis.

Chemical and Microbiological Analyses

The concentration of soluble and insoluble dietary fiber was measured (method 991.43; AOAC International, 2007) in experimental diets. Digesta samples were analyzed for dry matter content (method 990.15; AOAC International, 2007). Digesta pH was measured using a pH meter (model BASIC, Denver Instrument Company, Arvada, CO). Concentration of SCFA in digesta was measured following the procedures described by Erwin et al. (1961), using a gas chromatograph (Hewlett-Packard 5890A series II, Hewlett-Packard, Palo Alto, CA) standardized with a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA), and used nitrogen as carrier with a flow rate of 75 mL/min. Temperatures were set at 125°C in oven, 175°C in detector, and 180°C in injector.

Fecal samples were cultured in sheep blood agar plates and incubated overnight at 37ºC. Non-hemolytic and β-hemolytic colonies were scored separately on a scale from 0 = no growth through 10 = very heavy growth. Those colonies were further re-plated in differential agar media and incubated overnight at 37ºC to determine that were coliforms, and that the non-hemolytic colonies were non-pathogenic; the differential agar media used was MacConkey, Triple Sugar Iron, Lysine Iron Agar, Simmon's Citrate, and Motility Indole Ornithine (Thermo Fisher Scientific, Lenexa, KS). Thus, non-hemolytic colonies were interpreted to be commensal coliforms and β-hemolytic colonies were interpreted to be pathogenic coliforms.

The intestinal bacterial populations were analyzed by denaturant gradient gel electrophoresis (DGGE); this method separates the bacterial DNA in a gel and shows distinct bands for different bacterial species. Each band represents at least 1 bacterial species. The V3 region of bacterial 16S ribosome was replicated by polymerase chain reaction (Simpson et al., 1999). The resulting DGGE banding patterns were compared between pairs of pigs using Sorenson's similarity values (Cs) and analyzed as described by Collier et al. (2003). Briefly, the Cs values range from 0 to 100 to measure the similarity of DGGS banding patterns between 2 pigs; a Cs value of 100 indicates identical banding pattern between that pair of pigs, whereas a Cs value of 0 indicates no common bands. The Cs values were computed to measure the similarity intra-treatment (within treatments) and inter-treatment (between treatments). Thus, a greater intra-treatment Cs value indicated the DGGS banding pattern was more similar within pigs in that treatment. To determine whether the DGGS banding pattern differed between 2

treatments, their corresponding inter-treatment Cs value was compared against the intra-treatment Cs value mean of those 2 treatments; when those values were different, it was assumed the DDGS banding pattern changed between the 2 treatments.

Statistical Analysis

All analyses were facilitated using the MIXED procedure (SAS Inst., Inc., Cary, NC). The ANOVA for all data was conducted using a completely randomized design with a 4 (diets) × 2 (challenge treatments) factorial arrangement of treatments. The pig was the experimental unit. The 3 degrees of freedom sum of squares from both main effect of diet and diet × challenge treatment interaction were separated into single-degree-of-freedom sums of squares by contrast analysis for the linear, quadratic, and cubic effects. Treatment means were calculated by the LSMEANS procedure. The comparison of each inter-treatment Cs value against the mean of its 2 corresponding intra-treatment Cs values was done by contrast analysis. The changes in fecal coliforms over time were analyzed by repeated measures analysis (Littell et al., 1998). The Compound Symmetry covariance model was used to fit the structure of the data; the selection of covariance model was based on graphic visualization of the data structure, number of parameters estimated, values from fit statistics, and normal distribution of residuals. The linear and quadratic effects of time were analyzed by contrast. Normal distribution of the data and homogeneity of variance was determined by analysis of the residuals using the UNIVARIATE procedure.

Results

Dietary DDGS, challenge treatment, and time interacted ($P < 0.01$) in affecting the culture score of both non-hemolytic and β-hemolytic fecal coliforms (Table 4.2). In EC pigs, the culture score of non-hemolytic fecal coliforms gradually dropped to reach its lowest score between PI d 2 and 6, and then it gradually increased. This pattern of response had a quadratic time effect ($P < 0.001$). Also in EC pigs, the non-hemolytic culture score on PI d 2 was greater as more DDGS was included in the diet (diet linear × challenge interaction, $P < 0.001$). Simultaneously, in EC pigs the culture score of β-hemolytic fecal colonies gradually increased to reach its largest score between PI d 4 and 6, and then it gradually decreased. This pattern of response had both linear ($P < 0.01$) and quadratic ($P < 0.001$) time effects. Also in EC pigs, the β-hemolytic culture score on PI d 2 and 11 was smaller as more DDGS was included in the diet (diet linear × challenge interaction, $P < 0.001$). Dietary DDGS and challenge treatment also interacted in the overall culture score of non-hemolytic ($P = 0.04$) and β-hemolytic ($P = 0.02$). In

EC pigs, the non-hemolytic culture score increased and the β-hemolytic culture score decreased as more DDGS was included in the diet (diet linear × challenge interaction, $P < 0.01$).

The number of DGGE bands, as a measure of microbial diversity, was affected in cecum and colon by diet (Table 4.3). In cecum, pigs across challenge treatments fed either 5 or 10% DDGS diet had a larger bacterial diversity than those fed 20% DDGS diet: 23.9, 28.7, 27.5, or 21.1 DGGE bands (diet quadratic, $P < 0.01$; SEM = 1.75) for pigs fed 0, 5, 10, or 20% DDGS diets, respectively. In colon, pigs fed 5% DDGS diet had a larger bacterial diversity than those fed 20% DDGS diet: 25.8, 28.8, 25.5, or 22.6 DGGE bands (diet quadratic, $P = 0.08$; SEM = 1.64) for pigs fed 0, 5, 10, or 20% DDGS diets, respectively. Also in colon, Sham pigs had more ($P = 0.02$) DGGE bands than EC pigs (27.7 vs. 23.6 DGGE bands; SEM = 1.16). In contrast, Sham pigs had fewer ($P < 0.001$) DGGE bands than EC pigs in jejunum (24.9 vs. 32.1 DGGE bands; SEM = 1.33).

The intra-treatment Cs values, as a measure of bacterial homogeneity among pigs within treatments, showed that the inclusion of dietary DDGS interacted with the challenge treatment (Table 4.3). In cecum, only EC pigs showed a reduction in intra-treatment Cs values as the inclusion of dietary DDGS increased (diet linear × challenge interaction, $P < 0.01$). In colon, intra-treatment Cs values within Sham pigs were lower in those fed either 10 or 20% DDGS diets; however, within EC pigs, the Cs value was low only in pigs fed 20% DDGS and was high in those fed 10% DDGS diet (diet quadratic × challenge interaction, $P < 0.05$). In jejunum, intra-treatment Cs values within Sham pigs were greater in those fed either 10 or 20% DDGS diet; however within EC pigs, those fed 10% DDGS diet had the lowest Cs value (diet quadratic × challenge interaction, $P < 0.05$). No changes were observed in the number of DGGE bands or intra-treatment Cs values of ileum.

The analysis of inter-treatment Cs values showed that most of the changes in bacterial populations among treatments occurred between Sham and EC pigs. In the small intestine, only Sham pigs fed either 10 or 20% DDGS diets differed ($P < 0.05$) from EC pigs fed any diet (Tables 4.4 and 4.5). Cecum was the section where the most changes were detected (Table 4.6); all dietary treatments in Sham pigs differed ($P < 0.05$) from at least 1 dietary treatment in EC pigs. Moreover, pigs fed 0% DDGS diet differed ($P < 0.05$) from those fed 5% DDGS diet in both cases within and between challenge treatment; also within EC challenge treatments, pigs fed 5% DDGS differed ($P < 0.05$) from those fed 20% DDGS. The only change observed in colon

was within Sham pigs, in which pigs fed 5% DDGS differed ($P < 0.05$) from those fed 20% DDGS (Table 4.7).

The digesta dry matter content was observed to change only in jejunum (Table 4.8); it decreased as the inclusion of DDGS increased in the diet (diet linear effect, $P < 0.001$). Digesta pH in jejunum was lowest in Sham pigs fed the 10% DDGS diet, but was highest in EC pigs fed either 5% or 10% DDGS diet (diet quadratic × challenge interaction, $P = 0.01$). In the large intestine, EC pigs had lower digesta pH in cecum (5.07 vs. 4.70; SEM = 0.069; $P < 0.001$) and colon (5.51 vs. 5.13; SEM = 0.090; $P < 0.01$). The concentration of SCFA was detected to change only in ileum; as the inclusion of dietary DDGS increased, the concentration of SCFA in ileal digesta of Sham pigs increased, but in the EC pigs it decreased (diet linear × challenge interaction, $P = 0.02$). No other differences were observed among treatments in the digesta characteristics evaluated throughout the gastro-intestinal tract.

Discussion

The inclusion of DDGS in the diet of newly weaned pigs changed bacterial populations in both intestinal mucosa and feces; those changes were dependent on the concentration of DDGS and pig health status. Based on the results of fecal bacteria differential analysis, after inoculation with *E. coli* pathogenic coliforms gradually colonized the intestine by replacing commensal coliforms; then the population of commensal coliforms gradually recovered and replaced the pathogenic ones. This shift in fecal coliforms occurred in all EC pigs regardless of dietary treatment, but it was ameliorated as more DDGS was added to the diet. The inclusion of dietary DDGS delayed both the drop of fecal commensal coliforms and the colonization by pathogenic coliforms after EC inoculation. Also, dietary DDGS hastened the reduction of pathogenic coliforms in feces. The shift in fecal coliforms shows a competition for colonization, which suggests an infection and a recovery process. This pathogenic *E. coli* attaches to intestinal epithelial cells, so it needs to compete for sites to colonize (Bertschinger and Fairbrother, 1999). Clinical observations and growth data (Chapter III) also support an infection followed by a recovery process. The EC pigs grew less during the infection, but as much as the Sham pigs in the recovery phase. In fact, the severity of diarrhea in EC pigs decreased from PI d 8 to 11 as more DDGS was included in the diet, which matches the pattern of response in the reduction of fecal pathogenic coliforms reported here. The fast recovery of commensal coliforms post-infection was also facilitated by the absence of additional factors that may challenge the

intestinal bacterial populations and the immune system; the combination of such conditions contributed to the ultimate excretion of pathogenic *E. coli*, which explains the second shift in fecal coliforms during recovery.

Results of molecular analysis showed that changes in intestinal bacterial populations depended on dose of dietary DDGS, pig health status, and intestinal environment. Pigs fed either 5 or 10% DDGS had a larger bacterial diversity in cecum and colon, than those fed 20% DDGS. Also, Sham pigs had a larger bacterial diversity in colon than EC pigs, but that increment was basically driven by Sham pigs fed 5% DDGS. Similarly, chicks fed a diet with 10% DDGS showed a larger cecal bacterial diversity than those fed either 0 or 20% DDGS (Chapter VI). The DDGS has a large concentration of dietary fiber (Martinez-Amezcua et al., 2007); about 50% of that can be fermented in the gastrointestinal tract (Urriola and Stein, 2010). Fiber fermentation may promote microbial richness and diversity, but intensive fermentation may promote fewer bacterial species to become dominant (Pieper et al., 2008), perhaps partially explaining the reduction in bacterial diversity when pigs were fed 20% DDGS, as well as the larger bacterial diversity in colon of Sham pigs fed 5% DDGS.

The interactions between diet and challenge treatments on the homogeneity of bacterial populations (Cs values) in large intestine, suggest that DDGS may modulate the intestinal microbiota according to health status. In EC pigs, the bacterial population in cecum became less homogeneous within treatments (intra-treatment Cs values) as more DDGS was included in the diet. As the intra-treatment Cs values measure the bacterial homogeneity within a population in a given time, it is likely to decrease during a repopulation process, because each individual pig may be experiencing a different stage of the process. The mucosa samples for bacterial analysis were harvested on PI d 11, when pigs were experiencing an advanced recovery from infection. The lower digesta pH observed in the large intestine of EC pigs supports the notion of a recovery process from infection, because acidic conditions inhibit pathogenic *E. coli* (Bertschinger, 1999). Therefore, the described reduction in cecal bacterial homogeneity intra-treatments, suggests a repopulation process of bacterial populations being promoted as more DDGS was included in the diet. In colon, the bacterial intra-treatment homogeneity was reduced only in pigs fed the largest concentration of DDGS. Most of the pig fermentation occurs in colon, so its bacterial population is more complex and less prone to change than other sections of the intestine. Feeding diets with

20% DDGS, may have increased the fermentation enough to induce a re-adaptation of bacterial populations in colon.

We observed that the concentration of SCFA in ileal digesta of Sham pigs increased as DDGS increased in the diet, although the opposite effect was observed in EC pigs. More than 30% of the fiber in diets containing DDGS can be fermented in the small intestine of pigs (Urriola and Stein, 2010), and the bacterial fermentation of fiber increases the concentration of SCFA (Pluske et al., 2003). Thus, the fiber content in DDGS may have promoted the synthesis of more SCFA in Sham pigs. The reason for the opposite effect in ileum of EC pigs is unknown. However, the concentration of SCFA in digesta is affected by both the bacterial activity and the concentration of some bacterial populations (van Winsen et al., 2001; Franklin et al., 2002). Perhaps the disruption in the intestinal bacterial populations that we observed in EC pigs during the recovery process, affected the synthesis of SCFA. Our data showed no differences among treatments in the concentration of SCFA in large intestine. Similarly, Urriola and Stein (2010) reported that the inclusion of 30% DDGS to a conventional corn-soybean meal diet did not change the concentration of SCFA in large intestine. In the present study, the concentration of digesta SCFA in both cecum and colon was at least twice than that in ileum, which indicates a greater bacterial fermentation in large intestine (Franklin et al., 2002). The absorption of SCFA occurs very rapid in the large intestine because it is an important source of energy for its epithelial cells (Ruppin at al., 1980). In the present study, perhaps the rapid absorption of SCFA in large intestine partially contributed to the lack of differences in SCFA concentration among treatments. The lower digesta pH we observed in large intestine of EC pigs was not associated with a larger concentration of SCFA. However, the concentration of SCFA in digesta does not always correlate to digesta pH (Pluske et al., 2003; Nyachoti et al., 2006).

The comparison of bacterial population profiles between treatments (inter-treatment Cs values), showed that the only change observed in colon was between Sham pigs fed 5% DDGS and Sham pigs fed 20% DDGS. In contrast, cecal bacterial populations were more sensitive to change; however, most of those changes were between Sham and EC pigs, suggesting that the effect of *E. coli* infection was stronger than the diet effect in changing bacterial populations. The responses to diet and *E. coli* infection in small intestine were different from those observed in large intestine, perhaps because the ecology and micro-environment are different. No dietary effects on bacterial diversity were observed in small intestine, perhaps because fermentation is

far lower there than it is in large intestine. The larger bacterial diversity in jejunum of EC pigs, probably was a transient effect of a bacterial repopulation process after infection, in which more bacterial species are likely to be detected. In the absence of *E. coli* challenge, the bacterial populations in jejunum of Sham pigs were more homogeneous (intra-treatment Cs values) within pigs fed either 10 or 20% DDGS, and no changes in bacterial diversity were observed. It suggests that DDGS may stabilize the bacterial community not only in large intestine, but also in sites without intensive microbial fermentation, perhaps by changing the micro-environment. The experimental treatments affected the digesta DM and pH in jejunum, although it is unclear how those changes may contribute to developing a more homogeneous bacterial population. Pathogenic F18 *E. coli* colonize ileum and large intestine, and to a lesser extent jejunum (Bertschinger, 1999); thus, microbial populations in jejunum may overcome the effects of *E. coli* infection more quickly; so the observations in jejunum may reflect an advanced stage of recovery as compared with large intestine. Inter-treatment differences in bacterial populations of small intestine were only observed between Sham and EC pigs, suggesting a stronger *E. coli* effect than that from dietary DDGS.

Our data showed that increasing concentrations of DDGS in the diet for young pigs, may modulate the intestinal bacterial populations. The extent of those changes depended on the concentration of dietary DDGS, health status, and the intestinal environment. In healthy pigs, the inclusion of 5 or 10% DDGS in diet showed most of the effects that are considered to be beneficial for the intestinal health. However, pigs that experienced an *E. coli* enteric infection, showed signs of more advanced recovery when fed a larger concentration of DDGS. The bacterial community in the intestinal tract is very dynamic and adapts rapidly to the chemical and physical characteristics of the diet. As some characteristics of intestinal environment and its microbial populations are often use as indicators of intestinal health, the interpretation of those data should also consider whether they were measured in health, during an infection process, or during recovery from disease. Therefore, the interaction between diet and intestinal health should be evaluated over time, and especially during the course of enteric diseases.

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Tables

Table 4.1. Experimental diets (as-fed)

Ingredient, % of diet	Distillers dried grains with solubles (DDGS)			
	0%	5%	10%	20%
DDGS	0.00	5.00	10.00	20.00
Corn	39.30	36.15	31.92	23.45
Soybean meal, 47.5% CP	10.00	10.00	10.00	10.00
Whey, spray-dried	20.00	20.00	20.00	20.00
Lactose	7.00	7.00	7.00	7.00
Fish, Menhaden select[1]	9.44	6.43	5.28	2.98
Animal plasma, spray-dried[2]	5.00	5.00	5.00	5.00
Soy protein concentrate[3]	5.00	5.00	5.00	5.00
Soybean oil	2.99	3.34	3.47	3.73
Limestone, ground	0.36	0.57	0.69	0.92
Calcium phosphate, 21% P	0.16	0.53	0.62	0.80
Salt	0.40	0.40	0.40	0.40
Mineral and vitamin premix[4]	0.30	0.30	0.30	0.30
L-Lysine·HCl	0.000	0.154	0.200	0.294
DL-Methionine	0.038	0.076	0.072	0.077
L-Threonine	0.015	0.056	0.055	0.054
Chemical composition[5]				
ME, Mcal/kg	3.48	3.48	3.48	3.48
Lactose, %	21.00	21.00	21.00	21.00
CP, %	23.66	23.08	23.42	24.12
SID Lys, %[6]	1.45	1.45	1.45	1.45
Soluble dietary fiber, %[7]	1.10	0.60	0.60	1.80
Insoluble dietary fiber, %[7]	5.00	6.90	7.30	9.60

[1] Menhaden fish meal (Special Select Menhaden Fish Meal, Omega Protein Inc., Hammond, LA)
[2] Spray-dried animal plasma (AP-920, APC Co., Ankeny, IA).
[3] Soy protein concentrate (Soycomil K, ADM, Decatur, IL).

Table 4.1 (cont.)

[4] Supplied per kilogram of complete diet: Ca, 0.9%; available P, 0.55%; Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); Zn, 100 mg (ZnO); Fe, 90 mg ($FeSO_4 \cdot 5H_2O$); Mn, 20 mg (MnO); I, 0.35 mg (CaI_2); Se, 0.3 mg (Na_2SeO_3); NaCl, 3 g; retinyl acetate, 2,273 μg; cholecalciferol, 17 μg; DL-α-tocopheryl acetate, 88 mg; menadione sodium bisulfite complex, 4 mg; niacin, 33 mg; d-Ca-pantothenate, 24 mg; riboflavin, 9 mg; vitamin B12, 35 μg; choline chloride, 324 mg.
[5] Calculated analysis unless something different is reported.
[6] Standardized ileal digestible.
[7] Analyzed value.

Table 4.2. Culture score of fecal coliforms of young pigs fed increasing concentrations of distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1]

	Sham[2]				EC[2]						
	DDGS in diet[2]								*P*-value[3]		
Response[4]	0%	5%	10%	20%	0%	5%	10%	20%	Diet	Challenge	D×C
Non-hemolytic[5]											
PI d 0	7.8	9.3[6]	8.2	7.8	7.7[7]	7.3[7]	8.0[7]	8.2[7]	0.92	0.40	0.53
PI d 2	7.2	7.5	6.3	6.7	0.8	1.3	4.7	6.3	0.02	0.001	0.001[8]
PI d 4	7.3	5.2	7.5	7.0	2.7	2.7	3.7	1.8	0.23	0.001	0.42
PI d 6	7.2	5.8	6.5	6.2	0.5	2.7	2.0	1.7	0.94	0.001	0.25
PI d 8	7.2	6.3	7.0	7.2	2.0	3.8	3.2	4.2	0.65	0.001	0.42
PI d 11	7.5	6.2	6.7	6.2	6.0	8.0	7.3	8.0	0.98	0.24	0.16
Overall	7.4	6.7	7.0	6.8	3.3	4.3	4.8	5.0	0.38	0.001	0.04[9]
β-hemolytic[10]											
PI d 0	0.3	0.2	0.8	0.3[11]	0.2[12]	0.0[12]	0.0[12]	0.0[12]	0.97	0.48	0.97
PI d 2	0.0	0.3	0.5	0.8	7.8	7.8	3.7	4.3	0.02	0.001	0.001[8]
PI d 4	0.0	0.3	0.0	0.5	8.0	8.5	6.2	6.8	0.33	0.001	0.38
PI d 6	0.8	0.3	0.0	0.5	8.5	7.7	7.2	8.0	0.53	0.001	0.99
PI d 8	0.7	0.5	0.3	1.0	8.3	7.0	6.5	7.3	0.49	0.001	0.74
PI d 11	1.0	1.0	1.7	3.3	6.7	5.8	4.0	2.5	0.50	0.001	0.001[8]
Overall	0.5	0.4	0.6	1.1	6.6	6.1	4.6	4.8	0.16	0.001	0.02[9]

[1] Least square means of 6 pigs per treatment.

Table 4.2 (cont.)

[2] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or pathogenic *E. coli* at 10^{10} cfu per dose (EC).
[3] Repeated measures analysis in time to test the effects of dietary DDGS (Diet), challenge or not with EC (Challenge), Diet×Challenge interaction (D×C), and their interaction with time. In both non-hemolytic and β-hemolytic, time effect ($P < 0.001$) and time×D×C ($P < 0.01$) were significant. Linear and quadratic effects of Diet were explored in D×C, as well as the linear and quadratic effects of time in each treatment.
[4] Fecal samples were cultured in blood agar and the resulting colonies were scored from 0 (no growth) through 10 (very heavy growth). Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.
[5] The SEM for the individual day data analyzed over time is 0.85, and for the overall data is 0.42.
[6] Time linear ($P < 0.05$) and quadratic ($P < 0.01$) effects.
[7] Time quadratic effect ($P < 0.001$).
[8] Diet linear×Challenge interaction ($P < 0.001$).
[9] Diet linear×Challenge interaction ($P < 0.01$).
[10] The SEM for the individual day data analyzed over time is 0.74, and for the overall data is 0.44.
[11] Time linear effect ($P < 0.05$).
[12] Time linear ($P < 0.01$) and quadratic ($P < 0.001$) effects.

Table 4.3. Changes in intestinal mucosa microbiota of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1]

	Sham[2]				EC[2]							
	DDGS in diet[2]									*P*-value[3]		
Response	0%	5%	10%	20%	0%	5%	10%	20%	SEM	Diet	Challenge	D×C
Number of DGGE bands[4]												
Jejunum	24.8	25.3	25.7	23.8	31.8	29.5	32.3	34.7	2.66	0.90	0.001	0.66
Ileum	25.7	27.5	23.2	27.5	22.0	24.2	24.7	25.7	2.82	0.70	0.36	0.79
Cecum[5]	23.0	30.2	29.0	21.0	24.8	27.2	26.0	21.2	2.47	0.02	0.57	0.70
Colon[6]	27.2	34.0	25.7	23.8	24.3	23.5	25.3	21.3	2.31	0.08	0.02	0.15
Intra-treatment Cs value[7]												
Jejunum[8]	52.1	52.9	61.6	65.3	62.6	57.1	53.6	66.9	2.60	0.001	0.26	0.01
Ileum	60.5	56.1	55.5	57.3	56.2	52.4	52.8	59.2	2.51	0.16	0.22	0.59
Cecum[9]	62.1	58.8	60.6	61.9	64.2	62.6	56.8	54.4	2.03	0.06	0.34	0.02
Colon[8]	62.7	68.4	50.8	56.1	55.3	54.0	58.2	46.9	2.56	0.001	0.01	0.001

[1] Least square means of 6 pigs per treatment. Inoculation began on d 3 after weaning and samples were harvested on d 11 after first inoculation.
[2] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenge with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC).
[3] Effect of DDGS inclusion in diet (Diet), effect of Sham vs. EC challenge (Challenge), and Diet × Challenge interaction (D×C). The linear, quadratic, and cubic effects of Diet were tested.
[4] Number of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis (DGGE) bands to measure microbial diversity. Each band represents at least 1 bacterial species.
[5] Diet quadratic effect ($P < 0.01$).
[6] Diet quadratic effect ($P = 0.08$).

Table 4.3 (cont.)

[7] Sorenson's similarity (Cs) values to compare similarities of DGGE banding patterns (average number of bands in common) within (intra-) treatment. Each Cs value included 15 single comparisons between pairs of pigs within treatment.
[8] Diet quadratic × challenge interaction ($P < 0.05$).
[9] Diet linear × challenge interaction ($P < 0.01$).

Table 4.4. Sorenson's similarity values to compare the similarity of jejunal mucosa microbiota of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1,2]

		Sham[3]				EC[3]			
	DDGS[3]	0%	5%	10%	20%	0%	5%	10%	20%
Sham[3]	0%	52.1							
	5%	54.3	52.9						
	10%	54.8	59.1	61.6					
	20%	56.6	55.7	60.0	65.3				
EC[3]	0%	54.7	54.3	54.0[a]	57.1[a]	62.6			
	5%	52.6	51.3	53.6[a]	57.1	60.6	57.1		
	10%	51.9	53.5	54.0	54.1[a]	59.6	56.7	53.6	
	20%	53.2	56.7	58.1[a]	57.5[a]	65.3	58.0	61.6	66.9

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns (average number of bands in common), within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 15 single comparisons between pairs of pigs within treatment. Each inter-treatment value included 36 single comparisons between pairs of pigs between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 2.60), and the numbers below those are inter-treatment values (SEM = 1.68).

[3] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC). Samples were harvested on d 11 after first inoculation.

Table 4.5. Sorenson's similarity values to compare the similarity of ileal mucosa microbiota of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1,2]

		Sham[3]				EC[3]			
	DDGS[3]	0%	5%	10%	20%	0%	5%	10%	20%
Sham[3]	0%	60.5							
	5%	59.5	56.1						
	10%	54.8	54.3	55.5					
	20%	60.4	58.2	53.2	57.3				
EC[3]	0%	56.8	55.2	50.8[a]	56.4	56.2			
	5%	57.7	56.7	52.4	56.8	54.3	52.4		
	10%	56.6	55.4	45.5[a]	55.4	54.2	53.4	52.8	
	20%	59.1	58.9	52.1[a]	58.0	57.7	55.4	56.6	59.2

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns (average number of bands in common), within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 15 single comparisons between pairs of pigs within treatment. Each inter-treatment value included 36 single comparisons between pairs of pigs between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 2.51), and the numbers below those are inter-treatment values (SEM = 1.55).

[3] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC). Samples were harvested on d 11 after first inoculation.

Table 4.6. Sorenson's similarity values to compare the similarity of cecal mucosa microbiota of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1,2]

		Sham[3]				EC[3]			
	DDGS[3]	0%	5%	10%	20%	0%	5%	10%	20%
Sham[3]	0%	62.1							
	5%	55.9[a]	58.8						
	10%	58.0	60.7	60.6					
	20%	59.6	58.2	60.0	61.9				
EC[3]	0%	64.3	56.1[a]	57.5[a]	59.1	64.2			
	5%	57.8[a]	62.5	61.0	58.6	56.5[a]	62.6		
	10%	61.2	55.9	54.3[a]	55.1[a]	60.1	58.5	56.8	
	20%	59.8	51.0[a]	52.1[a]	53.8[a]	57.7	51.4[a]	57.5	54.4

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns (average number of bands in common), within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 15 single comparisons between pairs of pigs within treatment. Each inter-treatment value included 36 single comparisons between pairs of pigs between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 2.03), and the numbers below those are inter-treatment values (SEM = 1.47).

[3] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC). Samples were harvested on d 11 after first inoculation.

Table 4.7. Sorenson's similarity values to compare the similarity of colonic mucosa microbiota of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1,2]

		Sham[3]				EC[3]			
	DDGS[3]	0%	5%	10%	20%	0%	5%	10%	20%
Sham[3]	0%	62.7							
	5%	64.5	68.4						
	10%	55.2	56.3	50.8					
	20%	58.9	56.2[a]	52.8	56.1				
EC[3]	0%	58.2	60.5	53.4	54.2	55.3			
	5%	57.6	57.1	53.7	52.0	55.2	54.0		
	10%	59.9	58.7	56.1	56.4	56.7	54.6	58.2	
	20%	53.0	56.8	48.1	48.3	52.8	51.5	50.4	46.9

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns (average number of bands in common), within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 15 single comparisons between pairs of pigs within treatment. Each inter-treatment value included 36 single comparisons between pairs of pigs between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 2.56), and the numbers below those are inter-treatment values (SEM = 1.73).

[3] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenged with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC). Samples were harvested on d 11 after first inoculation.

Table 4.8. Digesta characteristics of young pigs fed increasing concentrations of dietary distillers dried grains with solubles (DDGS) and challenged or not with a β-hemolytic *E. coli* (Exp. 2)[1]

	Sham[2]				EC[2]							
	DDGS in diet[2]									*P*-value[3]		
Response	0%	5%	10%	20%	0%	5%	10%	20%	SEM	Diet	Challenge	D×C
Digesta DM, %												
Stomach	20.8	19.0	19.5	20.0	16.0	16.0	19.1	17.0	2.34	0.90	0.10	0.82
Jejunum[4]	23.8	22.9	17.2	15.6	20.2	21.3	20.7	11.7	2.60	0.01	0.44	0.46
Ileum	15.1	11.4	14.4	11.6	10.7	12.9	13.3	10.5	2.04	0.59	0.39	0.56
Cecum	11.6	13.3	9.3	13.0	11.0	11.6	11.2	12.1	1.37	0.23	0.70	0.52
Colon	18.9	21.1	18.9	21.2	18.7	20.2	13.5	18.8	2.32	0.20	0.15	0.65
Digesta pH												
Stomach	2.71	2.94	2.78	2.74	2.48	3.42	2.55	3.46	0.298	0.13	0.39	0.27
Jejunum[5]	5.50	5.45	5.03	5.33	4.91	5.26	5.38	5.12	0.157	0.73	0.16	0.04
Ileum	5.48	6.19	5.42	5.94	5.77	5.75	5.74	5.27	0.280	0.39	0.50	0.15
Cecum	5.04	5.28	4.99	4.98	4.72	4.85	4.66	4.54	0.138	0.16	0.001	0.96
Colon	5.61	5.74	5.34	5.34	5.11	5.18	5.21	5.04	0.180	0.48	0.01	0.62
SCFA, mmol × kg^{-1} of digesta DM[6]												
Ileum[7]	40.4	47.5	52.4	59.3	69.3	54.1	45.8	45.7	9.07	0.92	0.53	0.08
Cecum	186.9	171.6	205.1	164.3	215.1	206.1	208.2	165.5	25.12	0.32	0.32	0.85
Colon	89.7	106.1	142.2	104.1	132.5	89.5	129.7	101.6	17.57	0.12	0.81	0.25

Table 4.8 (cont.)

[1] Least square means of 6 pigs per treatment. Inoculation began on d 3 after weaning and samples were harvested on d 11 after first inoculation.
[2] Pigs were fed a diet with 0, 5, 10, or 20% DDGS and challenge with 3 consecutive daily doses of distilled water (Sham) or *E. coli* at 10^{10} cfu per dose (EC).
[3] Effect of DDGS inclusion in diet (Diet), effect of Sham vs. EC challenge (Challenge), and Diet × Challenge interaction (D×C). The linear, quadratic, and cubic effects of Diet were tested.
[4] Diet linear effect ($P < 0.001$).
[5] Diet quadratic × challenge interaction ($P < 0.01$).
[6] Short-chain fatty-acids.
[7] Diet linear × challenge interaction ($P = 0.02$).

CHAPTER V

INSOLUBLE DIETARY FIBER EXPEDITES RECOVERY FROM POST-WEANING COLIBACILLOSIS DIARRHEA IN PIGS

Abstract

To determine whether soluble or insoluble dietary fiber (DF), or their absence from the diet, is protective against colibacillosis diarrhea, an experiment was conducted with 80 pigs (21 d of age and 7.33 ± 0.21 kg of BW). The study was a randomized complete block design with a factorial arrangement of 4 diets × 2 challenge treatments: inoculation with 3 consecutive daily doses of either distilled water (Sham) or pathogenic *E. coli* to deliver 10^{10} cfu/dose (EC). Inoculation began on d 5 after weaning, considered post-inoculation (PI) d 0. Blocks were 2 replicates of the experiment. The experimental unit was the pig. Diets were: 1) standard weaning diet (CON); 2) CON + pectin (SF); 3) CON + cellulose (IF); 4) semi-purified corn starch-casein based diet with no dietary fiber (NF). Diet SF had 8% more soluble DF, and diet IF had 8% more insoluble DF, than the CON diet. Diarrhea was scored daily from 1= normal feces, through 5= watery diarrhea. Data were analyzed using the MIXED procedure of SAS; diarrhea score data were analyzed as repeated measures. Inoculation with EC reduced ($P < 0.001$) ADG from PI d 0 to 5 (153 vs. 85 g/d; SEM = 21) and increased ($P < 0.001$) the overall diarrhea score. In EC pigs, diarrhea score gradually increased up to PI d 6, and then gradually decreased (quadratic time effect, $P < 0.001$). Pigs fed IF diet showed less ($P < 0.05$) diarrhea at PI d 9 and 10 than those fed either CON or SF diets. Insoluble DF hastened the recovery from colibacillosis diarrhea.

Key words: colibacillosis, dietary fiber, insoluble fiber, microbial populations, soluble fiber, pig

Introduction

The effects of dietary fiber (DF) on susceptibility to colibacillosis and other enteric diseases in pigs are controversial, especially the contrast between the soluble and insoluble fractions of DF (Wellock et al., 2007). Soluble fiber is rapidly fermentable by microbes in the hindgut, which may promote the development of microbial communities. The development of microbial communities may in turn inhibit the colonization by pathogenic bacteria (Bauer et al., 2006). Insoluble fiber is only slowly fermentable, but is thought to be beneficial for intestinal health because of its effects on digestive physiology (Jin et al.,1994; Hedemann et al., 2006; Wilfart et al., 2007). Our laboratory has shown that the inclusion of barley or rice in the diet for pigs may improve their health (Perez et al., 2006). Those observations are difficult to explain

solely on the basis of DF because barley has a large content of DF, both soluble and insoluble, and rice has almost no fiber at all. It has been shown that low-fiber diets based on rice and animal proteins prevent susceptibility to enteric diseases (Pluske et al., 2003; Montagne et al., 2004). In addition, several studies suggest that fermentable fiber may be detrimental for pig health (Pluske et al., 1996; McDonald et al., 1999), whereas insoluble fiber may prevent post-weaning diarrhea (Mateos et al., 2006; Kim et al., 2008).

The ethanol by-product corn distillers dried grains with solubles (DDGS) is a feedstuff with a large concentration of insoluble DF (Martinez-Amezcua et al., 2007; Pahm et al., 2009). Previous observations from our laboratory (Chapters 3 and 4) have shown that the inclusion of DDGS in pig diets may hasten the recovery from colibacillosis diarrhea post-weaning. We hypothesized that the inclusion of insoluble DF in diets for newly weaned pigs can ameliorate colibacillosis diarrhea. The objective of this research was to determine whether the addition of either soluble or insoluble DF, or the absence of DF, in the diet for pigs may alleviate post-weaning colibacillosis diarrhea and promote recovery.

Materials and Methods

A disease challenge experiment was conducted to evaluate the effects of adding either soluble or insoluble DF to the diet, as well as their absence from the diet, on the prevention and recovery from post-weaning colibacillosis diarrhea. All procedures with animals were approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental design

The experiment was a randomized complete block design with 8 treatments in a factorial arrangement of 4 diets × 2 challenge treatments: inoculation with 3 consecutive daily doses of either distilled water (Sham), or pathogenic *E. coli* (EC) to deliver 10^{10} cfu per dose. Blocks were 2 replicates of the same experiment to achieve 10 pigs per treatment (6 and 4 pigs/treatment in the 2 blocks, respectively). The 4 experimental diets (Table 5.1) were defined by the addition of DF as follows: 1, control corn-soybean meal based diet (CON); 2, addition of 8% soluble fiber from pectin (SF); 3, addition of 8% insoluble fiber from cellulose (IF); 4, semi-purified corn starch-casein diet to completely avoid fiber (NF). The experimental unit was the pig. Litter of origin and sex were always balanced across treatments.

Animals, Housing, and Diets

All pigs were weaned at 21 d of age (7.3 ± 0.21 kg of BW) and housed in disease containment chambers. Each chamber housed 3 pigs to encourage proliferation of *E. coli* within chamber. The ventilation system kept a negative air pressure within chamber to reduce the chances of cross-contamination; animal biosafety level 2 protocols were followed after first inoculation. Each chamber had 4.65 m^2 of floor space and was equipped with a plastic coated expanded metal floor, a nipple drinker, and a stainless-steel 4-hole feeder. Temperature was set to remain constant at 28^{o}C; light period was set to 12 h per day and ventilation was automatically controlled. Feed and water were offered to allow *ad libitum* intake. Pigs were a crossbred of sire line 337 by dam line C-22 (Pig Improvement Company, Hendersonville, TN). All pigs received an intramuscular dose of 30 mg ceftiofur (Pfizer Inc., New York, NY) on d 3 of age; no more antibiotics were used before and during the experiment. Pigs were selected from litters with no β-hemolytic coliforms detected in feces of pigs or their dams 3 d before weaning.

Experimental diets were formulated using analyzed values of soluble and insoluble DF content in the batches of corn, soybean meal, and soy protein concentrate (Soycomil K, ADM, Decatur, IL) that were used in the diets. Those values on dry matter basis were as follows: corn, 2.43% soluble and 11.87% insoluble DF; soybean meal, 2.49% soluble and 17.94% insoluble DF; soy protein concentrate, 4.33% soluble and 17.54% insoluble DF. The DF values for pectin (Pectin LM 32, Tic Gums Inc., Belcamp, MD) and cellulose (Solka-floc 100 FCC, International Fiber Co., North Tonawanda, NY) used for formulation were those specified by the manufacturers. The pectin was 60% soluble DF and the cellulose was 100% insoluble DF, as-fed. The experimental diets were formulated to meet the same nutrient specifications across treatments, except that the estimated CP content in NF diet was lower than in the other diets. In all cases, diets met or exceeded the nutrient requirements estimated by NRC (1998). Chemical composition and digestibility percentages of feed ingredients were obtained from NRC (1998), except that the chemical composition and amino acid digestibility of soybean meal was taken from the Soy in Animal Nutrition Database (Kusina et al., 2008). The amino acids supply was calculated on the standardized ileal digestible basis (Stein et al., 2007), to meet the ideal amino acid profile estimated by Chung and Baker (1992).

Inoculation Procedures

The pathogenic *E. coli* used for inoculation was a β-hemolytic strain, serotype F18, that expressed 3 toxins: the heat-labile toxin (LT), the heat-stable toxin b (STb), and the Shiga-like toxin II (SLT2). The selection of *E. coli* strain and inoculation dose was based on the results of a preliminary experiment, which assessed its capacity to induce mild diarrhea in newly weaned pigs. The strain was isolated from a field outbreak by the Veterinary Diagnostic Laboratory, University of Illinois College of Veterinary Medicine. The pathogenic *E. coli* was grown on sheep blood agar plates (Thermo Fisher Scientific, Lenexa, KS) and incubated overnight at 37ºC. Then, colonies were collected and diluted in distilled water to reach a concentration of 10^{10} cfu per dose of 3 mL. All doses were prepared within 3 h before use. The inoculum was delivered in the back of the mouth ,using a 3-mL syringe with a 1-inch plastic tube attached at the end. Pigs received 3 doses delivered on 3 consecutive days. Inoculation began on d 3 after weaning (7.6 ± 0.26 kg of BW), considered post-inoculation (PI) d 0.

Sample Collection and Measurements

Pigs were weighed at weaning, and then on PI d 0, 5, and 10. The severity of diarrhea was assessed daily by 2 persons trained to visually score fecal consistency using the following scale: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea.

Pigs were anesthetized by intramuscular injection of Telazol:Ketamine:Xylazine (1:0.5:0.5 mg/10 kg of BW), and then euthanized by intra-cardiac injection of sodium pentobarbital (70 mg/kg of BW) on PI d 10. About 10 cm of tissue from ileum (20 cm before ileal-cecal junction) and middle colon were taken, longitudinally opened, and gently rinsed with distilled water to wash out the digesta; then, mucosal samples were taken by scraping the intestinal wall. Those samples were frozen in liquid nitrogen immediately after collection and then stored at -80°C until analysis. The concentration of immunoglobulin IgA was measured in the ileal mucosa.

Tissue samples from ileum and colon were also taken for histological measurements. Half of each sample for histology was cut longitudinally, so that one end was flat and the other end kept its round shape; samples were stored in 10% neutral buffered formalin to fix the tissue structure within 5 min post-euthanasia. Histological measurements were villus height, crypt depth, villus height to crypt depth ratio (VCR), and the number of lymphocytes infiltrated in mucosa layer of jejunum.

Laboratory Analyses

The experimental diets and selected ingredients were analyzed for their concentration of soluble and insoluble DF (method 991.43; AOAC International, 2007). The concentration of immunoglobulin IgA in ileal mucosa samples was measured in triplicates by enzyme linked immunosorbent assay. A pig IgA quantitation set (Bethyl laboratories Inc., Montgomery, TX) was used and the manufacturer's instructions were followed. Briefly, mucosa samples were homogenized and suspended in sample diluent (pH 8.0). The coating antibody was a goat anti-pig IgA in a dilution of 1:40,000 (working dilution range of 1:10,000 to 1:150,000). The absorbance was read at 450 nm after 30 min of incubation using an enzyme linked immunosorbent assay plate reader (ELX 808 IU; Biotek Instruments, Winooski, VT).

Tissue samples for histological measurements were embedded in paraffin and 2 cross-sections were obtained. Tissues were stained with hematoxylin and eosin stain. Histological analyses were performed using a slide scanning system (NanoZoomer 2.0 series; Hamamatsu Photonics, K.K., Japan) and its image analysis software (NPD.view).

Statistical Analyses

All data were analyzed as a randomized complete block design with a 4 (diet) × 2 (challenge) factorial arrangement of treatments. Blocks were 2 experiments and were used as a random variable in the model. All the statistical analyses were facilitated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The experimental unit was the pig and each treatment had a total of 10 pigs. Samples were harvested in 2 periods: PI d 5 and 10. Thus, each period had 5 replicates per treatment. The initial BW (weaning weight) was found to be different ($P < 0.05$) between Challenge treatments; therefore, initial BW was used as a covariable in the analysis of ADG data. Whenever differences were detected, means separation was facilitated by the LSMEANS procedure. The residuals were analyzed using the UNIVARIATE procedure to determine normality of the distribution and homogeneity of variance, and to identify outliers. A residual was considered an extreme outlier if the value was more than 3 SD away from the mean. Few extreme outliers were detected in the IgA data. Those extreme outlier observations were not considered in the ANOVA.

The diarrhea score data over time were analyzed by repeated measures analysis (Littell et al., 1998). The Autoregressive covariance model was used to fit the structure of the data; the selection of covariance model was based on graphic visualization of the data structure, number

of parameters estimated, values from fit statistics, and normal distribution of residuals. The linear and quadratic effects of time were analyzed by contrast.

Results

Inoculation with EC reduced ($P < 0.001$) the ADG in pigs from PI d 0 to 5 (153 vs. 85 g/d; SEM = 21), regardless of dietary treatment (Table 5.2). No differences among treatments were detected in ADG from PI d 5 to 10. Diet and challenge treatment interacted ($P = 0.08$) in the overall diarrhea score (Table 5.3). The inoculation with EC increased the overall diarrhea score; however, within EC pigs, those fed either IF or NF diets had the lowest overall diarrhea score. Time and challenge treatment interacted ($P = 0.07$) in the diarrhea score. In EC pigs, the diarrhea score gradually increased to reach its largest score by PI d 6, and then it gradually decreased. This pattern of response had a quadratic time effect ($P < 0.001$). Pigs fed the semi-purified NF diet did not defecate daily. Pigs fed the IF diet had a lower ($P < 0.05$) diarrhea score than pigs fed the CON or SF diets on PI d 9 and 10. The concentration of IgA in ileal mucosa was not different among treatments (Table 5.4).

In ileum, inoculation with EC increased the villus height and crypt depth ($P = 0.07$), and the number of lymphocytes per villus infiltrated in the mucosa layer ($P < 0.01$), on PI d 5 (Table 5.5). At the same time, pigs fed the IF diet had a greater ($P < 0.05$) ileal crypt depth than those fed either SF or NF diets. Within pigs fed the SF diet, those inoculated with EC had more ($P < 0.05$) lymphocytes per villus infiltrated in the ileal mucosa layer than the Sham pigs, on PI d 10. In colon, inoculation with EC increased the crypt depth ($P = 0.03$), on PI d 10. At the same time, pigs fed the NF diet had the least ($P < 0.05$) crypt depth in colon.

Discussion

The inoculation with EC induced diarrhea and reduced ADG from PI d 0 to 5. After d 6 PI, the diarrhea gradually decreased and no differences among treatments were detected in ADG from PI d 5 to 10. This pattern of response shows the course of disease from infection to recovery. Pigs fed the IF diet had a faster recovery from diarrhea, as their diarrhea score on PI d 9 and 10 was lower than that of pigs fed CON or SF diets. This effect agrees with previous observations from our laboratory, in which we observed that the inclusion of dietary DDGS (which has a large concentration of insoluble DF) reduced diarrhea in pigs during their recovery from colibacillosis (Chapters 3 and 4). Similarly, the inclusion of insoluble DF from oat hulls (20

g/kg of diet) to a rice-based diet ameliorated colibacillosis diarrhea in young pigs (Mateos et al., 2006; Kim et al., 2008).

The mechanisms through which insoluble DF protects against colibacillosis diarrhea are not fully understood. However, insoluble DF may have a washout effect on some enteric pathogens through its effects on the digestive physiology. Insoluble DF reduces viscosity (Dikeman et al., 2006) and transit time (Stanogias and Pearce, 1985) of digesta, and promotes intestinal development (Pluske et al., 2003), which may increase intestinal motility (Mateos et al., 2006). Insoluble DF also increases the enterocyte turnover rate (Jin et al., 1994). As the pathogenic *E. coli* colonize by attaching to the enterocyte (Bertschinger, 1999), a greater enterocyte turnover rate may help to hasten their excretion. Previous observations from our laboratory have shown a reduction in the concentration of fecal pathogenic coliforms in pigs fed DDGS, during their recovery from colibacillosis (Chapter 4). Mateos et al. (2006) suggested that the effect of insoluble DF on motility and transit time of digesta can also reduce the availability of substrate to support bacterial growth, which may also promote a faster recovery process.

Soluble DF increases viscosity of digesta (Hopwood et al., 2004; Dikeman et al,. 2006) and promotes microbial fermentation (Reilly et al., 2010). Both of those effects have been associated with the development of diarrhea due to colibacillosis and other enteric diseases (Pluske et al., 1998; McDonald et al., 1999; Hopwood et al., 2002). Our data on diarrhea score support this relationship because pigs fed the SF diet did not reduce their diarrhea as fast as those fed the IF diet. In contrast, others (Thomsen et al., 2007; Wellock et al., 2008) have observed that soluble DF from inulin protects from colibacillosis diarrhea. However, those observations are confounded with a prebiotic effect of inulin (Flickinger et al., 2003; Waltz et al., 2005).

Feeding pigs with low-fiber diets based on rice and animal proteins ameliorates the colibacillosis diarrhea and reduce colonization by *E. coli* (Pluske et al., 2003; Montagne et al., 2004). However, it is not clear whether that effect is due to the low concentration of dietary fiber or to the rice *per se*. Rice has a direct effect in reducing diarrhea (Macleod et al., 1995) through a compound that has been called *the rice factor* (Mathews et al., 1999). In our study, pigs fed the NF diet showed a low diarrhea score on PI d 3, 4, and 8. However, those pigs did not defecated every day, most likely because the diet is highly digestible. In fact, pigs fed the NF diet had the lowest crypt depth in colon, which suggest a lower digestive and bacterial activity because most nutrients were absorbed through the small intestine. The EC pigs fed the NF diet showed mild to

severe diarrhea when defecated. Therefore, our results did not support the notion that the absence of DF in the diet protects against colibacillosis diarrhea.

Changes in the intestinal microbiota can modulate the host immune response against pathogens (Magalhaes et al., 2007). The most important anti-body mediated immune response against pathogens in the intestinal mucosa is the production of IgA (Leser and Mølbak, 2009). We observed that the inclusion of insoluble DF from DDGS in pig and poultry diets changed their intestinal microbiota (Chapters 4 and 6). However, in the present study we did not detect any difference among treatments in the concentration of IgA in ileal mucosa.

Colibacillosis diarrhea damages the intestinal epithelium, which causes inflammation of adjacent tissue layers and a cell-mediated immune response in which lymphocytes migrate to the mucosal layer (Bertschinger and Fairbrother, 1999). In agreement with that, we observed that EC inoculation increased villus height, crypt depth, and infiltration of lymphocytes per villus in the mucosal layer of ileum on PI d 5. These changes were observed when the infection probably reached its most aggressive phase, but none of them remained during the recovery process on PI d 10. The larger crypt depth in ileum of pigs fed IF diet at PI d 5 can be explained by the increased enterocyte turnover rate promoted by insoluble DF (Jin et al., 1994). Similar effects of DF on intestinal morphology have been documented by others (Montagne et al., 2003; Hedemann et al., 2006). No more effects of DF were observed in the intestinal morphology, which also agrees with observations form McDonald et al. (1999).

In conclusion, the addition of 8% insoluble DF to a diet for newly weaned pigs can speed their recovery from colibacillosis diarrhea. This may be because of a washout effect that insoluble DF may exert on the intestinal bacterial populations through its effects on the digestive physiology. The inclusion of soluble DF and the absence of DF in the diet did not ameliorate the diarrhea caused by pathogenic *E. coli*.

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Tables

Table 5.1. Experimental diets (as-fed)[1]

Ingredient, % of diet	CON	SF	IF	NF
Pectin[2]	0.00	14.18	0.00	0.00
Cellulose[3]	0.00	0.00	9.93	0.00
Corn starch	0.00	0.00	0.00	40.84
Casein	0.00	0.00	0.00	10.04
Sand	0.00	0.00	0.00	8.12
Corn	40.67	17.16	22.44	0.00
Soybean meal	15.00	15.00	15.00	0.00
Whey, spray-dried	20.00	20.00	20.00	20.00
Lactose	7.22	7.22	7.22	7.22
Fish, Menhaden select[4]	9.16	11.99	11.35	11.12
Soy protein concentrate[5]	5.00	5.00	5.00	0.00
Soybean oil	1.00	8.11	7.58	1.00
Calcium phosphate, 21% P	0.63	0.26	0.34	0.56
Limestone, ground	0.22	0.09	0.12	0.05
Salt	0.40	0.40	0.40	0.40
Mineral and vitamin premix[6]	0.30	0.30	0.30	0.30
L-Lysine·HCl	0.168	0.063	0.087	0.000
L-Threonine	0.123	0.113	0.115	0.143
DL-Methionine	0.102	0.117	0.113	0.167
L-Tryptophan	0.010	0.005	0.006	0.045
Chemical composition[7]				
ME, Mcal/kg	3.34	3.34	3.34	3.34
Lactose, %	21.00	21.00	21.00	21.00
CP, %	22.52	22.28	22.33	18.86
SID Lys, %[8]	1.40	1.40	1.40	1.40
Soluble dietary fiber, %[9]	1.23	9.92	0.62	0.00
Insoluble dietary fiber, %[9]	8.31	5.63	15.31	0.00
Total dietary fiber, %	9.54	15.55	15.93	0.00

Table 5.1 (cont.)

[1] Experimental diets: CON, control; SF, soluble fiber; IF, insoluble fiber; NF, no fiber.
[2] Pectin (Pectin LM 32, Tic Gums Inc., Belcamp, MD).
[3] Cellulose (Solka-floc 100 FCC, International Fiber Co., North Tonawanda, NY).
[4] Menhaden fish meal (Special Select Menhaden Fish Meal, Omega Protein Inc., Hammond, LA)
[5] Soy protein concentrate (Soycomil K, ADM, Decatur, IL).
[6] Supplied per kilogram of complete diet: Ca, 0.9%; available P, 0.55%; Cu, 8 mg ($CuSO_4 \cdot 5H_2O$); Zn, 100 mg (ZnO); Fe, 90 mg ($FeSO_4 \cdot 5H_2O$); Mn, 20 mg (MnO); I, 0.35 mg (CaI_2); Se, 0.3 mg (Na_2SeO_3); NaCl, 3 g; retinyl acetate, 2,273 μg; cholecalciferol, 17 μg; DL-α-tocopheryl acetate, 88 mg; menadione sodium bisulfite complex, 4 mg; niacin, 33 mg; d-Ca-pantothenate, 24 mg; riboflavin, 9 mg; vitamin B12, 35 μg; choline chloride, 324 mg.
[7] Calculated analysis unless something different is reported.
[8] Standardized ileal digestible.
[9] Analyzed value.

Table 5.2. Growth rate of young pigs challenged or not with a pathogenic *E. coli* and fed different concentrations of dietary fiber[1]

	Sham[2]				EC[2]							
	Dietary treatments[3]									*P*-value[4]		
Response[5]	CON	SF	IF	NF	CON	SF	IF	NF	SEM	Diet	Challenge	D × C
ADG, g/d												
PI d 0 to 5	179	126	187	120	88	84	88	82	30	0.32	0.001	0.48
PI d 5 to 10	369	370	479	298	394	266	346	307	55	0.16	0.19	0.40

[1] Least square means of 10 (PI d 0 to 5) or 5 (PI d 5 to10) pigs per treatment.
[2] Pigs were challenged with 3 consecutive daily doses of distilled water (Sham) or pathogenic *E. coli* at 10^{10} cfu per dose (EC).
[3] Dietary treatments were: CON, control; SF, soluble fiber; IF, insoluble fiber; NF, no fiber.
[4] Effects of dietary treatment (Diet), Sham vs. EC challenge (Challenge), and Diet × Challenge interaction (D × C).
[5] Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.

Table 5.3. Diarrhea score of young pigs challenged or not with a pathogenic *E. coli* and fed different concentrations of dietary fiber[1]

	Sham[2]				EC[2]							
	Dietary treatments[3]									*P*-value[4]		
Response[5]	CON	SF	IF	NF	CON	SF	IF	NF	SEM	Diet	Challenge[6]	D × C
Diarrhea score												
PI d 1	1.30	1.20	2.00	1.99	2.30	1.60	1.80	1.92	0.61	0.45	0.32	0.36
PI d 2	1.70	1.40	2.00	1.62	2.80	2.55	2.70	2.54	0.48	0.74	0.001	0.92
PI d 3[7]	1.90	1.60	2.20	1.25	3.80	3.33	2.60	2.53	0.43	0.09	0.001	0.15
PI d 4[8]	1.70	1.60	2.60	1.18	3.30	3.60	3.00	2.50	0.43	0.07	0.001	0.15
PI d 5	2.00	1.60	2.00	1.62	3.50	3.74	3.30	2.59	0.44	0.34	0.001	0.45
PI d 6	2.10	2.01	1.91	1.33	3.57	4.26	3.25	3.22	0.72	0.42	0.001	0.77
PI d 7	2.14	1.71	2.06	1.17	3.40	3.93	3.23	3.41	0.77	0.82	0.001	0.61
PI d 8[7]	2.37	1.66	1.63	1.09	3.50	3.76	2.42	2.32	0.65	0.08	0.001	0.60
PI d 9[9]	1.79	2.03	1.82	1.05	3.45	3.38	1.61	1.84	0.79	0.06	0.03	0.26
PI d 10[9]	1.59	1.62	1.41	1.03	2.83	2.99	1.20	2.14	0.65	0.15	0.02	0.37
Overall	1.86	1.64	1.96	1.33	3.25	3.31	2.51	2.50	0.26	0.03	0.001	0.08

[1] Least square means of 10 (PI d 1 to 5) or 5 (PI d 6 to10) pigs per treatment.
[2] Pigs were challenged with 3 consecutive daily doses of distilled water (Sham) or pathogenic *E. coli* at 10^{10} cfu per dose (EC).
[3] Dietary treatments were: CON, control; SF, soluble fiber; IF, insoluble fiber; NF, no fiber.
[4] Repeated measures analysis in time to test the effects of dietary treatment (Diet), Sham vs. EC challenge (Challenge), Diet × Challenge (D × C), and their interaction with time. Time ($P < 0.001$) and time × challenge interaction ($P = 0.07$) were significant.
[5] Diarrhea was scored daily as follows: 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; and 5, watery diarrhea. Inoculation began on d 3 after weaning, considered post-inoculation (PI) d 0.

Table 5.3 (cont.)

[6] The time effect was quadratic ($P < 0.001$) in EC.
[7] Diarrhea score in NF was lower ($P < 0.05$) than in CON.
[8] Diarrhea score in NF was lower ($P < 0.05$) than in SF and IF.
[9] Diarrhea score in IF was lower ($P < 0.05$) than in CON and SF.

Table 5.4. Concentration of IgA in mucosa of ileum of young pigs challenged or not with a pathogenic *E. coli* and fed different concentrations of dietary fiber[1]

	Sham[2]				EC[2]							
	Dietary treatments[3]									*P*-value[4]		
Response[5]	CON	SF	IF	NF	CON	SF	IF	NF	SEM	Diet	Challenge	D × C
Ileal immunoglobulin, µg/g of wet mucosa												
IgA, PI d 5	0.38	0.56	0.36	0.38	0.32	0.47	0.47	0.46	0.27	0.55	0.89	0.76
IgA, PI d 10	2.12	1.73	1.54	1.15	1.52	1.07	1.52	1.19	0.98	0.81	0.52	0.93

[1] Least square means of 5 pigs per treatment.
[2] Pigs were challenged with 3 consecutive daily doses of distilled water (Sham) or pathogenic *E. coli* at 10^{10} cfu per dose (EC).
[3] Dietary treatments were: CON, control; SF, soluble fiber; IF, insoluble fiber; NF, no fiber.
[4] Effects of dietary treatment (Diet), Sham vs. CR challenge (Challenge), and Diet × Challenge interaction (D × C).
[5] Samples were harvested on post-inoculation (PI) d 5 and 10.

Table 5.5. Histological changes in the intestine of young pigs challenged or not with a pathogenic *E. coli* and fed different concentrations of dietary fiber[1]

	Sham[2]				EC[2]							
	Dietary treatments[3]									*P*-value[4]		
Response[5]	CON	SF	IF	NF	CON	SF	IF	NF	SEM	Diet	Challenge	D × C
Ileum, PI d 5												
Villus height, µm	331	352	337	300	375	365	366	369	53	0.86	0.07	0.80
Crypt depth, µm[6]	257	258	291	244	306	258	305	272	23	0.07	0.07	0.54
VCR[7]	1.28	1.37	1.19	1.21	1.23	1.44	1.20	1.37	0.16	0.42	0.58	0.88
Lymphocytes[8]	8.7	9.7	9.1	10.3	10.8	9.8	13.8	12.2	1.89	0.27	0.01	0.26
Colon, PI d 5												
Crypt depth, µm	377	371	424	351	395	349	427	444	32	0.24	0.31	0.31
Ileum, PI d 10												
Villus height, µm	409	406	366	408	414	368	415	393	36	0.76	0.99	0.36
Crypt depth, µm	301	278	300	268	296	310	324	297	28	0.42	0.11	0.71
VCR[7]	1.37	1.47	1.22	1.56	1.42	1.21	1.29	1.35	0.10	0.25	0.22	0.23
Lymphocytes[8]	18.0^{ab}	11.5^{b}	15.4^{ab}	12.7^{b}	11.9^{b}	21.6^{a}	18.3^{ab}	14.2^{ab}	2.72	0.54	0.27	0.04
Colon, PI d 10												
Crypt depth, µm[9]	404	364	373	315	417	448	403	340	24	0.01	0.03	0.45

[a,b] Within a row, means without a common superscript differ ($P < 0.05$).

[1] Least square means of 5 pigs per treatment.

Table 5.5 (cont.)

[2] Pigs were challenged with 3 consecutive daily doses of distilled water (Sham) or pathogenic *E. coli* at 10^{10} cfu per dose (EC).
[3] Dietary treatments were: CON, control; SF, soluble fiber; IF, insoluble fiber; NF, no fiber.
[4] Effects of dietary treatment (Diet), Sham vs. EC challenge (Challenge), and Diet × Challenge interaction (D × C).
[5] Samples were harvested on post-inoculation (PI) d 5 and 10.
[6] Within main effect of diet, IF was larger ($P < 0.05$) than SF and NF.
[7] Villus height to crypt dept ratio (VCR).
[8] Average of lymphocytes per villus infiltrated in the mucosa layer.
[9] Within main effect of diet, CON, SF, and IF was larger ($P < 0.05$) than NF.

CHAPTER VI

EFFECT OF CORN DISTILLERS DRIED GRAINS WITH SOLUBLES AND *Eimeria acervulina* INFECTION ON GROWTH PERFORMANCE AND THE INTESTINAL MICROBIOTA OF YOUNG CHICKS

Abstract

Young crossbred chicks were used to determine whether dietary corn distillers dried grains with solubles (DDGS) may prevent or ameliorate *Eimeria acervulina* (EA) infection. The experiment had a completely randomized design with a factorial arrangement of 3 diets (inclusion of 0, 10, or 20% DDGS) × 2 challenge treatments: inoculation with distilled water or 10^6 sporulated EA oocysts. Each treatment was replicated with 8 pens of 5 chicks each. Experimental diets were fed from 7 to 21 d of age. Inoculation occurred on d 10 of age, considered post-inoculation (PI) d 0. Feed intake and BW were measured on PI d 0, 7, and 14. Fecal samples were collected on PI d 0, 5 to 10, 12, and 14 to detect oocysts. On PI d 14, mucosal samples were collected for the analysis of bacterial populations by denaturing gradient gel electrophoresis, using the V3 region of bacterial 16S ribosome. The EA challenge reduced ($P < 0.001$) ADG by 17%, ADFI by 12%, and G:F by 6% from PI d 0 to 7, and by smaller percentages from PI d 7 to 14. Diet and challenge treatments did not interact in the chick performance. Feeding 20% DDGS reduced ADG from PI d 0 to 7 (diet linear, $P < 0.05$). Feeding 10% DDGS increased G:F from PI d 7 to 14 (diet quadratic, $P < 0.05$). Oocysts in feces were detected PI only in EA chicks and no dietary effects were found. Cecal bacterial population was changed ($P < 0.05$) by effect of dietary DDGS and EA infection. The cecal bacterial diversity and homogeneity within treatments were reduced by EA infection ($P = 0.02$ to 0.001, respectively), and increased by feeding 10% DDGS (diet quadratic, $P < 0.001$). In summary, feeding up to 20% DDGS to young chicks did not prevent or ameliorate EA infection. Changes in cecal microbiota of chicks fed 10% DDGS can be interpreted as beneficial for the intestinal health.

Key words: chick, coccidiosis, dietary fiber, distillers dried grains with solubles, intestinal microbiota.

Introduction

Coccidiosis is a pervasive problem in poultry production (Williams, 2005). In broilers, the disease ranges from light infections with no apparent reduction in chick health or

performance, to severe infections that cause clinically sick birds and mortality. However, the most common presentation of coccidiosis in the poultry industry is a subclinical infection that does not produce clinical signs of disease but reduces growth performance, causing economically important losses (Williams, 1999; Haug et al., 2008). Among the *Eimeria* species that cause subclinical coccidiosis in chicks, *Eimeria acervulina* (EA) appears to be one of the most prevalent (Williams, 2005; Haug et al., 2008; Martynova-Vankley et al., 2008). Vaccines against *Eimeria* (Williams, 2002) and in-feed anticoccidial drugs (Chapman, 2001), have been used successfully to reduce the impact of the disease. However, growing public concern about use of antibiotics in livestock production creates the need to search for other dietary strategies to maintain health in food animals (Pettigrew, 2006).

The ethanol by-product corn distillers dried grains with soluble (DDGS) is a nutritionally suitable ingredient for poultry diets, with a large content of dietary fiber (Martinez-Amezcua et al., 2007; Pahm et al., 2009). Dietary fiber appears to be important for maintaining intestinal health in non-ruminants animals (Montagne et al., 2003), because of its beneficial effects on digestive physiology (Jin et al.,1994; Hedemann et al., 2006; Wilfart et al., 2007) and microbial activity (Varel and Yen, 1997). Recent observations show that either DDGS or cellulose can speed recovery of young pigs from enteric disease after an experimental challenge with pathogenic *E. coli*. (Chapters 3 and 5). The objective of this research was to determine whether the inclusion of increasing concentrations of dietary DDGS can ameliorate the effects of EA infection in young chicks.

Materials and Methods

A 14-d disease challenge experiment with EA was conducted in young chicks fed increasing concentrations of dietary corn DDGS (Lincolnland Agri-Energy LLC, Palestine, IL). The growth performance, intestinal bacterial populations, digesta pH, and viscera weight were measured. All animal care and experimental procedures described in this paper were conducted in accordance with animal care and use protocols approved by the University of Illinois Institution Animal Care and Use Committee.

Experimental Design

The experiment had a completely randomized design with a factorial arrangement of 3 diets (inclusion of 0, 10, or 20% DDGS) × 2 challenge treatments: inoculation with a single dose

of either distilled water (Sham) or 10^6 sporulated oocysts of EA. Each treatment was replicated with 8 pens of 5 chicks each.

Animals and Husbandry

Chicks were New Hampshire × Columbian Plymouth Rock males. On d 7 of age, after a previous overnight period of feed removal, chicks were weighed, wing-banded, and allotted to pens in groups of 5 chicks, so that the pen weight was similar among treatments. Chicks were housed in thermostatically controlled starter batteries with raised wire floors. Batteries were located in an environmentally controlled room with light provided continuously.

Feed and water were provided to allow ad libitum access during the entire experiment. A corn-soybean meal based starter diet was fed to all chicks from d 1 to 6 of age. Experimental diets were fed starting on d 7 of age and were formulated to provide similar concentrations of nutrients among treatments (Table 6.1). All diets met or exceeded the nutrient requirements estimated for those chicks (NRC, 1994). Chemical composition of feed ingredients and their digestibility were taken from NRC (1994), except for the ME_n of DDGS. A value of 2.66 Mcal/kg was used for the ME_n of DDGS, which is an average of the NRC (1994) table value (2.48 Mcal/kg) and the value of 2.845 Mcal/kg reported by Waldroup (2007) as the mean TME_n value of 43 samples of DDGS evaluated in recent studies. No antimicrobial compounds were used before or during the experiment.

Inoculation procedures

Inoculation with either Sham or EA challenge treatments occurred on d 10 of age, considered post-inoculation (PI) d 0. The EA (Animal Parasitic Diseases Laboratory, USDA; Beltsville, MD) was diluted in distilled water to provide 10^6 sporulated oocysts per dose of 0.5 mL. The EA inoculation dose used in this experiment was determined in a preliminary experiment; the selected dose reduced ADG by about 20% as compared with chicks in the Sham treatment. All doses were prepared 1 d before delivering. Chicks were orally gavaged with the inoculum using a 1-mL syringe.

Measurements and Sample Collection

Body weight and feed intake were measured on PI d 0, 7, and 14. The ADG, ADFI, and G:F were calculated per pen and expressed per chick. Fecal samples were collected per pen on PI d 0, 5, 6, 7, 8, 9, 10, 12, and 14 for the detection of EA oocysts. The feces were placed in plastic tubes, transported on ice to the laboratory, and stored at -4^{o}C until analysis.

On PI d 14, chicks were euthanized by CO_2 gas inhalation. Digesta samples from ileum and cecum were pooled by pen to measure pH immediately after collection. The small intestine and ceca were emptied, longitudinally opened, gently rinsed with distilled water to wash out the contents, gently dried with paper towels, and weighed per pen. Then, a mucosa sample was taken by scraping the intestinal wall and pooled by pen. Mucosa samples were frozen in liquid nitrogen immediately after collection and then stored at -80°C until analysis.

Chemical and Microbiological Analyses

Experimental diets were analyzed to measure their concentrations of acid detergent fiber (ADF; method 973.18; AOAC, 2005) and neutral detergent fiber (NDF; Holst, 1973). Digesta pH was measured using a pH meter (model BASIC, Denver Instrument Company, Arvada, CO). The oocysts in feces were recovered by flotation in Sheather's suspension and microscopically counted in a McMaster counting chamber (Lillehoj and Ruff, 1987; Dalloul et al., 2003).

The intestinal bacterial populations were analyzed by denaturing gradient gel electrophoresis (DGGE). This method separates the bacterial DNA in a gel to show distinct bands for different bacterial species (each band represents at least 1 bacterial species) and uses the V3 region of bacterial 16S ribosome, which is replicated by polymerase chain reaction (Simpson et al., 1999). The resulting DGGE banding patterns were compared between pairs of pen replicates using Sorenson's similarity values (Cs) and analyzed as described by Collier et al. (2003). Briefly, the Cs values range from 0 to 100 to measure the similarity of DGGE banding patterns between 2 pen replicates; a Cs value of 100 indicates identical banding patterns between the 2 samples, whereas a Cs value of 0 indicates no common bands. The Cs values were computed to measure the similarity intra-treatment (within treatments) and inter-treatment (between treatments). Thus, a greater intra-treatment Cs value indicated the DGGE banding pattern was more similar among pen replicates in that treatment. To determine whether the DGGE banding pattern differed between 2 treatments, their corresponding inter-treatment Cs value was compared against the mean of the intra-treatment Cs values of those 2 treatments; when the inter-treatment value was lower, it was concluded that the DGGE banding pattern differed between the 2 treatments.

Statistical Analysis

All analyses were facilitated using the General Linear Model procedure of SAS (SAS Institute Inc., Cary, NC). The ANOVA for all data were conducted using a completely

randomized design with a 3 (diets) × 2 (challenge treatments) factorial arrangement of treatments. The pen with 5 chicks was the experimental unit for all measurements. The 2 degrees of freedom sum of squares from both main effect of diet and diet × challenge treatment interaction were separated into single-degree-of-freedom sums of squares for the linear and quadratic effects by contrast analysis. Treatment means were calculated by the LSMEANS procedure. The comparison of each inter-treatment Cs value against the mean of its 2 corresponding intra-treatment Cs values was done by contrast analysis. The concentration of oocysts in feces was analyzed by repeated measures in time (Littell et al., 1998). This analysis included data from only EA challenge chicks from PI d 5 through 14, because the rest of the data were zeros. The First-Order Autoregressive covariance model was used to fit the structure of the data; the selection of covariance model was based on graphic visualization of the data structure, number of parameters estimated, values from fit statistics, and normal distribution of residuals. The linear and quadratic effects of time were analyzed by contrast. Normal distribution of the data and homogeneity of variance were verified by analysis of the residuals using the UNIVARIATE procedure.

Results

The EA infection reduced growth performance, especially during the early PI period (Table 6.2). The EA challenge reduced ($P < 0.001$) ADG by 17%, ADFI by 12%, and G:F by 6% from PI d 0 to 7, as well as ADG and ADFI by smaller percentages from PI d 7 to 14, as compared to Sham chicks. Diet and challenge treatments did not interact in growth performance. The ADG from PI d 0 to 7 was reduced by feeding 20% DDGS: 26.1, 26.1, and 25.2 g/d for diets with 0, 10, and 20% DDGS, respectively (diet linear effect, $P < 0.05$; SEM = 0.3). The G:F from PI d 7 to 14 was increased by feeding 10% DDGS: 653, 676, and 654 g/kg for diets with 0, 10, and 20% DDGS, respectively (diet quadratic effect, $P < 0.05$; SEM = 8).

No EA oocysts were found in feces on PI d 0, nor in Sham chicks throughout the experiment. Therefore, the analysis of those data only include EA chicks from PI d 5 through 14 (Table 6.3). Shedding of EA oocysts in feces decreased over time ($P < 0.001$), but no diet effects were detected. No clinical signs as diarrhea, listless or recumbent birds were observed during the experiment.

Ileal digesta pH increased with the inclusion of dietary DDGS: 6.87, 7.25, and 7.17 pH for diets with 0, 10, and 20% DDGS, respectively (diet linear effect, $P < 0.05$; SEM = 0.099).

The EA infection increased small intestine weight in the absence of DDGS, but had little or no effect on the small intestine weight of chicks fed 10 or 20% DDGS diets (diet × challenge, $P < 0.01$) (Table 6.4).

The number of DGGE bands, as a measure of bacterial diversity, changed only in the cecum (Table 6.5). The EA challenge reduced ($P = 0.02$) cecal bacterial diversity (30.5 vs. 27.2 DGGE bands; SEM = 0.98), as compared with Sham chicks. Also, feeding 10% DDGS increased cecal bacterial diversity: 26.3, 33.8, and 26.4 DGGE bands for diets with 0, 10, and 20% DDGS, respectively (diet quadratic effect, $P < 0.001$; SEM = 1.21). Diet and challenge treatments did not interact in the bacterial diversity. The intra-treatment Cs values, as a measure of bacterial homogeneity within treatments, were reduced by DDGS only in EA infected chicks (diet × challenge, $P < 0.01$). In cecum, intra-treatment Cs values were reduced ($P < 0.001$) by EA challenge. Also, feeding 10% DDGS increased the intra-treatment Cs values, whereas feeding 20% DDGS reduced them: 81.8, 86.8, and 78.1 intra-treatment Cs values for diets with 0, 10, and 20% DDGS, respectively (diet quadratic effect, $P < 0.001$; SEM = 1.11). The analysis of inter-treatment Cs values in ileum showed (Table 6.6) that the bacterial populations in Sham chicks fed 0% DDGS differed ($P < 0.05$) from that in EA chicks fed 10% DDGS. In contrast, analysis of inter-treatment Cs values in cecum showed (Table 6.7) that most treatments differed ($P < 0.05$) from each other, suggesting that both EA and DDGS changed the bacterial populations.

Discussion

The EA challenge model used in this experiment was effective in causing subclinical coccidiosis, as shown by the reduction in growth performance without clinical signs of disease. These effects of coccidiosis on chick growth performance are similar to those previously observed when coccidiosis was either experimentally induced (Preston-Mafham and Sykes, 1970; Lillehoj and Ruff, 1987; Persia et al., 2006) or naturally occurring in commercial production (Williams, 2005; Haug et al., 2008). Poor growth performance during coccidiosis is associated with reduced nutrient digestion (Major and Ruff, 1978; Persia et al., 2006), reduced nutrient absorption (Preston-Mafham and Sykes, 1970; Ruff and Wilkins, 1980; Ruff and Edgar, 1982), and villus atrophy (Fernando and McCraw, 1973).

The inclusion of DDGS in chick diets did not prevent EA infection, as shown by the reduction in growth performance regardless of dietary treatment. Although we are aware of no

other reports of the effect of DDGS on chicks with enteric disease, there are parallel reports in pigs for comparison. In young pigs challenged with pathogenic *E. coli*, dietary DDGS did not prevent the infection. However, it reduced the diarrhea score during the recovery period (Chapter 3) and hastened both the excretion of pathogenic *E. coli* and the recovery of commensal coliforms (Chapter 4). In the present study, the fecal concentration of oocysts in EA infected chicks gradually dropped to undetectable levels by PI d 14. Moreover, the reduction in chick performance by EA infection observed from PI d 7 to 14 was smaller than that from PI d 0 to 7. Those effects suggest a recovery process was occurring. However, dietary DDGS did not affect those variables, providing no evidence that it promoted recovery from coccidiosis.

Feeding 20% DDGS reduced ADG only from PI d 0 to 7 (10 to 24 d of age) in both Sham and EA chicks. Similarly, Lumpkins et al. (2004) observed a reduction in BW gain by feeding 18% DDGS to chicks from 0 to 16 d of age, but no differences were detected from 17 to 42 d of age; those differences were related to a reduction in dietary lysine content when 18% DDGS was added. Parsons and Baker (1983) concluded that more than 25% DDGS can be used in chick diets if dietary lysine and energy content is maintained to support optimal growth. The observed reduction in ADG (10 to 24 d of age) from feeding 20% DDGS may have been due to reduced digestible lysine. Although the 20% DDGS diet was formulated to meet the total lysine requirement (NRC, 1994) of 1.10%, the digestibility of lysine in DDGS is lower than that in corn and soybean meal. With a similar magnitude of reduction in dietary lysine content, Lumpkins et al. (2004) observed that chick performance was reduced, whether it was in the presence or absence of dietary DDGS. Interestingly, chicks fed the 10% DDGS diet had the largest G:F from PI d 7 to 14. The inclusion of small amounts of fiber to chick diets may improve growth performance by increasing the digestibility and absorption of nutrients (Gonzalez-Alvarado et al., 2007; Jimenez-Moreno et al., 2009b). Also, fermentation of small amounts of fiber in chicks may release more energy available for the host, but large concentrations of dietary fiber may reduce the digestibility of nutrient (Jørgensen et al., 1996). That also can explain the lower G:F in chicks fed 20% DDGS, as compared with those fed 10% DDGS. The reason for the effect of DDGS increasing the small intestine weight, but not in cecum, is unknown. However, it may be possible that DDGS induced some fermentation in the small intestine as observed in pigs (Urriola and Stein, 2010), and that promoted the intestine to grow (Jørgensen et al., 1996; Jimenez-Moreno et al., 2009a).

In contrast to the lack of effect of DDGS on growth performance and pathogen excretion, DDGS had clear effects on bacterial populations associated with the cecal mucosa. The clearest evidence is in the low inter-treatment Cs values. The intermediate level of 10% DDGS also increased the diversity of the cecal microbiota and the similarity of microbial populations across chicks within treatment, in agreement with our results in Chapter 4, in which we found the same effects in the cecum and colon of pigs fed 10% DDGS. These effects are interpreted to indicate increased stability of the microbiota (Bhandari et al., 2008; Opapeju et al., 2009), although no association with clinical response appeared in this experiment. The source and amount of fiber in the diet can change the intestinal microbial communities (Pieper et al., 2008; Reilly et al., 2010), as the fiber is an important substrate to support the microbial activity, mainly through fiber fermentation. In the present study, all of the effects of DDGS in bacterial communities occurred in the ceca, where most fiber fermentation occurs. The EA infection also altered microbial populations as indicated by low inter-treatment Cs values, in agreement with Hume et al. (2006). It also reduced both bacterial diversity and homogeneity within treatment, suggesting the possibility of reduced stability. *Eimeria* infection may allow some pathogens, such as *Clostridium perfringens*, to become dominant (Williams, 2005), reducing the microbial diversity.

In conclusion, concentrations of DDGS up to 20% of the diet did not prevent EA infection nor promote a faster recovery. Chicks fed a diet with 10% DDGS had the largest G:F from PI d 7 to 14, as well as the largest bacterial diversity and homogeneity within treatment in the cecum. Both dietary DDGS and EA infection changed the bacterial populations in cecum.

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Tables

Table 6.1. Experimental diets (as-fed)

Ingredient, % of diet	Distillers dried grains with solubles (DDGS)		
	0%	10%	20%
DDGS	0.00	10.00	20.00
Corn	52.85	47.87	43.00
Soybean meal	37.50	33.70	28.57
Pork meal	2.00	0.00	0.00
Soybean oil	4.00	4.50	4.50
Limestone	1.10	1.45	1.45
Dicalcium phosphate	1.50	1.50	1.50
Sodium Chloride	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15
DL-Methionine	0.20	0.13	0.13
Choline chloride	0.10	0.10	0.10
Chemical composition[3]			
ME_n, Mcal/kg	3.09	3.09	3.07
CP	23.23	23.23	23.07
Lysine	1.31	1.20	1.11
Calcium	1.03	1.00	1.00
Available P	0.48	0.47	0.53
ADF[4]	3.20	4.00	4.86
NDF[5]	8.65	10.69	13.31

[1] Supplied per kilogram of complete 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; and menadione sodium bisulfite, 2.33 mg.
[2] Supplied as milligrams per kilogram of complete diet: Mn, 75 from MnO; Fe, 75 from $FeSO_4 \cdot 7H_2O$; Zn, 75 from ZnO; Cu, 5 from $CuSO_4 \cdot 5H_2O$; I, 0.75 from ethylene diamine dihydroiodide; and Se, 0.1 from Na_2SeO_3.
[3] Calculated analysis as a percentage of the diet, unless something different is specified.
[4] Acid Detergent Fiber. Analyzed value.
[5] Neutral Detergent Fiber. Analyzed value.

Table 6.2. Growth performance of young chicks challenged or not with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS) [1]

	Sham[2]			EA[2]				*P*-value[3]		
	Concentration of DDGS in the diet									Diet
Response[4]	0%	10%	20%	0%	10%	20%	SEM	Diet	Ch	× Ch
BW, g										
Initial	82.8	82.9	82.9	82.7	82.9	82.9	0.2	0.59	0.92	0.87
ADG, g/d										
PI d 0 to 7[5]	28.5	28.8	27.1	23.7	23.4	23.3	0.4	0.05	0.001	0.16
PI d 7 to 14	32.0	32.5	31.7	30.8	30.3	29.1	0.6	0.13	0.001	0.43
ADFI, g/d										
PI d 0 to 7	33.2	33.6	32.4	29.2	29.1	29.3	0.5	0.67	0.001	0.43
PI d 7 to 14	49.5	48.5	48.0	46.8	44.6	45.2	0.9	0.14	0.001	0.78
G:F, g/kg										
PI d 1 to 7	860	859	836	812	804	795	15	0.37	0.001	0.91
PI d 7 to 14[6]	647	670	662	659	681	645	11	0.08	0.81	0.36

[1] Least square means of 8 replicates of 5 chicks per treatment.
[2] Chicks were challenged with a single dose of distilled water (Sham) or 10^6 sporulated oocysts of *Eimeria acervulina* (EA).
[3] Effect of DDGS inclusion in diet (Diet), Sham vs. EA challenge (Ch), and Diet × Ch interaction. The linear and quadratic effects of Diet were tested.
[4] Inoculation occurred on d 10 of age, considered post-inoculation (PI) d 0.
[5] Diet linear effect ($P < 0.05$).
[6] Diet quadratic effect ($P < 0.05$).

Table 6.3. Fecal oocysts (10^5 oocysts per mL) in chicks challenged with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS) [1,2]

	DDGS in diet				
PI day[3]	0%	10%	20%	Effect	*P*-value[4]
5	16.3	13.4	16.9	Time	0.001
6	19.9	23.1	21.0	Diet	0.88
7	6.8	7.2	8.1	T × D	0.98
8	2.3	2.5	1.3		
9	0.8	1.1	1.1		
10	0.5	0.5	1.2		
12	0.1	0.3	0.2		
14	0.0	0.0	0.0		

[1] Least square means of 8 replicates of 5 chicks per treatment.
[2] All chicks were challenged with a single dose of 10^6 sporulated oocysts of *Eimeria acervulina*.
[3] Inoculation occurred on d 10 of age, considered post-inoculation (PI) d 0.
[4] Analysis of repeated measures in time (Time) to test the effect of dietary DDGS (Diet) and Time × Diet interaction (T × D). Pooled SEM of T × D was 1.65.

Table 6.4. Digesta pH and viscera weight of young chicks challenged or not with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS) [1]

	Sham[2]			EA[2]				*P*-value[3]		
	Concentration of DDGS in the diet									Diet
Response[4]	0%	10%	20%	0%	10%	20%	SEM	Diet	Ch	× Ch
Digesta pH										
Ileum[5]	6.72	7.40	7.17	7.02	7.10	7.17	0.169	0.02	1.00	0.11
Cecum	5.58	5.68	5.42	5.57	5.83	5.68	0.119	0.19	0.18	0.54
Viscera wt, g										
Small int.[6]	16.8	18.0	19.0	19.7	18.9	18.9	0.44	0.32	0.001	0.01
Cecum	2.78	2.89	2.98	2.84	2.82	2.78	0.09	0.73	0.33	0.34

[1] Least square means of 8 replicates of 5 chicks per treatment.
[2] Chicks were challenged with a single dose of distilled water (Sham) or 10^6 sporulated oocysts of *Eimeria acervulina* (EA).
[3] Effect of DDGS inclusion in diet (Diet), Sham vs. EA challenge (Ch), and Diet × Ch interaction. The linear and quadratic effects of Diet were tested.
[4] Inoculation occurred on d 10 of age and samples were harvested on d 14 post-inoculation.
[5] Diet linear effect ($P < 0.05$).
[6] Small intestine. Diet linear × Ch interaction ($P < 0.01$).

Table 6.5. Changes in intestinal mucosa microbiota of young chicks challenged or not with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS)[1]

	Sham[2]			EA[2]				*P*-value[3]		
	Concentration of DDGS in the diet									Diet
Response[4]	0%	10%	20%	0%	10%	20%	SEM	Diet	Ch	× Ch
Number of DGGE bands[5]										
Ileum	17.9	21.1	23.4	22.4	25.4	22.0	2.68	0.47	0.27	0.47
Cecum[6]	27.8	36.4	27.4	24.9	31.1	25.5	1.71	0.001	0.02	0.60
Intra-treatment Cs value[7]										
Ileum[8]	73.9	67.8	77.7	78.0	73.5	68.2	2.60	0.12	0.99	0.01
Cecum[9]	82.6	90.6	79.9	80.9	83.0	76.3	1.57	0.001	0.001	0.17

[1] Least square means of 8 replicates of 5 chicks per treatment.
[2] Chicks were challenged with a single dose of distilled water (Sham) or 10^6 sporulated oocysts of *Eimeria acervulina* (EA).
[3] Effect of DDGS inclusion in diet (Diet), Sham vs. EA challenge (Ch), and Diet × Ch interaction. The linear and quadratic effects of Diet were tested.
[4] Inoculation occurred on d 10 of age and samples were harvested on d 14 post-inoculation.
[5] Number of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis (DGGE) bands to measure microbial diversity. Each band represents at least 1 bacterial species.
[6] Diet quadratic effect ($P < 0.001$).
[7] Sorenson's similarity (Cs) values to compare similarities of DGGE banding patterns (average number of bands in common) within (intra-) treatment. Each Cs value included 28 single comparisons between pairs of replicates within treatment.
[8] Diet linear × Ch interaction ($P < 0.01$).
[9] Diet quadratic effect ($P < 0.001$).

Table 6.6. Sorenson's similarity values to compare the similarity of ileal mucosa microbiota of young chicks challenged or not with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS)[1,2]

		Sham[3]			EA[3]		
	DDGS[3]	0%	10%	20%	0%	10%	20%
Sham[3]	0%	73.9					
	10%	71.2	67.8				
	20%	73.7	71.3	77.7			
EA[3]	0%	74.4	71.9	76.7	78.0		
	10%	67.6[a]	69.8	76.6	74.8	73.5	
	20%	71.0	67.3	74.1	74.8	70.9	68.2

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns, within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 28 single comparisons between pairs of replicates (cage with 5 chicks) within treatment. Each inter-treatment value included 64 single comparisons between pairs of replicates between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 2.60), and the numbers below those are inter-treatment values (SEM = 1.66).

[3] Chicks were challenged with a single dose of distilled water (Sham) or 10^6 sporulated oocysts of *Eimeria acervulina* (EA) and fed a diet with either 0, 10, or 20% DDGS. Inoculation occurred on d 10 of age and samples were harvested on d 14 post-inoculation.

Table 6.7. Sorenson's similarity values to compare the similarity of cecal mucosa microbiota of young chicks challenged or not with *Eimeria acervulina* and fed increasing concentrations of distillers dried grains with solubles (DDGS)[1,2]

		Sham[3]			EA[3]		
	DDGS[3]	0%	10%	20%	0%	10%	20%
Sham[3]	0%	82.6					
	10%	78.1[a]	90.6				
	20%	77.5[a]	77.4[a]	79.9			
EA[3]	0%	66.0[a]	54.7[a]	60.4[a]	80.9		
	10%	79.0[a]	83.7[a]	81.6	62.1[a]	83.0	
	20%	75.0[a]	76.0[a]	70.7[a]	52.5[a]	73.7[a]	76.3

[a] Differs ($P < 0.05$) from the average of its 2 corresponding intra-treatment values.

[1] Least square means of Sorenson's similarity values to compare similarities of V3-16S rDNA PCR-Denaturing Gradient Gel Electrophoresis banding patterns, within (intra-) treatment group and among (inter-) treatment groups. Each intra-treatment value included 28 single comparisons between pairs of replicates (cage with 5 chicks) within treatment. Each inter-treatment value included 64 single comparisons between pairs of replicates between treatments.

[2] Numbers at the top of each column are intra-treatment values (SEM = 1.57), and the numbers below those are inter-treatment values (SEM = 1.04).

[3] Chicks were challenged with a single dose of distilled water (Sham) or 10^6 sporulated oocysts of *Eimeria acervulina* (EA) and fed a diet with either 0, 10, or 20% DDGS. Inoculation occurred on d 10 of age and samples were harvested on d 14 post-inoculation.

CHAPTER VII
OVERALL DISCUSSION

The disease challenge model developed as a result of this research allowed evaluation of dietary effects during infection and recovery processes. In all of the experiments, dietary treatments did not prevent pigs from developing post-weaning colibacillosis (PWC) or chicks from developing subclinical coccidiosis. However, inclusion of insoluble dietary fiber (DF) from either corn distillers dried grains with solubles (DDGS) or cellulose in the diet of pigs hastened their recovery from PWC. Dietary DDGS delayed the shift in fecal coliforms from commensal to pathogenics during the infection process and hastened the drop in concentration of pathogenic coliforms during recovery. This effect was observed in 2 consecutive experiments and also was associated with a reduction in diarrhea during the recovery phase. The magnitude of these effects was proportional to the concentration of DDGS in the diet, up to 20% the dietary concentration. When using purified sources of DF, only the inclusion of insoluble DF expedited the recovery from PWC diarrhea. These observations agree with those of Mateos et al. (2006) and Kim et al. (2008) where the inclusion of insoluble DF from oat hulls ameliorated PWC diarrhea in pigs.

In the present study, inclusion of a purified source of soluble DF did not ameliorate PWC diarrhea. This observation agrees with previous reports showing that soluble DF is rapidly fermentable and promotes diarrhea due to colibacillosis and other enteric diseases (Pluske et al., 1996, 1998; McDonald et al., 1999; Hopwood et al., 2002, 2004). In contrast, others have observed that using a source of soluble DF that does not increase viscosity of the digesta is actually protective against PWC (Wellock et al., 2008) and swine dysentery (Thomsen et al., 2007). However, the source of soluble DF in those studies was inulin, which has a prebiotic effect (Flickinger et al., 2003; Waltz et al., 2005). Thus, the protective effect in these cases is more likely to happen because of the prebiotic effect of inulin, rather than to the fermentation of the fiber.

We observed that pigs fed a diet depleted of fiber showed less diarrhea. However, these pigs did not defecate daily and, therefore, less diarrhea was observed. The lack of feces was because of the high digestibility of the semi-purified diet based on cornstarch and casein. When those pigs defecated, they showed mild to severe diarrhea. Thus, we cannot conclude that the absence of DF is protective against PWC diarrhea. This observation seems to be in contrast with others who observed that feeding pigs with low DF diets based on rice ameliorates PWC diarrhea

(Pluske et al., 2003; Montagne et al., 2004). However, it is possible that the beneficial effect is not because of the low concentration of DF but rather to the rice *per se*, because rice has a direct effect in reducing diarrhea (Macleod et al., 1995) through a compound that has been called *the rice factor* (Mathews et al., 1999).

The mechanisms by which insoluble DF protects against PWC diarrhea are not fully understood. However, insoluble DF reduces viscosity (Dikeman et al., 2006) and transit time (Stanogias and Pearce, 1985) of digesta, promoting intestinal development (Pluske et al., 2003) which, in turn, may increase intestinal motility (Mateos et al., 2006). Also, insoluble DF increases the enterocyte turnover rate (Jin et al., 1994). The sum of these effects can help in the recovery from PWC in 2 ways. First, the *E. coli* attached to the enterocyte can be excreted faster. Second, the intestinal morphology may be more conducive to absorbing fluids, thus preventing clinical diarrhea and dehydration. In addition, Mateos et al. (2006) suggested that the increased motility and transit time of digesta caused by insoluble DF can reduce the availability of substrate to support bacterial growth. In general, our data on the digestive physiology and bacterial populations suggest that insoluble DF from both DDGS and cellulose may promote intestinal health. However, those data are limited to the particular time when outcomes were measured. Typically, these measures were performed at the time of recovery and some of them at the peak of the disease. We observed that the transition throughout health to disease and recovery is a very dynamic process and its progress is inherent to the individual. Thus, it is not surprising that the data recorded more frequently throughout the infection to recovery process, i.e., diarrhea score and fecal coliforms, were more sensitive to the dietary effects.

All the beneficial effects that we observed in both pigs and chicks were not reflected in better growth performance. It is very likely that the environmental conditions in which these experiments were conducted allowed a fast recovery from disease, leaving a small opportunity for the diet to show any effect on growth performance. In typical conditions of pig production, PWC stays longer in the herd because pigs are constantly reinfecting each other. In such conditions, the effect of insoluble DF on the recovery from disease is more likely to affect growth performance. Although chicks were housed in groups of 5 within a pen, the use of batteries prevented contact with their own feces. Thus, the beneficial effects of DF on growth performance are more likely to be observed in broilers that are grown in floor pens.

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