Controlling Gut Health without the Use of Antibiotics

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During the past 50 years, the livestock and poultry industries have developed in several areas including nutrition, genetics, engineering, management, and communications to maximizing the efficiency of growth performance and meat yield. Now these industries must focus more attention on how animal agriculture affects the environment and food safety. As in many other industries, the global paradigm is shifting from an emphasis on productive efficiency to one of public security. Nothing demonstrates this paradigm shift more clearly than the issues concerning the use of antibiotic growth promoters. For the past 4 decades, antibiotics have been used in animal agriculture to improve the growth performance and protect animals from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. Now, antibiotics have come under increasing scrutiny because of the potential development of antibiotic-resistant human pathogenic bacteria after long use (Phillips, 1999; Ratcliff, 2000). In response to this "apparent threat", the European Union banned the use of sub-therapeutic levels of antibiotics to prevent disease or promote growth, starting with a ban on avoparcin in 1997 and a ban on virginiamycin, bacitracin, spiromycin, and tylosin in 1999. Antimicrobials scheduled to be banned by 2006 include avilamycin, bambermycin, salinomycin and monensin. In June of 2003, McDonald's Corp. announced that it would prohibit their direct suppliers from using antibiotics that are important in human medicine as growth promoters in food animals after 2004, and they created a purchasing preference for companies that work to minimize antibiotic use. Although banning antibiotic growth promoters may not be scientifically justified, the tide of public opinion is forcing animal agriculture to develop alternatives to antibiotic growth promoters, or at least substantially reduce the amount of antibiotics used to maintain production efficiency and produce safe meat and egg products. Some of these alternatives may include significant changes in husbandry practices or the strategic use of enteric microflora conditioners, including acidifiers, probiotics, enzymes, herbal products, microflora enhancers, and immunomodulators. The objective of this paper is to briefly review the use of antibiotic growth promoters as enteric conditions and discuss the potential of non-pharmaceutical alternatives.

Benefits of Feeding Antibiotics

Antibiotic usage in animal feeds has many benefits. It improves food safety by increasing animal health and reducing or eliminating certain pathogens. It reduces animal production costs and economic benefits are distributed along the food chain, including the feed industry, production animal agriculture, food processors, retailers, and consumers. Most of the cost savings attributed to antibiotics is from improved feed conversion, and this response is highest in fast-growing genetically improved animals reared in intensive production systems. Other cost savings come from faster growth rate, reduced mortality, greater resistance to disease challenge, improved reproductive performance, improved pigmentation, and better manure and litter quality. Rosen (1995) concluded from his review of 12,153 feeding studies that antibiotic growth promoters gave a positive response 72% of the time. `The magnitude of responses was dependent upon the type of animal management, disinfections procedures, age of the farm buildings, and quality of the feed. Finally, the use of antibiotic growth promoters has a positive impact on two important issues facing animal agriculture: animal welfare and environmental stewardship. Animal welfare is definitely improved in animals that are healthier due to the disease-suppressing effects of antibiotics. The improved utilization

of dietary nutrients by supplemental antibiotics results in significant reduction in nitrogen, phosphorus, and other nutrients excreted into the environment (Cromwell, 1999).

Antibiotic Modes of Action

Antibiotics are natural metabolites of fungi that inhibit the growth of bacteria. They function 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. Because of their elevated rate of growth and proliferation, bacteria are vulnerable to antibiotics that target active cellular metabolism. Limiting the growth and proliferation of certain bacteria and inhibiting the production of various toxins restricts the influence that the microbe has upon the host organism. This enables the host to grow and perform better than if grown under normal challenge conditions.

The term "Growth Promotor" has been used for years to describe the use of subtherapeutic levels of antibiotics to improve growth performance. "Growth Promotor" is an inappropriate term to describe this use of antibiotics because they do not promote growth as do anabolic hormones, such as growth hormone or estrogen-like compounds. This may be why the general public confuses this term with the use of anabolic hormones. The poultry industry does not use anabolic hormones as do the swine and cattle industries. Instead of calling them "Growth Promotors", they should be called "Growth Permitters" because they allow the animal to express their genetic potential for growth without compromise.

Antibiotics limit the growth of detrimental microbes, such as *Clostridium perfringens* (Truscott and Al-Sheikhly, 1977). They also limit the growth and colonization of numerous non-pathogenic species of bacteria in the gut, including *Lactobacilli*, *Bifidobacteria*, *Bacteroides*, and *Enterococci* (Tannock, 1997). Antibiotics reduce the production of antagonistic microbial metabolites, such as ammonia (Zimber and Visek, 1972), which adversely affect the physiology of the host animal. Subtherapeutic levels of antibiotics in the diet also reduce weight and length of the intestines (Visek, 1978; Postma et al., 1999). A thinner intestinal epithelium in antibiotic-fed animals may enhance nutrient absorption (Visek, 1978) and reduce the metabolic demands of the gastrointestinal system. The minimization of gastrointestinal bacteria may also ease the competition for vital nutrients between the bird and the microbes (Ferket, 1991). Finally, antibiotics may reduce the adverse effects of immunological stress on growth performance by lowering the enteric microbial load. Over-stimulation of the host immune system by the resident microflora could impair the optimum growth and performance of the bird (Cook, 2000; Klasing, 1988).

The Antibiotic-Resistance Debate

The use of growth promoting antibiotics (AGPs) has been criticized for their possible role in the occurrence of antibiotic resistant microbes. Numerous reports have been issued concerning the effects of agriculture-related antibiotics on the emergence of antibiotic resistance in human pathogens (SCAN Report, 1999; DANMAP, 2000). A complete ban on the use of sub-therapeutic doses of antibiotics in animal feed has not yet been enforced in many countries; this day may eventually come unless evidence comes forth to refute the effectiveness of the ban. There is evidence that the use of antibiotic growth promoters in animal and poultry feeds is associated with bacterial resistance in human disease therapy. Rapid selection for resistant bacteria when subtherapeutic levels of antibiotics are fed occurs because of the plethora of bacteria in the gut of animals, the high mutation rates among these bacteria, and the frequent transfer of genes including resistance genes. Mathew et al. (2002) demonstrated that selection for resistant bacteria can occur in as little as 2 days following administration of a feed-based antibiotic.

Wide use of AGPs in the poultry is one reason the public is placing some blame of antibiotic resistance of potential pathogens on the poultry industry. This blame may be partly justifiable. Antibiotic resistance has been displayed by field *Escherichia coli* isolates from commercial turkey farms in North Carolina, including resistance to Enrofloxacin (Fairchild et al., 1998). Although there are no specific claims that antibiotic growth promoters control disease (Gustafson and Bowen, 1997), the debate over resistance seen among Gram-negative bacteria, such as *E. coli* and *Salmonella,* has generated the strongest objection to antibiotic use. It has been reported that antibiotic resistance of indigenous *E. coli* of poultry has remained at a relatively high level since the 1950's (Gustafson and Bowen, 1997).

Can a ban on the use of AGPs reverse the trend in increasing antibiotic resistance of human pathogen? The Danish government thought so they instituted a voluntary ban on the use of AGPs along with a penalty tax for use in 1998. By 2000, the complete ban on the use of AGPs was in effect, but enteric disease problems and mortality rates began to mount and the therapeutic use of antibiotics began to rise sharply. By 2001, the total consumption of therapeutic antibiotics almost reached the same amount as the total consumption of AGPs before the ban was instituted. In effect, AGPs (avalimycin, virginiamycin, bacitracin, and tylosin) that are not typically used to treat human disease were replaced with therapeutic antibiotics (Ampicillin, Erythromycin, Streptomycin, Tetracycline, etc.) that are used to treat human disease pathogens (Hayes and Jensen, 2003). Tetracycline use in Denmark increased from 12,100 kg in 1998 to 27,000 kg in 2001. Now Denmark has mounting tretracyline resistance in human pathogens, such as *Salmonella typhimurium* and *Campylobactor jejuni* (DANMAP, 2001). Isn't it ironic that the policy against the use of AGPs actually results in an increase in resistance to antibiotics the public is most concerned about? A simple ban on AGPs will not solve the antibiotic resistance problem. We need to strategically use of different feed additives and management practices that will minimize the use of AGPs and therapeutic antibiotics.

General Strategies to control gut health without Antibiotics

Effective use of feed additives to manage gut health is dependent upon some degree of understanding of their mechanisms of action. Clearly, the modes of action of growth promoting antibiotics and their alternatives can differ considerably. Subtherapeutic 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. Because energy required to maintain the gut accounts for about 25% of the total basal metabolic needs of an animal (Croom et al., 2000), any reduction in gut tissue mass can have a significant impact on the amount of energy available for growth and caloric conversion efficiency. The reduced microbial load in the gut by subtherapeutic levels of antibiotics also reduces immunological stress, resulting in more nutrient partitioning towards growth and production rather than mechanisms of disease resistance. In contrast, most alternative compounds do not reduce overall microbial loads in the gut and thus will not promote growth by a mechanism similar to antibiotics. Instead, they alter the gut microflora profile by limiting the colonization of unfavorable bacteria while promoting the fermentation of more favorable species. Consequently, alternatives to antibiotics promote gut health by several possible mechanisms including: 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. Strategic use of these alternative compounds will help optimize growth provided they are used in a manner that complements their modes of action.

Sanitation and Pathogen Load Reduction

There is considerable evidence that subtherapeutic antibiotics or alternative compounds are most effective when fed to animals raised in unsanitary environmental conditions. Good barn sanitation, pest control, biosecurity practices, and litter or manure management are necessary to reduce pathogen load and exposure and minimize the need for antimicrobial therapy. Water must be clean and drinkers must be properly maintained to minimize spillage and prevent a bloom of pathogens in the litter and environment of the animals. Implementation of a good sanitation program is usually much less costly than any disease treatment.

Enhance Pathogen Colonization Resistance

Colonization of enteric pathogens is dependent upon the degree of resistance afforded by the stability of the resident microflora and the integrity of the intestinal mucin barrier in the animal. Older animals are much less susceptible to the colonization of enteric pathogens than young animals because they have a more stable and diverse gut microflora that competitively excludes pathogen colonization. In contrast, the ability of pathogens to colonize in the gut increases after antibiotic administration because of a loss of resident microflora. The stability of resident microflora can be enhanced by the administration of competitive exclusion cultures (probiotics) or feeding prebiotic compounds that feed the beneficial microflora. Hollister et al. (1999) reduced salmonella colonization in chicks by feeding a live cecal culture from salmonella-free poultry. Fedorka-Cray et al. (1999) has shown similar response to microbial cultures in young swine. Gram-positive bacteria, including *Lactobacillus, Enterococcus, Pediococcus, Bacillus*, and

bifidobacteria, and fungi of the *Saccharomyces* (yeast) genus are often fed after antibiotic therapy as a means of re-introducing a beneficial flora to the gut of affected animals. Beneficial bacteria inhibit the colonization of pathogens by producing volatile fatty acids that reduce the pH of the brush-boarder microenvironment or they can block the attachment of pathogens. Organic acids have strong antibacterial effects, especially to gram-negative pathogens. Blomberg et al. (1993a) also demonstrated that undefined compounds in a culture of lactobacilli inhibit the attachment to intestinal components of pigs by pathogenic K88 *E. coli*. They suggested that compounds produced by the lactobacilli or the lactobacilli themselves bound to the receptor of K88 *E. coli* in pig intestine, thereby preventing the colonization by the *E. coli*.

Mucins and glycoproteins associated with the intestinal brush boarder serves a very important barrier protecting the delicate absorptive surface from the abrasive action of feedstuffs, bacteria colonization, and toxins. Mucin, produced by goblet cells, is secreted in response to the degree of insult on the absorptive surface of gut. Glycoproteins of gut mucins specifically bind pathogens and reduce their colonization by serving as alternative binding sites to receptors on host enterocytes. For example, pathogenc *E. coli* K88 adhesins were found to bind to ileal mucus from pigs, and Blomberg et al. (1993b) concluded that the intestinal mucus might intercept these pathogens before they can attach to intestinal tissues and cause disease. Dietary factors that result in increased mucus secretion may thus indirectly enhance an animal's ability to resist pathogen colonization.

There is a complex balance between the gut ecosystem and intestinal mucins, and this balance can be altered by enteric health conditions and the diet. Although intestinal mucins and glycoproteins have a protective function, they also serve as a nutritional substrate for some bacteria that thrive on galactose-rich environment, such as bifidobacteria (Roy et al., 1991). In pigs, Petova et al. (2000) observed significant decrease in intestinal mucins following weaning, and this was partially prevented by the inclusion of galactose in the post-weaning diet. Apparently, the lack of galactose in the postweaning high starch diet increasing the scavenging of galactosyl units in mucins by some microflora, thus promoting the degradation of the protective mucin barrier. Dietary inclusion of compounds that feed beneficial bacteria, such as bifidobacteria, should alleviate their attack on the protective mucins. Such compounds include oligosaccharides or enzymes that liberate galactose from galactosyl polymers, such as galacto-mannans. More research must be done in this area of interest.

Immune Response Augmentation

The immune system is the primary defense mechanism of the animal to fight infectious disease. Augmentation of humoral and cell-mediated immunity will increase an animal's ability to resist disease. Although there is a small nutrient costs in the production of immunoglobulins, good antibody titer levels indicate a far more efficient capacity to resist disease by humoral immune responses than an active inflammatory response (Humphrey et al., 2002). A pro-inflammatory innate immune response is associated with the mobilization of nutrients away from growth and suppression of feed intake. Thus, dietary immunomodulators or vaccines that enhance humoral immunity and minimize immunological stress will affect growth performance most positively.

Although there is now a considerable amount of knowledge about systemic immunity, knowledge about gut-associated immunity is still primitive. The gut is a major interface where the immune system can sample the potential disease antigens in the animal's environment and mount a defensive strategy to resist disease. Therefore, the resident microflora will have a marked effect on the amount and profile of immune factors, such as immunoblobulins. Perdigon et al. (1991) observed that specific lactobacilli fed to mice resulted in enhanced protection against Salmonella typhimurium and E. coli by increasing IgA production. IgA, predominantly found in the mucus secretions in the respiratory tract and gut, function to attenuate antigens and present them to lymphocytes for degradation and stimulation of the production of specific antibodies. Dietary supplementation of phosphorylated mannanoligosaccharide has also been shown to enhance IgA titers in the plasma of poultry (Savage et al., 1996) and sow's milk (O'Quinn et al., 2000).

An alternative to feeding dietary factors that stimulate gut-associated humoral immunity may be feeding specific antibodies that neutralize pathogenic organisms. To produce the specific antibodies, laying hens are exposed to particular antigens to stimulate the production of immunoglobulins, which are deposited in

the egg. These immunoglobulins are then harvested from the eggs and fed to susceptible young animals. There may be some limitations to this technology, since these immunoproteins are sensitive to heat treatment during feed processing and the digestive process of the animal.

Nutritional Strategies and Feed Additives

Diet digestibility and Enzyme supplementation

Gut health and enteric disease resistance is often dependent upon the digestibility of feed components and feed formulation. Poorly digested protein meals due to improper heat processing causes the proliferation of putrifying bacteria in the hindgut, which increases toxic metabolites (ammonia and biogenic amines) that compromise gut health. In agreement, antibiotics are most effective in birds fed diets containing high levels of indigestible protein (Smulders et al., 2000). Similarly, poultry fed diets containing high levels of poorly digested non-starch polysaccharides from wheat, barley or rye are more susceptible to enteric disease, such as necrotic enteritis (Riddell and Kong, 1992; Kaldhusdal and Skjerve, 1996). Langhout (1999) observed that dietary NSP significantly increases gut populations of pathogenic bacteria at the expense of beneficial bacteria. However, the digestibility of wheat, barley, rye, triticale and even corn-based diets can be significantly improved through use of exogenous enzymes including xylanases, phytases and ß-glucanases. The response to dietary enzyme supplementation is greater when antibiotics are not used than when they are, but the performance responses do not approach the level that is observed when diets contain enzymes and antibiotics together (Bedford, 2000b; Elwinger and Teglof, 1991; Danicke et al., 1999). In a comprehensive literature review, Rosen (2001) concluded that the effect of enzymes was nearly equivalent to the effects of antibiotics on gain and FCR, and that in combination there was improved, but less than the sum of the two. Enzymes are perhaps the most extensively reviewed products that seem to be capable of limiting the performance losses associated removal of antibiotic growth promoters.

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. The use of enzymes has been shown to alter the gut microflora populations in the small intestine and caeca (Choct et al., 1996; Hock et al., 1997; Bedford, 2000a) and reduce mortality rates (Rosen, 2001). Such a benefit is brought about by a more rapid digestion and absorption of starch, protein and fat from the small intestine, which effectively limits available substrate for the resident flora. In general, the improvement in nutrient digestibility achieved for the host by the use of an appropriate enzyme is much smaller than the concomitant loss of substrate experienced by microflora resident in the large intestinal. This starch and protein removal effect is coupled with the production of exogenous enzyme for fiber-derived oligomers, which serve as substrate for specific populations of bacteria that seem to benefit the host (Bedford, 2000a).

Acidifiers and Organic Acids

Clostridia and pathogenic coliform bacteria often associated with enteric disease do not grow well in media of low pH, so any means to reduce gut pH should improve an animal's resistance to enteric disease. Because organic acids have strong bacteriostatic effects, they have been used as salmonella-control agents in feed and water supplies for livestock and poultry. Organic acid blends have also been used as acidifiers in baby pig diets to reduce enteric disease, but the benefit for poultry seems to be less conclusive. Dietary accidifiers may work better in baby pig diets because they have more limited hydrochloric acid production than chicks. Moreover, dietary organic acids are easily neutralized in the duodenum unless they are delivered to the ileum and below by adsorbent vehicles.

Herbs, Spices, and Essential Oils

Herbs, spices, and plant extracts have been used to make humans foods more appetizing for centuries, and many of them are recognized for their health benefits. Some of these compounds stimulate appetite (e.g. menthol from peppermint), provide anti-oxidant protection (e.g. cinnamaldehyde from cinnamon), or suppress microbial growth (carvacol from oregano). These plant-based antimicrobials compounds, which function fundamentally similar to antibiotic compounds produced by fungi, could be used to replace some

antibiotic growth promoters. To be most effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural source. 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.

Essential oils from Oregano are showing the greatest potential as an alternative to antibiotic growth promoters. Oregano contains phenolic compounds, such as carvacrol, that have antimicrobial activity (Akagul and Kivanc, 1988). Like antibiotics, Oregano essential oils modify the gut microflora and reduce microbial load by suppressing bacteria proliferation. There are some claims that Oregano oil can replace anticoccidial compounds, not because they inactivate coccidia, but because they increase the turnover of the gut lining and prevent coccidial attack by maintaining a more healthy population of gut cells (Bruerton, 2002). This mode of action would increase the animal's maintenance energy requirement because enterocyte turnover is a major proportion of the basal metabolic rate.

Oligosaccharides

Oligosaccharides are promising alternatives to antibiotic growth promoters because they facilitate and support the symbiotic relationship between host and microflora. Fructooligosaccharide (FOS) and mannanoligosaccharide (MOS) are two classes of oligosaccharides that are beneficial to enteric health, but they do so by different means.

Fructooligosaccharides (FOS)

FOS compounds are inulin-type oligosaccharides of *D*-fructose attached by β-(2→1) linkages that are attached to a *D*-glucosyl residue at the end of the chain (Yun, 1996). A sucrose unit attached to one additional fructose residue is commonly referred to as 1-kestose. Nystose contains two additional fructose units, and three additional fructose units are designated as 1^F- β-fructofuranosyl (Hidaka and Hirayama, 1991). Fructooligosaccharides are found in numerous plants such as the onion, Jerusalem artichoke, garlic, banana, chicory, asparagus, and wheat.

FOS influence enteric microflora by "feeding" the "good" bacteria, which competitively excludes the colonization of pathogens. Dietary supplementation of FOS provides selective enrichment of *Lactobacilli* (Mitsuoka et al., 1987) and *Bifidobacteria* (Hidaka et al. 1991; Hopkins et al., 1998). Patterson et al. (1997) found that cecal *Bifidobacteria* concentrations were increased 24-fold and *Lactobacilli* populations increased 7-fold in young broilers fed the FOS-enriched diets. Fructooligosaccharides are well utilized by the majority of *Bifidobacteria* strains (*longum, brevis, and infantis*) with the exception of *Bifodobacterium bifidum* (Hidaka and Hirayama, 1991). The *Bacteroides* group also showed a tendency to utilize FOS as a growth source, while *Lactobacillus fermentum*, *E. coli*, and *Clostridium perfringens* failed to utilize FOS as a fermentative carbohydrate source. *Bifidobacteria* readily ferment FOS because of the innate secretion of a β-fructoside enzyme. *Bifidobacteria* may inhibit other microbes because of its acidic surroundings from the high production of VFA's or the secretion of bacteriocin-like peptides. The improvement in gut health conditions by dietary FOS supplementation often results in improved growth performance. Ammerman et al. (1988) demonstrated that the addition of either 0.25% or 0.50% dietary FOS improved feed efficiency from 1 to 46 days of age and reduced mortality when fed at the higher level (0.50%). FOS-treated birds also had less air sac lesions at day 46.

Mannanoligosaccharide (MOS)

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

Inhibition of pathogen colonization by MOS

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 Oyofo et al. (1989a) to inhibit the *in vitro* attachment of *Salmonella typhimurium* to intestinal cells of the day old chicken. Then Oyofo et al. (1989b) provided evidence that dietary D-mannose was successful at inhibiting the intestinal colonization of *Salmonella 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 swine.

In an effort to confirm that MOS inhibits pathogen 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. choleraecuis*, *S. pullorum*, and *Campylobacter* did not lead to agglutination. Although MOS does not bind clostridia, it does reduce clostridial numbers in some trials, possibly by enhancing the mucin barrier or stimulating gut associated immunity.

Enhancement of Immune Function by MOS

MOS has been shown to have a positive influence on humoral immunity and immunoglobulin status. As mentioned above, a good humoral immune response is nutritionally more efficient means to resist disease than an active inflammatory response (Humphrey et al., 2002). Savage et al. (1996) reported an increase in plasma IgG and bile IgA in poults 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 their colonization leading to disease, yet allowing them to be presented to immune cells as attenuated antigens. In deed as MOS facilitates the secretion of IgA into the gut mucosa layer, pathogenic agents become more labile to the phagocytic action of gut-associated lympocytes.

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, amino acids, 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 that by intraperitoneal injection of LPS from *Salmonella typhimurium* strain SL 684. The poults were fed either 1 kg BioMos/tonne, 20 g virginiamycin/tonne, or control diet from day of age. Cloacal temperatures were measured eight hours after the LPS injection, and then body, liver, 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 BioMOS-fed birds showed no fever response 8 h 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 VM-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.

Effect of MOS on Gut Microflora Fermentation and Dietary Energy Utilization

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 volatile fatty acid (VFA) 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 dietary supplementation of BioMos® and antibiotics reduced total VFA content of jejunum digesta by about 40%. Most of this effect was attributed to a reduction in proprionic acid, which is the major fermentation product of microflora that uses starches and sugars as their primary substrate. Therefore, BioMos® may improve dietary energy availability by reducing the microflora-host competition for available starches and sugars. In deed, apparent metabolizable energy of the diet was increased by about 3% when BioMos® or virginiamycin was supplemented to the diet. Another benefit to dietary inclusion of BioMos® was a decrease in jejunum digesta pH and ammonia concentration in comparison to the antibiotic-fed birds. Lower gut pH suppresses the proliferation of putrifying bacteria that excrete ammonia as their fermentation byproduct, and ammonia has a detrimental effect on the integrity of gut tissues.

Effect of MOS on Gut Tissue Integrity and Health

The beneficial effects of MOS on the gut microflora, nutrient utilization, and growth performance may be associated brush boarder morphology and how it influences enteric disease resistance. To test this hypothesis, Ferket (2002) conducted an experiment to ascertain effects of MOS and VM on jejunum villi morphology. Commercial Hybrid® poults were fed a corn-soy control diet or diets supplemented with 1 kg BioMos®/tonne or 20 g virginiamycin/tonne starting a 1 day of age. At 14 days of age, 8 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 treatments. 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 researchers also observed MOS to significantly increase protein/DNA of jejunal mucosa, as well as increases in the brush boarder enzymes maltase, leucine aminopepidase and alkaline phosphatase. Turkeys receiving MOS in our experiment 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 major 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 lipopolysacchharides, 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

In response to consumer demands and government regulations, today's intensive animal agriculture industry must adapt to producing animals in a world without antibiotic growth promoters. This paper presented several alternatives to antibiotics to manage gut health. Although no single alternative may be as effective as antibiotics, use of a combination of strategies and feed additive can be used to achieve good gut health and growth performance. The key to selecting the most cost effective approach will depend upon the production requirements of each company, and the type of production challenges they face.

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