

## **Dietary Factors that Affect Gut Health and Pathogen Colonization**

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The poultry industry has undergone remarkable change and growth over the last 30 years, and it will continue to expand in the coming years to meet higher demand for low-cost, healthy and convenient products. In addition to the expanding market, commercial poultry is being genetically selected for ever increasing growth performance and efficiency. This selection for increased growth rate has resulted in changes in gastrointestinal development during growth of the animal (Tottori *et al.*, 1997). Apparently, young poultry are more susceptible to enteric pathogens today than they were 30 years ago. These pathogens have been of concern to the poultry industry because of lost productivity, increased mortality, and contamination of poultry products for human consumption (Patterson and Burkholder, 2003).

Prophylactic and therapeutic uses of antibiotics have been the main tools used by the poultry industry to prevent or to treat disease due to enteric pathogens. However, the voluntary or legislated limits on the use of antibacterial feed additives for poultry is motivating poultry scientists to better understand the role of commensal gut microflora and how it can be modified or stabilized by various “natural” alternatives to antibiotics. Some of these natural alternatives include prebiotics, probiotics, enzymes, acidifiers, herbs, essential oils, and immunomodulators. Many enteric pathogens do not develop mechanisms of resistance to these natural dietary components and they have been shown to shift the intestinal microflora to more stable and healthier state (Lan *et al.*, 2005). Understanding the role of gut microorganisms in nutrition and health is vital for achieving future sustainability and for improving the efficiency and environmental acceptability of poultry production. The objective of this paper is to review nutritional strategies to modulate gut health and control pathogen colonization with emphasis on potential natural alternatives to antibiotics.

### **Intestinal Tract Ecosystem**

The major purpose of the digestive system is to assimilate nutrients required for energy, maintenance, growth, and reproduction. Digestion consists of a number of physical and chemical processes. Feed is ingested, broken down into smaller particles, macerated, mixed with digestive enzymes, and propelled through the digestive tract by the muscular activities of the tract. Salivary, gastric, pancreatic, biliary, and intestinal secretions collectively provide enzymes that aid in digestion, watery medium, and optimal pH required for digestion. Digestive enzymes hydrolyze carbohydrates, protein, and lipids into a limited number of much smaller compounds suitable for absorption. Mucus, secreted onto the mucosal lining, protects and lubricates the gastrointestinal tract. Microorganisms, indigenous to the digestive tract, can provide additional nutrients by breaking down structural carbohydrates that are not subject to digestion by endogenous enzymes and by synthesizing amino acids and vitamins essential to the host animal.

The gastrointestinal tract the main portal through which pathogens invade the body and cause disease; but, the animal has natural protective barriers to prevent this invasion. Indeed, there are more bacterial cells in the digestive tract of most animals than there are cells of the animal itself.

Pathogenic bacteria and viruses are discouraged from entering the body by physical barriers such as skin, intestinal mucosa, and intestinal flora. If pathogens breach these barriers, the immune system and its associated defenses mechanisms spring into action in order to minimize systemic infection. These natural defenses rely on precise physiological mechanisms that are genetically predetermined. However, the expression and efficacy of these mechanisms is dependent upon the presence of specific external elements, including dietary nutrients that meet the metabolic demands for maintenance and growth.

The intestinal ecosystem contains a very diverse community of microbial cells that influence the host in various ways. According to Lan et al. (2005), some intestinal microflora have beneficial effects on the host, such as: 1) promotion of gut maturation; 2) enhancement of gut integrity; 3) antagonisms against pathogens (competitive exclusion); and 4) immune modulation. The microflora also plays a significant role in maintaining intestinal immune homeostasis and prevent inflammation. The strongest determinant of the gut microbial ecosystem is the diet of the host. Apajalahti *et al.* (2001) surveyed the microbial community of broilers raised at eight commercial poultry farms in Finland and fed different commercial wheat-based diets, some with locally added whole wheat. This survey covered different seasons (spring and fall) and years (1997, 1998, and 2000). They found that diet was the strongest individual determinant of the total microbial community structure in the ceca of broiler chickens, whereas profiles of individual farms with identical feed regimes hardly differed from each other. There was also no significant variation of the colonic microbial community due to season or year. Therefore, it should be possible to shift the microbial community from pathogenic to beneficial bacteria by changing the dietary composition of ingredient (Gibson and Roberfroid, 1995; Collins and Gibson, 1999).

The balance in this ecosystem is also imposed by the animal host responses to control the bacterial proliferation in their intestines using several different physiological mechanisms. These mechanisms include chemical processes (e.g. acid and bile production), highly competitive rate of nutrient absorption, high passage rates of digesta that washes out free bacteria, continuous sloughing of the epithelial cells and mucus that washes out adhered bacteria, and secretion of immunoglobulin A. Any unbalance caused by changes in general immunity of the bird, temperature stress (heat or cold), diet composition, or any other inefficiency of the host to maintain low numbers of bacteria can cause a disbacteriosis and/or enteritis with lower absorption of nutrients by the host.

There are multiple causes for secondary enteritis. Some of the most common reasons are bad litter quality, poor hygiene and ventilation, and low quality of feed or high variability in its composition. Any of these factors or a combination of several of them can trigger a gut health problem. Coccidiosis is a common parasitism in poultry and one of the more common causes of enteric problems. *Coccidia* infection causes reduced weight gain and poor feed conversion efficiency, reduced feed and water intake, increased intestinal passage time, decreased digesta viscosity and nutrient digestion, villous atrophy, intestinal leakage of plasma proteins, and increased intestinal acidity (Williams, 2005).

Coccidial infection has been shown to increase broiler chicken susceptibility to necrotic enteritis, which suggests that the parasite changes the rules of bacterial competition in the gastrointestinal tract for the advantage of *Clostridium perfringens* (*Cl. perfringens*) (Van Immerseel et al., 2004; Williams, 2005). Apajalahti (2004) presented data that indicates that *Eimeria maxima* infection changes microbial fermentation in the gastrointestinal tract of broilers. Presumably, the damage to the mucosa following *Eimeria* challenge is incremental with time until the immune system

deals with the pathogen or the damage to the intestinal wall has been healed. Initial changes in gut environment chemistry would therefore be limited to the site of infection in the small intestine. As the *Eimeria* challenge reaches peak, sloughed cells and undigested feed would reach the caeca, which may explain why the short chain fatty acid levels in the caeca of the challenged and unchallenged birds appear to diverge after those of the ileum. Apalajahti (2004) suggested that under commercial conditions, the changes in MC caused by *E. maxima* infection are continuous and less acute, since oocyst recirculation and the host immune and physiological responses affect the gut environment. He also presented data that showed that MC shifts caused by *E. maxima* challenge are temporary. Oviedo et al.(2005a, b, c, d) have observed in several studies that vaccination against coccidia with viable oocysts by itself causes small changes on intestinal microflora, and that stresses and coccidia challenge result in drastic shifts in microbial communities that can be modulated with feed additives.

The responses to all feed additives including the growth promoting antibiotics are very variable. Extensive literature reviews (Thomke et al., 1998; Huyghebaert, 2003) have concluded that the average benefit of growth promotants is 3 to 4% with a range that goes from no benefits at all to 8% maximum.

## **Current Antibiotic Debate**

Antibiotics are natural metabolites of fungi that inhibit the growth of bacteria by altering certain properties of bacterial cellular metabolism resulting in impaired growth or death. Some antibiotics interfere with the building and maintenance of the cell wall, while others interrupt proper protein translation at the ribosomal level (Ferket, 2003). Unfortunately, the long term and extensive use of antibiotics for medical and veterinary purposes have resulted in selection of resistant bacterial strains, and genes encoding for this resistance have been also transferred to other formerly susceptible bacteria, thus posing a threat to both animal and human health (Montagne *et al.*, 2003). The unifying concept of resistance is that the microorganism under stress will sense a deteriorating environment and undergo a programmed molecular response by which specific, stress-inducible proteins are synthesized. These proteins presumably act to prevent or repair macromolecular damage caused by the stress. Some stress proteins are induced by various stress conditions, while others are induced in response to a specific stress.

Antibiotic usage by the food animal industry has come under increasing scrutiny by some scientists, consumers, and government regulators because of potential development of antibiotic-resistant bacteria, including pathogenic strains. Controlling gram-negative bacteria, like *E. coli* and *Salmonella* spp. have generated the strongest objection to antibiotic use (Gustafson and Bowen, 1997). For example, Nayak and Kenney (2002) showed that 25% of the *Salmonella* isolates from turkey flocks in West Virginia were resistant to one or more antibiotics, including gentamicin, spectinomycin, streptomycin, tetracycline, tobramycin, and sulfamethoxazole. Consequently, some countries have banned (Sweden started on January 1986) or limited (European Union started on January 2000 and total withdrawal will start on January 2006) the general use of antibiotics as growth promotants in livestock feeds. As a result of this ban on antibiotic growth promotants is a rise in the incidence of colibacillosis and necrotic enteritis caused by *Clostridium perfringens* in poultry (Truscott and Al-Sheikhly, 1997; Ferket, 2003). Prohibiting antibiotic use has caused a decrease in performance, nutrient uptake, gut health, and economical losses in livestock production. Lastly, the antibiotic ban has increased the susceptibility of foodborne pathogen colonization in the poultry intestine and contamination of poultry products for human consumption.

## Nutritional Strategies to Modulate Gut Health and Pathogen Colonization

Several strategies have been proposed as a means to manage gut health. Effective use of feed additives to manage gut health is dependent upon some degree of understanding of their mechanisms of action (Figure 1). Growth-promoting antibiotics work in part by decreasing the microbial load in the gut, resulting in a reduction in energy and protein required to maintain and nourish the intestinal tissues; thus, more nutrients are partitioning toward growth and production. In contrast, most natural feed additives do not reduce overall microbial loads. Instead, they alter the gut microflora profile by limiting the colonization of unfavorable bacteria, which promote the activity or growth of more favorable species. These natural feed additives promote gut health by several possible mechanisms: altering gut pH; maintaining protective gut mucins; selection for beneficial intestinal organisms or against pathogens; enhancing fermentation acids; enhancing nutrient uptake; and increasing the humoral immune response.

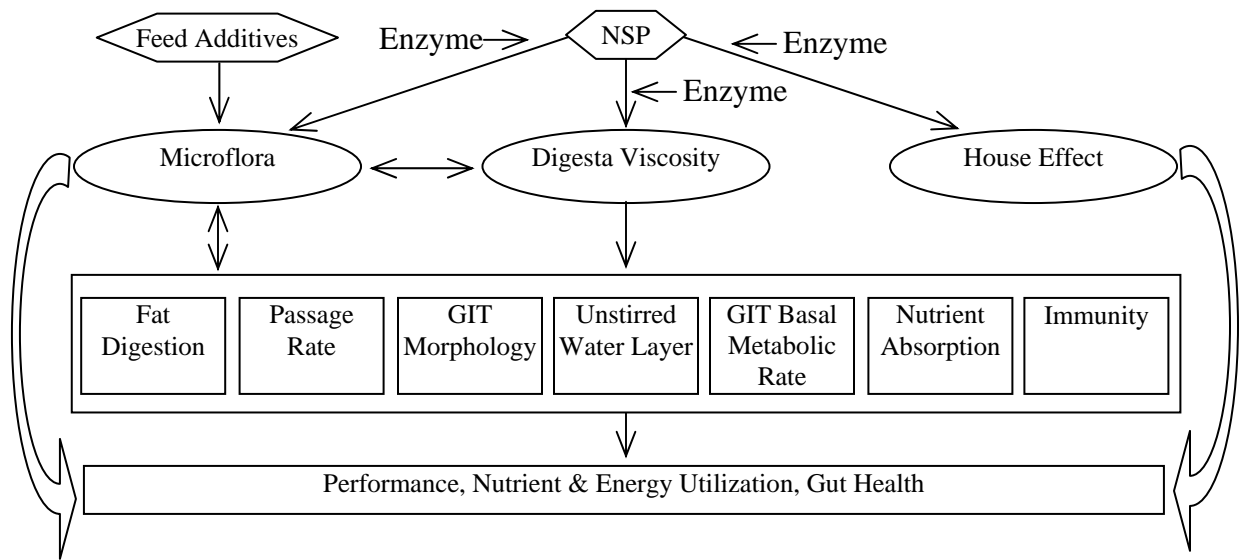


FIGURE 1: Flowchart of the modes of action of feed additives, non-starch polysaccharides, and enzyme supplementation.

## Enzyme Supplementation

Supplemental enzymes have become common additives to poultry and swine feeds to improve nutrient utilization and production performance characteristics. Enzymes are organic catalysts that initiate or accelerate reactions occurring in organic matter that would not otherwise proceed at an appreciable rate (Schaible, 1970). Even the simplest living organisms contain multiple copies of nearly a thousand different enzymes. With the development of enzyme products targeting specific substrates, the use of enzymes to improve the nutritional value of feed has received increased attention. Since the 1920s, researchers have observed beneficial effects from enzyme supplementation in poultry feeds, particularly feeds that contain grains with a high fiber component (Hastings, 1946; Moran Jr. and McGinnis, 1968; Pettersson and Aman, 1989; Santos *et al.*, 2004a). Supplemental enzymes in the feed are used to achieve one or all the following objectives: 1) increase the animal's own supply (Schaible, 1970); 2) alleviate the adverse effects

of antinutritional factors, such as arabinoxylans,  $\beta$ -glucans, etc; 3) render certain nutrients more available for absorption and enhance the energy value of feed ingredients (Classen and Bedford, 1991; Lyons, 1993); and 4) indirectly modify enteric microflora ecosystem. Consequently, enzyme supplementation improves performance parameters.

Commercial enzyme products are typically a blend of several different enzymes that are effective on a wide variety of substrates. The enzymes with proven efficacies for animal husbandry include xylanase, arabinoxylanase,  $\beta$ -glucanase, cellulase, phytase (Choct and Kocher, 2000), proteases (Odetallah *et al.*, 2003), and phospholipase (Santos *et al.*, 2004b). The following is a brief description of NSP-enzymes that has shown positive effects on gut health and pathogen control.

### ***Non-Starch Polysaccharide (NSP) Enzymes***

Today, pentosanase (NSP-enzymes) are used in virtually all poultry and swine diets comprised of mainly of small grains, such as wheat, barley, oats or triticale. Supplementing cereal-based diets with microbial enzyme preparations capable of hydrolyzing endosperm cell walls, may improve dietary nutrient availability by several means. Endoxylanase for example, degrades the xylan backbone of arabinoxylan into smaller units, which has several beneficial consequences. It renders the xylose units more available to monogastrics (Odetallah, 2000). It disrupts the water holding capacity of the NSP (Scott and Boldaji, 1997) and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct *et al.*, 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes enabling the bird to digest and absorb more nutrients (Pawlik *et al.*, 1990). Endoxylanase releases entrapped nutrients for the digestion by the endogenous enzymes of the bird (Chesson, 2000). Endoxylanase inhibits the proliferation of the fermentative microorganisms in the small intestine by increasing the digesta passage rate and nutrient digestion (Choct *et al.*, 1999). Thus, nutrient utilization is improved by reducing the competition between the host and its enteric microflora (Santos *et al.*, 2005).

Several studies about the effects of NSP and NSP-enzymes on bacterial community have been carried in our laboratory. We recently showed that higher concentration of plant cell polysaccharides in the diet shifted the intestinal microflora of poult to a healthier state through decreased cecal *Salmonella* spp. population, improved animal performance (Santos *et al.*, 2005) and improved gut morphology (data not published yet). In addition, we showed that turkeys fed diets high in plant cell wall polysaccharides supplemented with a blend of xylanase, protease and amylase enzymes had lower cecal *Salmonella* spp. population than turkeys on the same diet without enzyme supplementation (Santos *et al.*, 2005). NSP-enzymes hydrolyze the polysaccharides to oligosaccharides. Polysaccharides are not selectively fermented and persist through the distal colon, although symbiotic bacteria in the ceca can ferment oligosaccharides formed from the hydrolysis of NSP. In agreement with the literature, most of the commercial prebiotics have rather low molecular weight and they are generally fermented in the proximal colon (Rastall and Maitin, 2002).

Cecal symbiotic bacteria have important and specific metabolic, trophic and protective functions. Metabolic functions include fermentation of non-digestible dietary residue and endogenous mucus, which is important for the recovery of energy as short-chain fatty acids (SCFA), production of vitamin K, and absorption of ions. Symbiotic bacteria influences epithelial cell proliferation and differentiation due to their production of SCFA, and they also positively influence the development and homeostasis of the immune system. Symbiotic microflora also protects the host against pathogens through a variety of mechanisms. The attachment of non-

pathogenic bacteria to the brush border of intestinal epithelial cells can prevent the attachment and subsequent entry of pathogens. Symbiotic bacteria also competitively exclude pathogenic bacteria by competing for available nutrients, by producing bactericins, or maintaining their habitat by consuming resources of the gut and secreting compounds that are inhibitory to pathogens. Therefore, oligosaccharides formed from the breakdown of NSP molecules by enzymes discourages the colonization of enteric pathogens by increasing the resistance of the resident symbiotic microflora.

Because supplemental enzymes mediate their beneficial effects primarily by enhancing feed digestibility and nutrient availability to the host, it must be assumed that they also influence the gut microbial ecosystem by limiting substrate for the resident flora. Rapid digestion and absorption of starch, protein and fat from the small intestine effectively limits available substrate for the resident flora. Because enzyme supplementation reduces the microbial population in the small intestine (Choct *et al.*, 1995; Dunn, 1996), the entire gut ecosystem changes. These conditions in the gut alter the composition and activity of intestinal microflora (Vukic-Vranjes and Wenk, 1996). As dietary enzyme supplementation changes the gut microflora, there is a decrease in the adverse effects associated with microbial fermentation, such as 1) deconjugation of bile salts reducing fat digestion (Langhout, 1999); 2) competition between the host and the microflora for nutrients (Bedford, 1995; Choct *et al.*, 1996; Langhout *et al.*, 2000); and 3) atrophy of the intestinal villi and enlargement of digestive organs (Brenes *et al.*, 1993a,b; Viveiros *et al.*, 1994). Dietary enzyme supplementation has been shown to significantly reduce enterobacteria and gram-positive cocci in the small intestine of chicken, while counts of lactic acid bacteria increase in the same animal (Vahjen *et al.*, 1998).

Commercial NSP enzymes products are typically a blend of several different enzymes. For example, Allzyme Vegpro<sup>TM</sup> (Alltech Biotechnology, Inc.) is a solid-state fermentation product that contains a natural blend of enzymes, including protease, cellulase, pentosanase, alphagalactosidase, and amylase. Kumprencht *et al.* (1998) evaluated the performance of Ross broiler chicks fed diets containing Allzyme Vegpro<sup>TM</sup> at rates of 0, 325, 650, 1000, 1350 or 1650 g/ton of complete diet. After 42 days, birds supplemented with Allzyme Vegpro<sup>TM</sup> had increased body weight and improved feed conversion. It was determined that the optimum level of Allzyme Vegpro<sup>TM</sup> addition was 1000 g/tonne. These results were similar to findings reported by Prabhakar and Darur (1998) in India when feeding laying hens diets receiving 0 or 1 kg of Allzyme Vegpro<sup>TM</sup> per tonne of feed. Recently in Brazil, Teixeira *et al.* (2001) conducted a trial to evaluate the potential of using large amounts of soya with added Allzyme Vegpro<sup>TM</sup> in pre-starter pig diets. Allzyme Vegpro<sup>TM</sup> (4 to 6 kg/tonne) increased weight and feed consumption and improved the feed conversion ratio (FCR) of piglets at 28 days of age. Similarly, Fibrozyme, another enzyme preparation produced by Alltech Biotechnology Inc. has shown positive results in animal performance. Fibrozyme contains xylanase and cellulase, which has been shown to improve growth performance and carcass characteristics of pigs (Landblom *et al.*, 2005) and steers (Gomez *et al.*, 2003).

Taking in consideration that enzymes can modify the intestinal microflora to more favorable populations for the host growth, Parker *et al.* (2004) evaluated the utilization of a combination of amylase, protease, and xylanase designed for corn-soybean meal diets (Avizyme<sup>®</sup> 1502) as a feed additive to improve the responses of broilers vaccinated against coccidia in broiler starter diets with different levels of crude protein (CP). The normal variations in proportions of corn and soybean necessary to obtain three dietary protein levels were used to observe the efficacy of the enzyme under different substrates. There were 12 treatments in a 3 x 3 factorial, plus 3 negative

controls (No additives-No challenge) within each CP level were distributed. The CP levels (19, 21, 23%) and the anticoccidial control programs (Cocci-Vaccine=CV, Antibiotic + Ionophore, and Cocci-vaccine+Enzyme=CV+E) were evaluated as main effects. The chickens in the CV and CV+E groups were vaccinated against coccidia at 1 d of age with Advent<sup>®</sup>. All chickens, except those in negative control treatments were gavaged at 17 d with *E. acervulina*, *E. maxima*, and *E. tenella*. The enzyme product improved BW in vaccinated birds at 19 and 21% crude protein diets, but not at 23% CP. Lesion scores were affected ( $P<0.05$ ) by anticoccidial programs. Vaccinated chickens fed diets with enzyme had the lowest lesion scores in the caecum, while the antibiotic+ionophore group had the lowest lesion scores in midgut.

Coccidia challenge reduced the ileal digestibility of CP and amino acid (Oviedo et al., 2005f) by 7%. The enzyme addition did not improve significantly the ileal digestibility of coccidian vaccinated broilers. The modifications in microbial communities were measured by %G+C profiles and IgA concentrations were measured with ELISA (Oviedo et al., 2005a). Microbial responses were dependent on dietary CP level. The Coccidia challenge alone had very small effect on microbial numbers in the ileum, but suppressed IgA production. The addition of enzyme increased ileal microbial numbers at the lower CP level. In the caecum, the coccidia challenge reduced microbial numbers when compared with the non-challenged controls. CV+E resulted in microbial numbers and %G+C profiles similar to the control; especially at 19 and 21% CP.

These results indicated that the beneficial effects of this complex enzyme combination over BWG (5.6 and 17.0 %) and FCR (4.9 and 8.6%) 7 days after mixed coccidia challenge, in diets containing 18.5 and 20.1% of CP, respectively, might be due to changes in carbohydrate substrates available to microflora communities in the ceca. This has beneficial effects against *E. tenella*, and finally in live performance.

## Herbs, Spices and Essential Oils

Herbs, spices and essential oils (EO) have been used to make human foods more appetizing for centuries, and many of them are recognized for their health benefits. It is difficult to distinguish between them, because an EO is a mixture of fragrant, volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated (Oyen and Dung, 1999). The term 'essential' was adapted from the theory of 'quinta essentia' proposed by Paracelsus, who believed that this quintessence was the effective element in a medical preparation (Oyen and Dung, 1999). Because the term, 'essential oil' is a poorly defined concept from medieval pharmacy, the term 'volatile oil' has been proposed by Hay and Waterman, (1993) to be more appropriate. However, the name of 'essential oil' is still preferentially used. Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable. For example, the concentrations of two predominant components of thyme EO (i.e. thymol and carvacrol) have been reported to range from as low as 3% to as high as 60% of total EO (Lawrence and Reynolds, 1984). Cinnamaldehyde, a main component of cinnamon essential oil, amounts to approximately 60 to 75% of the total oil (Duke, 1986). Because of the large variation in composition, the biological effects (Schilcher, 1985; Janssen *et al.*, 1987; Deans and Waterman, 1993), if any, of EO may differ.

Essential oils have long been recognized for their anti-microbial activity (Lee *et al.*, 2004a), and they have gained much attention for their potential as alternatives to antibiotics. Lee and Ahn

(1998) found that cinnamaldehyde, derived from the cinnamon EO, strongly inhibits *Clostridium perfringens* and *Bacteroides fragilis* *in vitro* and moderately inhibits *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human. Also, a wide range of *in-vitro* anti-microbial activities of essential oils derived from cinnamon, thyme and oregano have been published (Deans and Ritchie, 1987; Lee *et al.*, 2004a). The exact anti-microbial mechanism of EO is poorly understood, it may be associated with their lipophilic property and chemical structure (Lee *et al.*, 2004a).

Helander *et al.* (1998) investigated how two isomeric phenols, carvacrol and thymol, and the phenylpropanoid, cinnamaldehyde, exert their antibacterial effects on *E. coli* O157 and *S. typhimurium*. Both carvacrol and thymol disintegrated the membrane of bacteria, leading to the release of membrane-associated materials from the cells to the external medium. Conversely, cinnamaldehyde exhibited its antibacterial activity due to its lipophilicity of terpenoids and phenylpropanoids, which can penetrate the membrane and reach the inner part of the cell and impair bacterial enzyme systems. Therefore, these plant-based have antimicrobial effects similar to antibiotic compounds produced by fungi. As with antibiotics, continued use of these plant-based antimicrobials may result in the development of resistance in some pathogenic bacteria. However, more research is necessary to confirm this risk. In addition, to be as effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural state, which may cause some economical disadvantage.

The EO supplementation has been tested in numerous studies with variable responses, depending upon the type of basal diet used, field or induced disease challenge, and other stress factors (Table 1). The most consistent positive response to EO blends has been observed under coccidia challenge. Oviedo *et al.* (2005d) evaluated the effects of two specific essential oil (EO) blends Crina<sup>®</sup> POULTRY (CP) and Crina<sup>®</sup> ALTERNATE (CA) on mixed *Eimeria* spp. infection in chickens vaccinated or not against coccidia with Advent<sup>®</sup>. The results 7 days after the mixed coccidia challenge with pathogenic viable oocysts showed that the non cocci vaccinated chickens fed the EO blend CA, and the cocci vaccinated chickens fed diets without feed additives had similar feed conversion ratios and body weight gains to the unmedicated-uninfected control broilers. The EO CP help to reduce coccidia lesion scores in duodenum and the EO CA reduced lesions in the caecum. The dietary supplementation of these EO blends did not improve any response in broilers vaccinated at first day of age.

The same treatments were evaluated (Oviedo *et al.*, 2005e) in a floor pen trial with used litter and previous *Eimeria* oocysts presence. Non-cocci-vaccinated chickens fed Crina<sup>®</sup> POULTRY had better feed conversion ratio (FCR) than the unmedicated control treatment in the starter period. The same EO improved FCR in coccidia vaccinated birds in the finisher period in comparison to the negative control group, but those responses were not significantly different from other treatments, or significant at 49 d of age. No significant differences were observed in coccidia lesion scores at 37 d. Diets supplemented with the growth promotant antibiotic (BMD) and the ionophore monensin supported consistently the best BWG and FCR in each period, and entire grow-out. No significant beneficial or deleterious effects on live performance were observed due to these specific EO blends in coccidia vaccinated broilers. It was concluded that these two specific EO blends differ in their efficacy to promote growth and the BMD+monensin combination was more effective on promoting better live performance.

**Table 1.** Literature review of the effects of specific essential oil blends on live performance, carcass traits and digesta parameters.

Essential oil composition	Diet, husbandry and challenge conditions	Effect of EO	Reference
Blend non specified	+ 20 mg/kg virginiamycin. Three experiment in cages	No effect on BW and FCR	Vogt, 1990, 1991
Crina <sup>®</sup> Poultry	Floor pens	No effect on BW and FCR	Ristic and Damme, 2001
Oregano (carvacrol – thymol)	Wheat-soybean meal basal diet – floor pens	No effect on BW and FCR	Botsoglou et al., 2002
Carvacrol and cinnamaldehyde combination		Negative effects as compared to the two fed individually	Lee et al., 2004c
EO blends		Improve antioxidant activity in carcasses	Dorman et al., 1995; Saricoban and Ozcan, 2004; Basmacioglu et al., 2004
Apacox <sup>®</sup>		No effect on BW and FCR, retarded lipid oxidation in meat	Botsoglou et al., 2004
Genex <sup>®</sup> (EO + organic acids) 500 – 1000 ppm		Growth promoting effect compared to flavomycin	Spais et al., 2002
Capsaicin, carvacrol, cinnamic aldehyde, 150 – 300 ppm	Wheat-barley diet	Improvement in BW (5.4, 8.1%) and feed conversion (3.1 and 7.1%). Reduced <i>E. coli</i> and <i>C. perfringens</i>	Jamroz et al., 2003
Crina <sup>®</sup> Poultry; or thymol and cinnamaldehyde	Maize-maize starch-soybean meal diet in wire floored cages	No effect of BW and FCR. Amilase activity improved in Crina <sup>®</sup>	Lee et al., 2003
Cinnamaldehyde	Diet with carboxymethyl cellulose, rye	Reverse negative effects of high viscosity diets	Lee et al., 2004 a, b
Blend from Turkey	Corn-soybean meal diets with wheat and sunflower meal in floor pens	Improved BW, FCR and carcass yield	Alçiçek et al., 2003
Crina <sup>®</sup> Poultry and combinations with antibiotics and lactic acid		Improve BW, FCR Combination with antibiotics or lactic acid did not show any effect	Suk et al., 2003
EO blend plus lactic acid		Improve BWG and increase digestive enzyme activities of the pancreas nad intestinal mucosa	Jang et al., 2004
Oregano (carvacrol and thymol)		Reduced feed intake and significantly improve feed efficiency	Halle et al., 2004
EO blend		Improve BWG, FCR and carcass yield	Alçiçek et al., 2004
RepaXol <sup>™</sup> (oregano, cinnamon, thyme, and capsicum) 100 ppm to 300 ppm.	Corn soybean meal diets in floor pens and cages from 1 to 42 d	No effect on BWG, FCR or FI. Improvement of final carcass yield and Feed efficiency at 14 d but not over the course of the experiment	Zhang et al., 2005

Crina <sup>®</sup> Poultry and Crina <sup>®</sup> Alternate	Corn soybean meal diets in floor from 1 to 49 d. Cocci-vaccinated birds	Improvement of FCR 13 d but not over the course of the experiment. No effect on cocci-vaccinated birds	Oviedo et al., 2005, e
Oregano (carvacrol and thymol)	Eimeria tenella infection - cages	Improve responses	Giannenas et al., 2003
Oregano (carvacrol and thymol)	Mixed Eimeria vaccination	Improvement in BWG and FI	Waldenstedt, 2003
Oregano (carvacrol, thymol)	Mixed Eimeria infection and induced necrotic enteritis	Improve BWG, lesion scores	Saini et al., 2003
Thymol, carvacrol, eugenol, curcumin, and piperin.	12 field trials to test Clostridium perfringens numbers	Reduced Clostridium perfringens colonization	Mitsh et al., 2004
Crina <sup>®</sup> Poultry and Crina <sup>®</sup> Alternate	Mixed Eimeria vaccination and infection	Crina Alternate but not Crina Poultry improved BWG, FCR and lesion scores post-infection. No effect on cocci-vaccinated birds	Oviedo et al., 2005, b, c, d,

Oviedo et al. (2005c, d) also measured the dynamics of intestinal microbial communities (MC) by DGGE and analyses of similarity coefficients (SC), in broilers vaccinated at first d of age with live oocysts of *Eimeria spp* and fed diets supplemented with the EO blends CP and CA. These broilers were challenged with pathogenic oocysts at 19 days of age. The dendrograms of amplicon patterns indicated MC differences due to intestinal location, feed additives, and cocci infection. The EO blends CP and CA did affect MC in all gut sections. The cocci-infection caused drastic MC population shifts in duodenal, ileal, and cecal sections (36.7, 55.4, and 36.2% SC, respectively). The CP supplemented birds had higher SC between pre- and post-challenge MC in duodenum and ileal (73.3, 81.8 %) than cocci-vaccinated chickens fed diets without feed additives (66.4, 66.5 %). However, cocci-vaccinated broilers had the smallest changes in cecal MC after challenge (79.5% SC). We concluded that cocci-vaccination causes small changes in intestinal MC, but challenge causes drastic shifts. The EO blend supplementation modulates MC in cocci-vaccinated broilers avoiding drastic shifts after a mixed challenge. The microbial ecology dynamics of the cecal compartment seems to be more related to the final broiler performance under conditions of stress.

## Acidifiers and organic acids

Acidifiers and organic acids have been used for decades in feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feeds (e.g. silages). Because organic acids have strong bacteriostatic effects, they have been used as salmonella-control agents in feed and water supplies for livestock and poultry. The most common organic acids in animal nutrition are citric acid, propionic acid, fumaric acid, lactic acid, formic acid and benzoic acid. Additionally, some other available acidifiers and organic acids have been shown to have some antimicrobial activity (Russell, 1992).

Experiments with pigs have shown that several organic acids, including citric acid, fumaric acid, formic acid, and propionic acid, have a positive influence on growth performance (Partanen and Mroz, 1999). It has been reported that the nutritive effect of organic acids is most pronounced in

weaning pigs (Gabert & Sauer, 1994; Roth & Kirchgessner, 1998), which often suffer from digestive disturbances resulting in diarrhea related to infections with *E. coli*. Problems at weaning may be triggered by an insufficient production of hydrochloric acid and digestive enzymes, and the feeding of a pre-starter diet with high protein content (Eidelsburger, 1997). Dietary acidification increases gastric proteolysis and protein and amino acid digestibility. The acid anion has been shown to complex with Ca, P, Mg and Zn, which improves the digestibility of these minerals. Furthermore, organic acids serve as substrates in the intermediary metabolism (Kirchgessner & Roth, 1988).

The use of organic acids has not gained as much attention in poultry production as it has in pig production, partly because limited positive responses in weight gain and feed conversion (Langhout, 2000). However, Vogt *et al.* (1982) reported a positive influence on either feed conversion ratio or growth performance by dietary supplementation of fumaric acid, propionic acid, sorbic acid and tartaric acid. Organic acids have mainly been used to sanitize the feed and reduce *Salmonella* colonization in poultry (Iba and Berchieri, 1995; Thompson and Hinton, 1997). An objective of dietary acidification is to inhibit of intestinal bacteria competing with the host for available nutrients, and reduce toxic bacterial metabolites (e.g. ammonia and amines).

The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu. The antibacterial activity increases with decreasing pH-value. Organic acids are lipid soluble in the undissociated form, and they easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. Once in the cell, the organic acid releases the proton H<sup>+</sup> in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism, inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to export the excess of protons H<sup>+</sup>, ultimately resulting death by starvation. In the same matter, the protons H<sup>+</sup> can denature bacterial acid sensitive proteins and DNA. Generally lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to organic acids than other bacterial species, such as *E. coli* and *Salmonella*. Lactic acid bacteria, like other gram-positive bacteria, have a high intracellular potassium concentration, which counteracts acid anions (Russell & Diez-Gonzalez, 1998).

As with antibiotics, continued use of acidifiers and organic acids may result in the development of resistance in some pathogenic bacteria. Inducible resistance (adaptation or tolerance) to acidic environments is recognized as an important survival strategy for many prokaryotic and eukaryotic microorganisms. In addition, different microorganisms have developed different acid survival strategies. Inducible acid resistance has been observed in many gram-negative and gram-positive microorganisms. Kwon and Ricke (1998) suggested that SCFA in the gastrointestinal tract of a host animal or in food materials might contribute to the enhancement of the virulence of *S. typhimurium* by increasing acid resistance. Although bacteria under *in vitro* conditions are known to adapt to acids, it is not known whether this also occurs in GIT of animals fed organic acids.

## Probiotics

A probiotic is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). *Lactobacillus* and *Bifidobacterium* species have been used most extensively in humans, whereas species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most common organisms used in livestock. Live yeast is a probiotic, but yeast cell-wall is prebiotic (discussed later). Probiotics have a similar mode of

action as prebiotics, because both increase the colonization of symbiotic bacteria at the lower gut. Probiotic microorganisms inhibit growth of potentially pathogenic microorganisms by: 1) lowering the pH through production of lactate, lactic acid and SCFA; 2) competing for gut lining attachment and available nutrients; 3) producing bactericins; and 4) stimulating the gut associated immune system. Thus, probiotics have been shown to improve performance, decrease mortality, and improve FCR of poultry and swine.

Most commercial probiotic products are composed of pure cultures of one or more microorganisms. Competitive exclusion products originating from adult intestinal microbiota are usually inoculated to 1-day-old chicks in order to control of *Salmonella* contamination. Combinations of prebiotics and probiotics are known as synbiotics (Patterson and Burkholder, 2003). Availability of prebiotics specifically targeting specific bacterial strains would enable the development of a symbiotic product blend. A symbiotic blend is especially important for strains of probiotic microorganisms with poor survival properties, and to enhance of the effect of the probiotic as a treatment of enteric disease.

Probiotics have several disadvantages in comparison to other modulators of enteric microflora. Relatively few species of microorganisms can be considered for use in probiotics products due to their limited knowledge of culturability. Probiotics have a short shelf-life and most are labile to excessive heat and pressure during feed processing. Some probiotic microorganisms may be reduced or eliminated by the low pH in the gizzard, and thus have little effect in the lower intestinal tract where pathogens pose problems. If a probiotic is added to the drinking water, the chlorine sanitizer may adversely affect its survivability. Acidification would be a better sanitizer than chlorine when delivering a probiotic via the drinking water. Coating technology has helped with some of these concerns, but more research is needed.

## Prebiotics

Prebiotics are dietary components that are not indigestible to the host, but are readily fermented by many bacteria, predominant those that produce SCFA. Prebiotics have several advantages over probiotics, where culture viability needs to be maintained. Many important commensal bacteria that are present in a “healthy gut” cannot be cultured, so they cannot be used in commercial probiotic products. However, dietary supplementation of prebiotics has been shown to stimulate these unculturable bacteria in humans (Rastall *et al.*, 2005), and pigs (Konstantinov *et al.*, 2003). Moreover, prebiotics have the advantage of being more stable to the heat and pressure incurred during feed processing. Prebiotics also have an economical advantage because some of the best prebiotics are derived from inexpensive food processing by-products (Playne and Crittenden, 1996).

Prebiotics have great potential to modulate colonic microflora and discourage the colonization of enteric pathogens. Any food or feed ingredient that enters the large intestine is a potential prebiotic, but it must be fermented by microorganisms that benefit the host to be an effective prebiotic (Lan *et al.*, 2005). Most current attention and successes have been derived using non-digestible oligosaccharides, especially those that contain fructose, xylose, galactose, glucose and mannose (Gibson and Roberfroid, 1995; Gibson, 1998). It has been reported that oligosaccharides and polysaccharides are preferentially utilizable by *Bifidobacteria* (Yazawa *et al.*, 1978). A prebiotic substrate is selectively utilized by symbiotic bacteria of the gut but not potential pathogens, such as toxin-producing clostridia, proteolytic bacterioides and toxigenic *E. coli*. In this manner, a “healthier” microflora composition is obtained whereby the

*Bifidobacterium* and/or *Lactobacillus* become predominant in the intestine and exert possible health-promoting effects.

The dominant prebiotics are fructooligosaccharide products (FOS, oligofructose, and inulin). However, trans-galactooligosaccharides, glucooligosaccharides, glycooligosaccharides, lactulose, lactitol, maltooligosaccharides, xylo-oligosaccharides, stachyose, raffinose, and sucrose thermal oligosaccharides have also been investigated (Monsan and Paul, 1995; Orban *et al.*, 1997; Patterson *et al.*, 1997; Piva, 1998; Collins and Gibson, 1999). Although mannan-oligosaccharides (MOS) have been used in the same manner as the prebiotics listed above, they do not selectively enrich for beneficial populations. Instead, they act by binding and removing pathogens from the intestinal tract and stimulation of the immune system (Spring *et al.*, 2000).

In humans and animals, prebiotics has been widely studied on their ability to improve resistance to pathogens. A recent study in mice has shown that dietary supplementation of fructooligosaccharides and inulin was protective against enteric and systemic pathogens and tumor inducers (Buddington *et al.*, 2002). This includes the verocytotoxin strain of *Escherichia coli* O157:H7 and *Campylobacter*. Likewise, we have recently observed that a high concentration of plant cell wall polysaccharides in wheat-based turkey diets shifted the intestinal microflora of poults to a healthier state, and decreased *Salmonella* spp. population in the cecum (Santos *et al.*, 2005). The major effect of selective-fermentation prebiotics is that they increase lactic acid producing bacteria and short chain fatty acids (SCFA) in the ceca, which decrease the gastro-intestinal tract (GIT) pH. In effect, fermentative-prebiotics indirectly have the antimicrobial effect of an organic acid on susceptible gram-negative organisms as described above. Swanson (2002) observed that prebiotics affected the immune function of human and dogs by stimulating lactic acid bacteria. The rise in intestinal lactic acid bacteria stimulated phagocytic activity (cellular immune response) and/or IgA secretion (humoral immune response) that may affect the colonization of pathogens, such as *Salmonella* and rotavirus (Manning and Gibson, 2004).

### ***Mannan oligosaccharide (MOS)***

Unlike fermentative-prebiotics, MOS is not used as a substrate in microbial fermentation, but it still exerts significant growth-promoting effect by enhancing the animal's resistance to enteric pathogens. Bio-Mos<sup>®</sup> (Alltech<sup>®</sup>, Nicholasville, Kentucky, USA) is a commercial source of MOS that has been used in most of the published research literature. Based on the scientific literature, Bio-Mos<sup>®</sup> enhances an animal's resistance to enteric disease and promotes growth by the following means: 1) it inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining; 2) it enhances immunity; 3) it modifies microflora fermentation to favor nutrient availability for the host; 4) it enhances the brush border mucin barrier; 5) it reduces enterocyte turnover rate; and 6) it enhances the integrity of the gut lining.

Mannan oligosaccharides, derived from mannans on yeast cell surfaces, act as high affinity ligands, offering a competitive binding site for a certain class of bacteria (Ofek *et al.*, 1977). Gram-negative pathogens with the mannose-specific Type-1 fimbriae attach to the MOS instead of attaching to intestinal epithelial cells and they move through the gut without colonization. Dietary MOS in the intestinal tract removes pathogenic bacteria that could attach to the lumen of the intestine (Newman, 1994). Mannose was shown by Oyofe *et al.* (1989a) to inhibit the *in vitro* attachment of *S. typhimurium* to intestinal cells of the day-old chicken. Then Oyofe *et al.* (1989b) provided evidence that dietary D-mannose was successful at inhibiting the intestinal colonization of *S. typhimurium* in broilers. The ability of MOS to interfere with the attachment of

pathogenic bacteria in the gut raises the possibility that it could also inhibit the binding between bacteria that is required for plasmid transfer via conjugation. This kind of inhibition of plasmid transfer in the digestive tract of mice colonized with human microflora has been described using lactose (Duval-Iflah, 2001). Lou (1995) demonstrated that dietary MOS supplementation decreased the proportion of specific groups of Gram-negative antibiotic-resistant fecal bacteria in pigs.

In an effort to confirm that MOS inhibits pathogens colonization, Spring *et al.* (2000) screened different bacterial strains for their ability to agglutinate mannanoligosaccharides in yeast cell preparations (*Saccharomyces cerevisiae*, NCYC 1026). Five of seven strains of *E. coli* and 7 of 10 strains of *Salmonella typhimurium* and *S. enteritidis* agglutinated MOS and *Sac. cerevisiae* cells. However, strains of *S. choleraeuis*, *S. pullorum*, and *Campylobacter* did not lead to agglutination. Although MOS does not bind clostridia, it does reduce clostridia numbers in some trials, possibly by enhancing the mucin barrier or stimulating gut-associated immunity.

MOS has been shown to have a positive influence on humoral immunity and immunoglobulin status. Good immune response is a nutritionally more efficient mean to resist disease than an active inflammatory response (Humphrey *et al.*, 2000). Savage *et al.* (1996) reported an increase in plasma IgG and bile IgA in poult fed diets supplemented with 0.11% MOS. An increase in antibody response to MOS is expected because of the ability of the immune system to react to foreign antigenic material of microbial origin. Portions of the cell wall structure of the yeast organism, *Saccharomyces* contained in MOS has been shown to elicit powerful antigenic properties (Ballou, 1970). However, MOS may also enhance humoral immunity against specific pathogens by preventing the colonization leading to disease, yet allowing them to be presented to immune cells as attenuated antigens. Indeed as MOS facilitates the secretion of IgA into the gut mucosa layer, pathogenic agents become more labile to the phagocytic action of gut-associated lymphocytes.

All animals reared under commercial field conditions are subjected to immunological stress depending on the pathogen load in their environment and the vaccination program. The release in cytokines associated with inflammation and the innate immune response results in fever (which reduces appetite), causes the mobilization of body reserves (glucose, aminoacids, and minerals) away from liver, muscle and bone, suppresses nutrient absorption in the gut, and increases body fluid losses as diuresis and diarrhea. The positive growth-performance effects observed among animals fed MOS may be partly due to its effect on acute immunological stress. Although MOS may enhance humoral immunity, there is some evidence that it may suppress the pro-inflammatory immune response that is detrimental to growth and production. To test this hypothesis, Ferket (2002) induced an acute immune stress in 14 day-old turkey poults by intraperitoneal injection of lipopolisaccharides (LPS) from *Salmonella typhimurium* strain SL 684. The poults were fed either 1 kg Bio-Mos<sup>®</sup>/tonne, 20g virginiamycin/tonne, or control diet from day of age. Cloacal temperatures were measured 8 hrs after the LPS injection, and then body, spleen, bursa of Fabricius, and intestinal tract weights were recorded 24 hours post-injection. In contrast to the control and the antibiotic-fed birds, the Bio-Mos<sup>®</sup>-fed birds showed no fever response 8 hrs post-injection, even though liver and intestine weights were increased. In other words, the MOS-fed birds retained normal body temperature after exposure to a pro-inflammatory antigen, while the controls and virginiamycin-fed birds expressed elevated body temperature. Under commercial conditions where birds are subjected to chronic immunological stress, MOS may help reduce the pro-inflammatory response and associated depression in feed intake and growth.

Even though the ceca are the primary site of gut microflora fermentation, microbial fermentation in the jejunum has a greater influence on digestion and nutrient absorption. Measurement of SCFA content and pH of the jejunum digesta is one way to evaluate the influence of feed additives on microbial fermentation. In a study with turkeys, Ferket (2002) observed that dietary supplementation of Bio-Mos<sup>®</sup> and antibiotics reduced total SCFA content of jejunum digesta by about 40%. Most of this effect was attributed to a reduction in propionic acid, which is the major fermentation product of microflora that uses starches and sugars as their primary substrate. Therefore, Bio-Mos<sup>®</sup> may improve dietary energy availability by reducing the microflora-host competition for available starches and sugars. Indeed, apparent metabolizable energy of the diet was increased by about 3% when Bio-Mos<sup>®</sup> or virginiamycin was supplemented to the diet. Another benefit to dietary inclusion of Bio-Mos<sup>®</sup> was a decrease in jejunum digesta pH and ammonia concentration in comparison to the antibiotic-fed birds. Lower gut pH suppresses the proliferation of putrefying bacteria that excrete ammonia as their fermentation byproduct, and ammonia has a detrimental effect on the integrity of gut tissues.

The beneficial effects of Bio-Mos<sup>®</sup> on the gut microflora, nutrient utilization, and growth performance may be associated with the brush border morphology and how it influences enteric disease resistance. To test this hypothesis, Ferket (2002) conducted an experiment to ascertain effects of Bio-Mos<sup>®</sup> and virginiamycin on jejunum villi morphology. Commercial Hybrid<sup>®</sup> poult chicks were fed a corn-soya control diet or diets supplemented with 1kg Bio-Mos<sup>®</sup>/tonne or 20g virginiamycin/tonne starting a 1 day of age. At 14 days of age, eight birds per treatment pen were sampled for morphometric measurements, including villus height, crypt depth, muscularis thickness, and goblet cell number. MOS had the greatest effect on villi morphology. Although MOS did not affect villus height, a decrease in crypt depth approached significance and villi height: crypt depth ratio was significantly greater than the control or VM treatment. Iji *et al.* (2001) also observed an increase in jejunal villi height: crypt depth ratio by MOS supplementation in broilers, but this was due to a significant increase in villi height rather than crypt depth. These researches also observed MOS to significantly increase protein/DNA of jejunal mucus, as well as increase in the brush border enzymes maltase, leucine aminopeptidase and alkaline phosphatase. Turkeys receiving MOS in our experiments also exhibited a thinner muscularis layer and increased the number of goblet cells per mm of villus height as compared to control birds.

The mucus gel layer coating the surface of the intestinal epithelium is the first barrier to enteric infection. Hence, the production of mucus, as indicated by the number of goblet cells, is an important feature in the protective scheme against pathogens. Feeding MOS resulted in an increased proliferation of goblet cells into the surface of the villus membrane. The innate immune system recognizes key molecular structures of invading bacteria, including lipopolysaccharides, peptidoglycans, and possibly the mannose structures in the cell walls of yeasts. Oligosaccharides containing mannose have been shown to affect the immune system by stimulating liver secretion of mannose-binding protein. This protein, in turn, can bind to bacteria and trigger the complement cascade of the host immune system (Newman, 1994). Intestinal microbes might influence goblet cell dynamics by releasing bioactive compounds or indirect activation of the immune system (Bienenstock and Befus, 1980).

## Conclusion

The intestinal tract is a major organ of the body, which contributes to critical functions of human and animal health, including the digestion and absorption of nutrients and the defense of the body's exposed surface against the external environment. The gastrointestinal tract is also host to billions of resident microflora that serve overall health that directly and favorably affect gut structure and function. Poultry are susceptible to the colonization of pathogens that are of concern to the human health. In response to consumer demands and government regulation, today's intensive animal agriculture industry is searching for alternatives to antibiotics. A combination of feed additives strategies and feed additives can be used to achieve good gut health and growth performance. Considering their flexibility of use and consistency in efficacies, mannan oligosaccharides supplied by a product such as Bio-Mos<sup>®</sup> with or without an enzyme preparation blend such as Allzyme Vegpro<sup>™</sup> are feasible alternative to promote gut health and growth performance.

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