Application of Prebiotics and Probiotics in Poultry Production

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ABSTRACT

The intestinal microbiota, epithelium, and immune system provide resistance to enteric pathogens. Recent data suggest that resistance is not solely due to the sum of the components, but that cross-talk between these components is also involved in modulating this resistance. Inhibition of pathogens by the intestinal microbiota has been called bacterial antagonism, bacterial interference, barrier effect, colonization resistance, and competitive exclusion. Mechanisms by which the indigenous intestinal bacteria inhibit pathogens include competition for colonization sites, competition for nutrients, production of toxic compounds, or stimulation of the immune system. These mechanisms are not mutually exclusive, and inhibition may comprise one, several, or all of these mechanisms. Consumption of fermented foods has been associated with improved health, and lactic acid bacteria (lactobacilli and bifidobacteria) have been implicated as the causative agents for this improved health. Research over the last century has shown that lactic acid bacteria and certain other microorganisms can increase resistance to disease and that lactic acid bacteria can be enriched in the intestinal tract by feeding specific carbohydrates. Increased bacterial resistance to antibiotics in humans has caused an increase in public and governmental interest in eliminating sub-therapeutic use of antibiotics in livestock. An alternative approach to sub-therapeutic antibiotics in livestock is the use of probiotic microorganisms, prebiotic substrates that enrich certain bacterial populations, or symbiotic combinations of prebiotics and probiotics. Research is focused on identifying beneficial bacterial strains and substrates along with the conditions under which they are effective.

(Key words: intestinal microbiota, poultry, prebiotic, probiotic)

INTRODUCTION

Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (human food safety). With increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage in Europe and the potential for a ban in the United States, there is increasing interest in finding alternatives to antibiotics for poultry production. Probiotics and prebiotics are two of several approaches that have potential to reduce enteric disease in poultry and subsequent contamination of poultry products. Probiotic, which means “for life” in Greek (Gibson and Fuller, 2000), has been defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). Prebiotics are defined as “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995). Combinations of prebiotics and probiotics are known as synbiotics.

Probiotic and prebiotic foods have been consumed for centuries, either as natural components of food, or as fermented foods. Interest in intestinal microbiology and the dietary use of prebiotics and probiotics blossomed in the late 1800s and early 1900. The growing enthusiasm was motivated Escherich’s isolation of Escherichia coli in the late 1800s, as well as active research on the benefits of feeding lactic acid bacteria and lactose near the turn of the 20th century (Rettger and Cheplin, 1921). Metchnikoff noticed the longevity of Bulgarians who consumed yogurt, and in 1907, he proposed that the indigenous bacteria were harmful and that ingestion of lactic acid bacteria in yogurt had a positive influence on health (Stavric and Kornegay, 1995; Rolfe, 2000). Numerous in vivo and in vitro studies since then have shown that the commensal intestinal microbiota inhibit pathogens, that disturbances of the intestinal microbiota can increase susceptibility to infection, and that addition of prebiotics and probiotics increase resistance to infection (Stavric and Kornegay, 1995; Rolfe, 2000).

Intestinal pathogens encounter a multifaceted defense system composed of low gastric pH, rapid transit through sections of the intestinal tract, as well as the intestinal microbiota, epithelium, and immune systems. Although
not reviewed here, there is extensive information on the mucosal immune system (Schat and Myers, 1991; Kitagawa et al., 1998; Mayer, 1998; Muir, 1998; Hershberg and Mayer, 2000; Shanahan, 2000; Erickson and Hubbard, 2000; Jeurissen et al., 2000; Spellberg and Edwards, 2001; Toms and Prowrie, 2001), the intestinal epithelium (Glick, 1995; Fontaine et al., 1996; Dai, et al., 2000; Freitas and Cayuela, 2000; Deplancke and Gaskins, 2001; McCracken and Lorenz, 2001) and their interactions. Stress also has detrimental effects on the immune system and intestinal epithelium (Blecha, 2000; Matteri et al., 2000; Maunder, 2000; Soderholm and Perdue, 2001; Tache et al., 2001) and the neuro-endocrine system is intimately involved in the response of immune and epithelial systems to stress (Cook, 1994; Kohm and Sanders, 2000; Levite, 2001; Petrovsky, 2001). Additionally, there is information on cross-talk between pathogens and epithelial tissues, resulting in extensive rearrangement of epithelial cells upon colonization by pathogens (Goosney et al., 2000; Sansonnetti, 2001). Recently, Hooper et al. (2001) have shown that cross-talk between Bacteroides thetaiotaomicron and the epithelium results in epithelial secretion of specific glycans, which are utilized by the bacterium. It is probable that other intestinal bacteria, including probiotic bacteria, may interact with the epithelium in a similar manner to enhance the ability of these microorganisms to colonize the mucosal lining.

Intestinal microbial populations have been characterized using classical plating techniques (Savage, 1987; Vahjen et al., 1998; Van der Wielen et al., 2000). Although Bacteroides and Bifidobacterium predominate in the human intestine, Ruminococcus and Streptococcus tend to predominate in the chicken intestinal tract (Apajalahti et al., 1998; Van der Wielen et al., 2000). However, recent molecular techniques indicate that only 20 to 50% of the bacterial species present in the intestinal tract have been cultured. Molecular approaches identifying changes in specific bacterial populations or general changes in microbial community structure should enhance our understanding of intestinal microbial ecology, including the influence of probiotics and prebiotics (Apajalahti et al., 1998; Netherwood et al., 1999; Gong et al., 2002; Zhu et al., 2002).

The concept of a balanced intestinal microbiota enhancing resistance to infection and reduction in resistance when the intestinal microbiota is disturbed is important in understanding the microbe-host relationship. What constitutes the balanced and disturbed populations is not clear; however, lactobacilli and bifidobacterial species seem to be sensitive to stress, and these populations tend to decrease when a bird is under stress. Proposed mechanisms of pathogen inhibition by the intestinal microbiota include competition for nutrients, production of toxic conditions and compounds (volatile fatty acids, low pH, and bacteriocins), competition for binding sites on the intestinal epithelium, and stimulation of the immune system (Fuller, 1989; Gibson and Fuller, 2000; Rolfe, 2000). These are not mutually exclusive mechanisms, and some microorganisms may effect change with a single mechanism, whereas others may use several mechanisms.

### Table 1. Characteristics of ideal probiotics and prebiotics1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Probiotics</th>
<th>Prebiotics</th>
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<tbody>
<tr>
<td>Be of host origin</td>
<td>Non-pathogenic</td>
<td>Be neither hydrolyzed or absorbed by mammalian enzymes or tissues</td>
</tr>
<tr>
<td>Non-pathogenic</td>
<td>Withstand processing and storage</td>
<td>Selectively enrich for one or a limited number of beneficial bacteria</td>
</tr>
<tr>
<td>Withstand gastric acid and bile</td>
<td>Resist gastric acid and bile</td>
<td>Beneficially alter the intestinal microbiota and their activities</td>
</tr>
<tr>
<td>Adhere to epithelium or mucus</td>
<td>Adhere to epithelium or mucus</td>
<td>Beneficially alter luminal or systemic aspects of the host defense system</td>
</tr>
<tr>
<td>Persist in the intestinal tract</td>
<td>Persist in the intestinal tract</td>
<td></td>
</tr>
<tr>
<td>Produce inhibitory compounds</td>
<td>Produce inhibitory compounds</td>
<td></td>
</tr>
<tr>
<td>Modulate immune response</td>
<td>Modulate immune response</td>
<td></td>
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<tr>
<td>Alter microbial activities</td>
<td>Alter microbial activities</td>
<td></td>
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1Adapted from Simmering and Blaut, 2001.

# PROBIOTICS AND PREBIOTICS

Characteristics and effects of ideal probiotics and prebiotics are shown in Tables 1 and 2. Proposed mechanisms by which probiotics and prebiotics act include competition for substrates, production of toxic compounds that inhibit pathogens, and competition for attachment sites.

Extensive research conducted with humans and rodent models has shown a reduction in pathogen colonization, alteration of microbial populations, alteration of the immune system, prevention of cancer, and reduction of triglycerides, cholesterol, and odor compounds (ammonia, skatole, indole, p-cresol, and phenol) associated with probiotic and prebiotic use (Walker and Duffy, 1998; Gibson and Fuller, 2000; Simmering and Blaut, 2001). More research and commercial application of probiotics and prebiotics has occurred in Japan and Europe than in the United States.

A variety of microbial species have been used as probiotics, including species of Bacillus, Bifidobacterium, Enterococcus, E. coli, Lactobacillus, Lactococcus, Streptococcus, a variety of yeast species, and undefined mixed cultures. 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 (Simon et al., 2001). However, there has been a recent increase in research on feeding Lactobacillus to livestock (Gusils et al., 1999; Pascual et al., 1999; Jin et al., 2000; Tellez et al., 2001).

The dominant prebiotics are fructooligosaccharide products (FOS, oligofructose, inulin). However, trans-galactooligosaccharides, glucoooligosaccharides, glycoooligosaccharides, 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 mannano oligosaccharides (MOS) have been used in the same manner as the prebiotics listed above, they do not selectively enrich for beneficial bacterial populations. In-
instead, they are thought to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system (Spring et al., 2000).

The competitive exclusion approach of inoculating 1-d-old chicks with an adult microflora successfully demonstrates the impact of the intestinal microbiota on intestinal function and disease resistance (Nisbet, 1998; Stern et al., 2001). Although competitive exclusion fits the definition of probiotics, the competitive exclusion approach instantaneously provides the chick with an adult intestinal microbiota instead of adding one or a few bacterial species to an established microbial population. Inoculating 1-d-old chicks with competitive exclusion cultures or more classical probiotics serves as a nice model for determining the modes of action and efficacy of these microorganisms. Because of the susceptibility of 1-d-old chicks to infection, this practice is also of commercial importance. By using this model, a number of probiotics (Owings et al., 1989; Jin et al., 1998; Line et al., 1998; Nisbet, 1998; Netherwood et al., 1999; Fritts et al., 2000) and prebiotics (Chambers et al., 1997; Fukata et al., 1999) have been shown to reduce colonization and shedding of Salmonella and Campylobacter.

Studies with probiotics have been difficult to assess because many of the earlier studies were not statistically analyzed, experimental protocols were not clearly defined, microorganisms were not identified, and viability of the organisms was not verified (Stavric and Kornegay, 1995). In many cases the environmental and stress status of the birds was neither considered nor reported. Diet and feed withdrawal have been shown to increase pathogen colonization (Bailey et al., 1991; Line et al., 1997; Craven, 2000). Bailey et al. (1991) clearly showed the importance of stress on reduction of Salmonella colonization by fructooligosaccharides. In this study, unstressed birds and fructooligosaccharide-treated stressed birds had low levels of colonization, whereas stressed control birds had high levels of Salmonella. Orban et al. (1997) using mild heat stress showed that temperature and level of trace minerals and vitamins influences performance responses to sucrose thermal oligosaccharide caramel.

Using an organ culture challenge model, we (Burkholder and Patterson, unpublished data) have shown that fasting for 24 h increases attachment of Salmonella to the ileum by 1.5 logs. Although horizontal transfer of pathogens to uninfected birds has been clearly demonstrated (Gast and Holt, 1999), little concern has been shown for horizontal transfer of probiotic organisms to untreated birds. Thus, frequently birds on control and probiotic treatments are caged adjacent. Fritts et al. (2000) indicate that probiotic organisms can be horizontally transferred to control birds unless birds are physically separated.

Sub-therapeutic antibiotics are discussed in detail elsewhere; however, it is important to note that sub-therapeutic antibiotics not only influence intestinal microbial populations and activities but also affect animal metabolism and specifically alter intestinal function (Anderson et al., 2000). As would be expected, antibiotics are more effective when the animal is producing well below its genetic potential and may have only statistically significant improvements in performance 80% of the time (Rosen, 1995). Because stress status is important in detecting growth performance responses, it is important to include growth promotant antibiotics as a positive control treatment in probiotic and prebiotic studies. Studies in which there is no response to the growth promotant antibiotic should not be considered negative for the probiotic or prebiotic treatments.

**SUMMARY**

Pathogens have to overcome numerous obstacles in order to colonize the intestinal tract and cause an infection. In addition to the physical restraints of low gastric pH and rapid transit time in the small intestine, pathogens have to overcome the inhibitory effects of the intestinal microbiota, the physical barrier of the epithelium, and the response of host immune tissues. The concept that cross-talk between these systems and between pathogens and the epithelium occurs is well established. Recent data demonstrate that at least some species of non-pathogenic intestinal microbiota also communicate with the epithelium and immune system, modulating tissue physiology and ability to respond to infection. Probiotics and prebiotics alter the intestinal microbiota and immune system to reduce colonization by pathogens in certain conditions. As with growth promotant antibiotics, environmental and stress status influence efficacy of prebiotics and probiotics. These products show promise as alternatives for antibiotics as pressure to eliminate growth promotant antibiotic use increases. Defining conditions under which they show efficacy and determining mechanisms of action under these conditions is important for the effective use prebiotics and probiotics in the future.

### TABLE 2. Beneficial effects of probiotics and prebiotics

<table>
<thead>
<tr>
<th>Effect of Probiotics</th>
<th>Effect of Prebiotics</th>
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<tbody>
<tr>
<td>Modify intestinal microbiota</td>
<td>Increase production of VFA</td>
</tr>
<tr>
<td>Stimulate immune system</td>
<td>Increase biomass and stool bulking</td>
</tr>
<tr>
<td>Reduce inflammatory reactions</td>
<td>Increase B vitamin synthesis</td>
</tr>
<tr>
<td>Prevent pathogen colonization</td>
<td>Improve mineral absorption</td>
</tr>
<tr>
<td>Enhance animal performance</td>
<td>Prevent cancer</td>
</tr>
<tr>
<td>Decrease carcass contamination</td>
<td>Lower serum cholesterol</td>
</tr>
<tr>
<td>Decrease ammonia and urea excretion</td>
<td>Lower skatol, indole, phenol, etc</td>
</tr>
</tbody>
</table>

1 Adapted from Stavric and Kornegay (1995); Jenkins et al. (1999); Monsan and Paul (1995); Piva (1998); Simmering and Blaut (2001).
REFERENCES


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