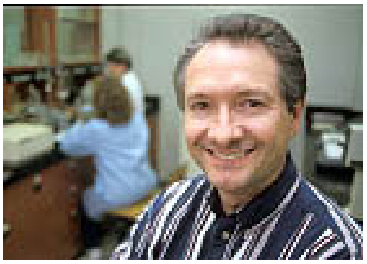

Raising Drug-Free Poultry – What are the Alternatives?

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During the past 50 years, the livestock and poultry industries have made great strides in several areas including nutrition, genetics, engineering, management, and communications thereby maximizing the efficiency of growth performance and meat yield. These industries are now expected to focus more attention on how animal agriculture affects the environment and food safety. Currently, 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 (AGPs). For the past 4 decades, antibiotics have been used in animal agriculture to improve the growth performance and to protect animals from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. Recently, the use of antibiotics, have come under increasing scrutiny because of the potential development of antibiotic-resistant human pathogenic bacteria after long use. The tide of public opinion is forcing animal agriculture to develop alternatives to antibiotic growth promoters. 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 discuss the potential of non-pharmaceutical alternatives to antibiotics. Alternatives to antibiotics promote gut health by several possible mechanisms including: altering gut pH, maintaining protective gut mucins, selecting 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.

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 restoring equilibrium, by re-introducing a beneficial flora to the gut of affected animals. Beneficial bacteria inhibit the colonization of pathogens by producing volatile fatty acids (VFAs) that reduce the pH of the brush-boarder microenvironment and by blocking the attachment of pathogens. Organic acids have strong antibacterial effects, especially to gram-negative pathogens.

Immune Response Augmentation

The immune system is the primary defense mechanism of the animal in its fight against infectious disease. Augmentation of humoral and cell-mediated immunity will increase an animal's ability to resist disease. Although there is a small nutrient cost in the production of immunoglobulins, good antibody titer levels indicate a far more efficient capacity to resist disease by humoral immune responses than by 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 have a more positive effect on growth performance. An alternative to feeding dietary factors that stimulate gut-associated humoral immunity, may be the feeding of 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 processes of the animal.

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 cause the proliferation of putrifying bacteria in the hindgut, which increases toxic metabolites (ammonia and biogenic amines) that compromise gut health. Similarly, poultry fed diets containing high levels of poorly digested non-starch polysaccharides (NSPs) 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 NSPs significantly increase 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. 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 performance, although this was less than the sum of the two effects. Enzymes are perhaps the most extensively reviewed products that seem to be capable of limiting the performance losses associated with the removal of antibiotic growth promoters (AGPs).

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).

Organic Acids, Herbs, Spices, and Essential Oils

Many compounds that have bacteriostatic effects can serve as alternatives to AGPs, which work in similar fashion. Organic acids have been used as salmonella-control agents in feed and water supplies for livestock and poultry, and they are most effective in young poultry because they have limited acid production in the proventriculus. Herbs, spices, and plant extracts may be useful because they could stimulate appetite (e.g. menthol from peppermint), provide anti-oxidant protection (e.g. cinnamaldehyde from cinnamon), or suppress microbial growth (carvacol from oregano). Essential oils from Oregano is 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, the essential oils of Oregano modify the gut microflora and reduce microbial load by suppressing the proliferation of bacteria. These plant-based antimicrobials compounds, which function fundamentally similar to antibiotic compounds produced by fungi, could be used to replace some antibiotic growth promoters. 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.

Oligosaccharides

Oligosaccharides are promising alternatives to AGPs, 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)

Fructooligosaccharides are found in numerous plants such as the onion, Jerusalem artichoke, garlic, banana, chicory, asparagus, and wheat. They 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). 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.

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 a 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. 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 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 positive growth performance effects observed among animals fed MOS, may be partly due to the effect of MOS on reducing acute immunological stress and associated inflammation 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 lipopolysaccharides (LPS) from *Salmonella typhimurium* strain SL 684. The poults were fed either, 1 kg BioMos/tonne, 20 g virginiamycin /tonne, or control diet from one 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 the virginiamycin (VM)-fed birds expressed elevated body temperatures. 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 Tissue Integrity and Health

The beneficial effects of MOS on the gut microflora, nutrient utilization, and growth performance may be associated with 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 by 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, the combination of strategies and feed additives 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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