Dietary Influence on the Challenged Gut

Audrey McElroy, PhD
Professor and Extension Specialist
Department of Poultry Science
Texas A&M University, College Station, TX

Take-Home Message

Intestinal responses to enteric pathogens, the host-pathogen interaction, and the ability to minimize the impact of these on growth and performance in commercial poultry are important areas of research. The intestinal environment, including the mucosal structure and function, is influenced by many factors including dietary ingredients, non-antibiotic alternatives, dietary composition, and diet quality among others. Intestinal integrity is based on the system functioning properly for digestion, absorption, secretion, and immunity. Subsequently, when disruption of this system occurs, major consequences in terms of bird health and growth follow. The poultry industry is increasingly in need of non-antibiotic alternatives to improve gut health or decrease enteric pathogen impacts in commercial broilers. Options being investigated include among many, enzymes, and prebiotics and probiotics. Additionally, there may be numerous opportunities to preserve a healthy intestinal environment by alterations in existing nutritional strategies including adjustment in levels of amino acids, dietary minerals, or protein. While for years dietary enzymes have been used to improve nutrient availability and bird performance, less research has been done to investigate modulation of intestinal health and gut integrity by dietary enzymes to improve intestinal response during a disease challenge in relation to its ability to function optimally for immune defense, digestion, absorption, secretion and transport. Dietary nutrient levels may also play a pivotal role in intestinal health during enteric disease challenges, and altering of levels could influence the response to and severity of the disease. Lastly, the usage of dietary supplements, including probiotics, can alter the host response to intestinal challenges depending on the timing of application, the specific challenge, and other factors in the intestinal environment. This is a complex area of research as the response to enteric disease involves many other factors influencing the dynamic intestinal environment including dietary ingredients and form, pathogen challenge, availability of nutrients for both the host and microbial population, and inclusion of other dietary supplements.

Introduction

Intestinal integrity is based on the system functioning properly for digestion, absorption, secretion, and immunity. Subsequently, when disruption of this system occurs, major consequences in terms of bird health and growth follow. In poultry, the gastrointestinal tract accounts for twenty percent of the energy expenditure of the whole body (Choct, 2009). This energy requirement is variable and dependent on numerous factors influencing the highly dynamic nature of the intestinal environment. Maintaining intestinal integrity is a daily challenge in today's poultry industry, and it will likely become even more challenging with increasing environmental considerations, changing feed ingredients, and reduced medication usage. These increasing constraints demand the industry continually seek new opportunities or reconsider adopted strategies for maintaining a healthy and optimally functional intestinal mucosa.

Common Intestinal Challenges for Disruption of Intestinal Integrity: *Eimeria* and Necrotic Enteritis

If homeostasis in the intestinal bacterial community in commercial poultry is disrupted, over proliferation of indigenous bacteria can cause diseases, such as necrotic enteritis (NE) (Van Immerseel et al., 2004). *C. perfringens,* the bacteria responsible for NE, are naturally occurring in the intestines of chickens; however, its presence alone is not a determining factor for disease development. In situations of impaired intestinal function, a shift in commensal intestinal microflora is thought to result in rapid proliferation of *C. perfringens* bacteria, which in turn produce extracellular toxins that damage the intestinal wall and lead to development of necrotic lesions. When intestinal conditions are not favorable, even highly virulent *C. perfringens* strains fail to produce disease. However, there are some intestinal physiopathological circumstances that favor the development of NE, such as intestinal stasis, crude protein level and source (Drew et al., 2004; Palliyeguru et al., 2010), changes in gastrointestinal pH, and damage to the intestinal mucosa such as occurs with coccidiosis (Williams, 2005; Cooper and Songer, 2009; Lee et al., 2011).

One of the precursors to NE occurrence can be infection by *Eimeria* resulting in coccidiosis. Available options for control of coccidia in the commercial poultry industry include dietary anticoccidial drugs, vaccination, or natural (non-antibiotic) dietary products. Historically, dietary anticoccidials have provided effective strategies of control; however, *Eimeria* has the ability to become resistant to these products and efficacy declines. As a result of this development of resistance, alternative strategies are being sought for management and prevention of the intestinal damage from this disease. Vaccination is one option for treatment, but there can be challenges associated with this approach. In order to generate complete immunity from vaccination, the vaccine must contain live oocysts to induce infection. The infection then results in intestinal damage and potential impacts on performance prior to establishment of immunity. Evaluating strategies to preserve intestinal integrity during the response to vaccination are critical to optimal vaccine usage.

Both coccidiosis and NE, independently or in combination, result in mild to extensive damage to the intestinal mucosa and intestinal function. Sub-clinical cases of these diseases may be as detrimental to commercial poultry production as more severe occurrences due to daily losses in bird performance that aren't attributed to a particular cause. With more producers considering alternatives to the use of in feed antibiotics, controlling these two diseases are central to the maintenance of intestinal integrity.

Dietary Approaches to Maintenance of Intestinal Health

Research on the daily contribution of management factors to intestinal integrity and the host-pathogen interaction with specific diseases is necessary to decrease their impact on bird productivity and industry profits. The poultry industry is increasingly in need of non-antibiotic alternatives to improve gut health or decrease enteric pathogen impacts in commercial broilers. Options being investigated include among many, enzymes, and prebiotics and probiotics. Additionally, there may be numerous opportunities to preserve a healthy intestinal environment by alterations in existing nutritional strategies including adjustment in levels of amino acids, dietary minerals, or protein.

Exogenous enzymes are added to poultry feed with the objective to improve nutrient availability and digestibility, which ultimately results in improvements in bird performance. The mechanisms by which these enzymes improve bird performance have been extensively researched in healthy birds. However, limited literature is available on the use of exogenous enzymes,

including widely used phytases, during intestinal disease challenges. Enzymes can influence availability of nutrients for both the host and microbial population with performance and/or intestinal health alterations, and the response elicited could be different in a compromised intestinal environment as compared to a healthy one.

With the widespread commercial usage of phytase and its known effects on availability of nutrients, our laboratory has been interested in the effect of phytase during *Eimeria* and/or NE infections. Initially, the supplementation of diets with phytases targeted improvements in P availability and digestibility by its action on phytate (Selle et al., 2009). However, research has shown that phytase supplementation also has a significant impact on the availability of other nutrients, such as proteins and amino acids, minerals, and carbohydrates (Cowieson et al., 2009). Among the minerals greatly impacted by phytate and phytase supplementation is Ca. Calcium solubility in the intestine is closely related to Ca level in the diet, small intestinal pH (around 6.0), phytate concentration, and phytase activity (Cowieson et al., 2009). In the intestinal lumen, Ca must be soluble in order to be absorbed. Phytate can precipitate with Ca, particularly at conditions within the small intestine, creating insoluble Ca-phytate complexes (Selle et al., 2009). When pH is close to neutrality, phytate forms mineral chelates that are highly insoluble (Sebastian et al., 1996; Tamim et al., 2004; Selle et al., 2009) and maximum insolubility of phytate-mineral chelates occurs between pH 4 and 7 (Wise and Gilburt, 1981).

In order to minimize the detrimental effects of phytate, phytases have been used by poultry nutritionists since 1991 to formulate diets for broilers (Bedford, 2003). As phytase hydrolyzes phytate, it increases nutrient availability and digestibility, which results in improved bird performance (Cowieson et al., 2006). Several authors have reported that elevated levels of Ca in the diet decreased phytase efficacy (Applegate et al., 2003; Tamim et al., 2004; Plumstead et al., 2008). Applegate and collaborators (2003) reported that a dietary Ca level commonly used in broiler diets (0.9%) resulted in a reduced intestinal phytase activity and apparent ileal phytate phosphorus hydrolysis compared with a lower level of Ca (0.4%). A high Ca to total P ratio appeared to exacerbate this effect, by increasing the formation of mineral-chelate complexes and decreasing phytase activity (Qian et al., 1997; Tamim et al., 2004). In a study by Sebastian et al. (1996), the optimal growth performance and retention of P and Ca was achieved at the lowest level of dietary Ca tested (0.6% total Ca). Similar performance benefits were observed by several other authors (Tamim et al., 2004; Powell et al., 2011). A healthy intestine is able to digest and absorb nutrients released by phytase from the phytate molecule resulting in improvements in bird performance. However, when the gastrointestinal tract is colonized by enteric pathogens, damage to the intestinal lining impairs function of the intestinal mucosa and birds' utilization of nutrients, and these nutrients could potentially become available to enteric pathogens. Among the factors identified that influence the onset of NE including is high dietary Ca (Titball et al., 1999). The mechanism by which Ca favors *C. perfringens* is unknown, but it has been theorized that excess Ca may increase intestinal pH (Selle et al., 2009; Walk et al., 2012) and favor *C. perfringens* growth in a more neutral environment (Wages and Opengart, 2003; Williams, 2005). Considering the improved performance with lower Ca levels and the proposed involvement of Ca in NE pathogenesis, we designed experiments to evaluate the effects of dietary Ca, P and phytase levels on broiler performance, gastrointestinal morphology, mineral digestibility and bone mineralization during a natural NE outbreak.

Research to understand risk factors, pathogenesis, prevention and control of NE has been hindered by the necessity to "induce" NE infection artificially through challenge models, often with manipulations of diet and other factors, which make results of limited applicability to the industry. For the past 5 years, our laboratory has been able evaluate naturally occurring NE, without dietary or challenge manipulations, in our bird rearing facilities. Broilers with no administered challenge or dietary manipulation have a *C. perfringens* associated natural

outbreak of NE with mortality ranging from 10 to 40%. The NE is repeatable and consistent between pens and timing of appearance in the flock. We have focused our research on this natural NE occurrence to evaluate dietary factors in relation to the intestine's ability to function optimally for immune defense, digestion, and transport. In repeated trials we have evaluated the effects of dietary levels of Ca (0.60% and 0.90%), Ca sources (limestone or a highly soluble source of calcified seaweed), and 2 levels of an *E. coli* phytase (0 and 1,000 FTU/Kg) on the development and pathogenesis of naturally occurring NE (Paiva et al., 2013). In our research, feeding lower levels of Ca (0.6% compared to 0.9%) in the diet resulted in improvements in bird performance and mineral digestibility, as shown in previous publications (Sebastian et al., 1996; Selle et al., 2009). Interestingly, mortality was significantly higher when broilers were fed 0.9% Ca diets (20% mortality) compared to 0.6% Ca(5% mortality) with the highly soluble calcified seaweed source, while no differences in mortality occurred between birds fed 0.9% (11% mortality) or 0.6% Ca (8% mortality) from limestone. Given that the main difference in these Ca sources is solubility, we theorize that the involvement of Ca with NE pathogenesis is related to either the available soluble Ca or undigested Ca in the intestine. Although the pathogenesis of NE is not completely elucidated, it is believed that the toxins produced by *C. perfringens* are responsible for lesion production. Researchers suggest that the most important toxins in the pathogenesis of NE are NetB and alpha-toxin, and both of these have been shown to have Ca dependent modes of action. NetB forms pores in the cellular membrane causing an influx of ions (Ca, Na, Cl, etc.) that eventually lead to osmotic cell lysis (Keyburn et al., 2010). In addition to osmotic lysis, another mechanism involving Ca has been suggested that would induce a special type of cell death. Kennedy et al. (2009) reported that the alpha-toxin of *C. septicum* (pore-forming toxin) forms Ca permeable pores, which increase intracellular Ca. It was believed that this Ca influx induced a cascade of events consistent with programmed necrosis since it was associated with calpain activation and release of cathepsins from lysosomes. Evidence from our research involving a natural occurrence of NE, suggests that the presence of greater amounts of soluble Ca in the intestine with 0.9% dietary Ca compared to 0.6% Ca significantly aggravated the enteritis caused by *C. perfringens*. Furthermore, a more soluble Ca source favored the incidence and severity of NE. Collectively, the results support the involvement of Ca in the development and pathogenesis of a naturally occurring NE episode and indicate the need for further research to evaluate Ca requirements in growing broilers, particularly during this enteric disease.

Maintaining a Community of Beneficial Bacteria

The intestines of poultry house a diverse community of bacteria, which are important for growth performance and protection against pathogenic bacteria (van der Wielen et al., 2002). The gastrointestinal system is not only the major site for nutrient digestion and absorption, but also works as the largest immunological organ in the animal, protecting the host against pathogens. Bacteria in the intestine form a natural defense barrier and exert numerous protective, structural, and metabolic effects on the epithelium. Feed additives, such as enzymes, probiotics and prebiotics, can alter intestinal microbiota and ultimately impact bird performance and animals' susceptibility to diseases.

The obesity epidemic in humans has lead researchers to focus on the microbe gut interaction. It has been shown that in obese individuals the gut microflora is different than in normal weight individuals (Ley et al., 2005), obese mice have significantly lower amounts energy remaining in their feces than lean individuals (Turnbaugh et al., 2006). This research indicates that intestinal microbial populations may play a role in body condition and performance. While these studies have not been replicated in poultry, studies have shown that bacteria increase energy utilization from the diet (Muramatsu et al., 1991; 1994). Intestinal development is not the only thing that

benefits from early establishment of bacteria as immune function and protection of intestinal integrity is upregulated as well (Kelly et al., 2007).

Yeast products can promote the growth of beneficial bacteria in the intestine, which ultimately can enhance gut health and the immune response (Sauerwein et al., 2007). Most of these products are derived from the cell wall (CW) of yeast, and three of the most commonly used products are the whole yeast CW, mannanoligosaccharides (mannans or MOS), and ß-glucans. Products derived from CW have been shown to have a number of effects, such as improved feed conversion, in commercial poultry (Santin et al., 2001; Zhang et al., 2005; Gao et al., 2008). There have been differing results regarding the effect of CW on BW with no significant differences seen in some studies (Zhang et al., 2005; Gao et al., 2008) but improvement in others (Santin et al., 2001). These discrepancies may be due to differences in the intestinal environment, disease exposure, and therefore, potential benefit from CW products. Mannans, like CW, alter the gut microflora in poultry (Yang et al., 2008). Pathogenic bacteria modify the energy utilization of the birds making it less efficient and thus reducing growth (Muramatsu, et al., 1994). Mannans may help to offset energy loss by binding to pathogenic bacteria, thereby preventing their binding to host cells and reducing the need for the immune system to respond to them. β-glucans are another component of CW that can improve bird performance as well as the immune response to pathogenic bacteria. Chae et al. (2006) found that β-glucans improved BW and increased immune cell counts in poultry. β-glucans can also alleviate the negative response to enteric diseases. In *E. coli*-infected birds, β-glucans improved feed conversion and overall BW (Huff et al., 2006).

Our group has conducted a number of studies to investigate the effect of yeast derived products on intestinal integrity in broilers and turkeys during *Eimeria* infections. A series of trials were performed with various yeast derivatives in commercial broilers with the objectives to 1) evaluate the effect of feeding cecal droppings collected from heavy (HW) or low weight (LW) broilers on performance and 2) to determine if dietary supplementation with yeast derivatives would affect growth and gut morphology in broilers in both a challenge and non-challenge setting (VanWyhe et al., 2009a; VanWyhe et al., 2009b). Day-old Cobb 500 broiler chicks were given a standard commercial diet and raised to 28 d of age. At d 28, birds in the top 10% (HW) and bottom 10% (LW) of the population based on body weight (BW) were moved into battery cages, and cecal droppings were collected for a period of 72 h. Day-old Cobb 500 chicks were split into two groups and given access to the collected droppings from the HW or LW birds for a period of 48 h. After 48 h, chicks from each microflora treatment were weighed and placed in floor pens. Diets in included control, control+ 0.1% CW, and control +0.1% mannanoligo-saccharides. Birds fed the control diet had the lowest BW of all treatments on d 16 and d 28. There were no differences in performance due to microflora treatments nor were there any performance interactions between dietary and microflora treatments. There was a cecal droppings and diet interaction on intestinal morphology. The interaction was most evident in differences in villi height, and interactions varied across age and intestinal section. The results suggested that in a non-challenge setting, early feeding of dietary yeast products affected intestinal morphology and bird performance and early feeding of microflora from HW and LW broilers effected intestinal morphology; however, consistent beneficial responses were not seen with either treatment. In another trial, we evaluated the intestinal response of broilers to hydrolyzed yeast cell wall (CW) products during exposure to an environmental coccidial challenge. Day-old Cobb 500 (n=3024) chicks were placed in floor pens on litter seeded with coccidia and fed 1 of 9 diets. Dietary treatments included 1) control (C); 2) cell wall (C+0.1% CW); 3) mannanoligosaccharides (C+0.1% M); 4) glucans (C+0.1% G); 5) nucleotides (C+0.1% N; 6) peptides (C2 + 0.1% P); 7) M+G (C+0.1% M+0.1% G); 8) P+N (C2 + 0.1% P+0.1% N); and 9) all products (P+N+M+G, C2 + P+0.1% M+0.1% G+0.1% N). There were no significant differences between any of the diets for BW, BW gain, FI, feed conversion, or intestinal lesion

score. There were significant interactions of diet and age for VH, CD, and VCR measurements in all intestinal sections. The results suggested that these yeast products did effect intestinal growth or recovery during the exposure to coccidia and may be section specific or have a limited time of effectiveness.

A more specific study was conducted with the yeast derived β-glucan (Auxoferm YGT) to investigate the effects of this derivative on broiler chick performance, lesion scores, and immune-related gene expression (Cox et al., 2010). Broiler chicks were fed diets containing 0, 0.2 or 0.1% YGT and divided into either non-challenged or challenged replicates, which received a mixed *Eimeria* inoculum. Dietary supplementation had no effect on broiler performance, but gross lesion severity was reduced in birds supplemented with 0.1% YGT. Auxoferm YGT also altered immune-related gene expression profiles, favoring an enhanced T-helper type-1 response during the coccidia infection.

Our group has also evaluated the response of commercial turkeys to the inclusion of a *Saccharomyces cerevisiae* fermentation product, Original XPC™, in antibiotic free diets following coccidia vaccination (Paiva et al., 2010). The experiment was a 4 x 2 factorial design with 4 dietary treatments and two coccidia vaccination treatments (vaccinated and non-vaccinated). Vaccinated and non-vaccinated birds were given one of the four diets with different inclusion levels of XPC™ (no XPC™ = negative control; 0.0625% = 0.5X, 0.125% = 1X; 0.250 = 2X). XPC™ supplementation significantly increased BW and BWG during d 0 to 28 and d 0 to 42, but did not have statistically significant effects on BW and BWG during the cumulative d 0 to 63 trial. Stress from coccidia vaccination on d 0 likely made the benefit of XPC™ more obvious in the early growth periods while the turkeys responded immunologically to the vaccination and low level of coccidia infection to generate protective immunity. The mechanism by which XPC™ supplementation improved performance likely involves modulation of the immune response by improved cellular immune response and increased levels of antibodies (Gao et al., 2009; Gao et al., 2008), which eased the stress and severity of coccidia infection from the vaccination. It is expected that during the peak of infection with *Eimeria* villi would be shorter in infected birds (vaccinated) than in non-infected birds, but the opposite happened in birds supplemented in the diet with 0.0625% XPC™, with vaccinated birds having longer villi than non-vaccinated birds at both d 14 in the jejunum and d 28 in the duodenum. This would suggest that in the challenge environment, the 0.0625% level of XPC™ was beneficial to either protection of gut integrity and/or promotion of immune response to the infection. The only main effects of diet on intestinal morphology were observed at d 63 in the duodenum. Dietary XPC™ at 0.065% resulted in increased CD as compared to all other treatments and a smaller VH:CD ratio than observed with 0.25% XPC™. Interestingly, at d 14 and d 28 there were interactions between diet and vaccination, but at d 63 only effects of diet, which again suggests a different responsiveness when the birds are undergoing a response to an intestinal challenge.

Conclusion

The intestine is a dynamic environment that is continually in a state of maintenance and turn-over. The function of the intestine at any given point in time is a reflection of the integrity of the villus and crypt balance, enterocyte maturity, secretion type and abundance, pH, microbial community, and digestive and absorptive capability. Certainly diet and intestinal health contribute substantially to the integrity of intestinal function and ultimately determine the success and efficiency of broiler and turkey performance. With changing dietary influences and pathogen challenges in today's commercial industry and in the years ahead, alternative approaches and perhaps adjustments to standard adopted practices must be evaluated for identification of opportunities to maintain intestinal integrity and bird performance.

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Notes