

Prebiotic Feed Additives: Rationale and Use in Pigs

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■ Introduction

Disease has always been a critical issue in pig production, affecting not only animal health and well-being, but also the physical and economic health of the producer. Growth promotant antibiotics have been fed to livestock since the 1940's and have generally enhanced pig performance (Cromwell, 2000). Growth promotant antibiotics act by a variety of mechanisms to alter the intestinal microbiota, with subsequent direct and indirect effects on the pig (Anderson, et al., 2000, Gaskins et al., 2000). Enteric disease issues are coming to the forefront as governmental and public concerns about preharvest food safety and microbial antibiotic resistance increase. The European Union is phasing out use of growth promotant antibiotics and there is increasing pressure to do so in North America.

Thus, there is increasing interest in alternatives to growth promotant antibiotics. Fundamental to developing alternatives to growth promotant antibiotics is the enhancement of our understanding of defence systems used to inhibit pathogens, their interactions and regulation.

■ Host Defenses Against Infection

The pig's defence against pathogens includes a combination of physical processes (gastric acidification, rapid transit through the small intestine), as well as the epithelial lining of the intestine, the mucosal immune system and the intestinal microbiota (Gaskins, et al., 2000, Mackie, et al., 1999). Effective defence against pathogens requires that all of these systems are functioning properly. Factors affecting these systems include genetics, pathogen load and stressors. We are just starting to learn about the extensive amount of cross-

talk between these systems (Deplancke and Gaskins, 2001, Hooper, et al., 2002, McCracken and Lorenz, 2001, Sonnenburg, et al., 2004, Xu and Gordon, 2003).

Symbiotic Microbiota

The microorganisms that are frequently associated with the majority of animals are called by a variety of names (normal, commensal, indigenous, symbiotic) and symbiotic will be used here. Opportunistic pathogens may be a small component of this microbiota, but only become pathogenic in response to specific environmental conditions. Studies using germ-free, gnotobiotic (colonized by selected bacterial species) and conventional animals clearly show that the symbiotic bacteria provide protection against enteric pathogens. Although the intestinal microbiota is complex and the role of most of the bacteria in protecting the pig is not clear, bacterial species of the genera *Lactobacillus* and *Bifidobacterium* have been shown to provide protection against enteric infections. These two genera have also been shown to be suppressed when animals or humans are stressed. Recent research is unfolding a picture of how the symbiotic bacteria inhibit enteric pathogens and how they synergistically interact with the intestinal epithelium and mucosal immune system.

The intestinal tract is sterile at birth and undergoes a succession of bacterial populations before a stable microbial ecology is established. Facultative organisms, such as *E. coli*, initially colonize the intestinal tract. As the animal ages, the intestinal tract is subsequently colonized by relatively aerotolerant lactic acid bacteria (lactobacilli and bifidobacteria), bacteroides and other anaerobic bacteria (Mackie, et al., 1999, Tannock, 1997). The intestinal microbial community structure becomes increasingly complex after weaning and with time, the microbial community structure becomes relatively stable at the genus level. However, there is increasing evidence that the species composition within a genus is dynamic and varies between individual animals. Thus, subtle changes in bacterial species may alter host resistance to pathogens. The microbial community structure is thought to be altered when the animal is stressed and this altered microbiota, along with altered epithelial and immune function, provide pathogens with an increased opportunity for colonization. Although the mechanisms of this decrease in bacterial resistance and specific microbial population changes involved are not known, lactobacilli and bifidobacterial populations have been shown to decrease in humans and these or similar beneficial populations may be decreased in pigs.

The symbiotic intestinal microbiota inhibit pathogens through a variety of mechanisms. The mechanism, or combination of mechanisms used depends upon the microbial species and the ecosystem within which they are residing.

Possible mechanisms are:

- competition for nutrients
- production of toxic conditions or compounds (low pH, fermentation acids, bacteriocins, etc.)
- competition for binding sites on epithelial surfaces, or in the tightly adherent mucus layer
- stimulation of the immune system

The intestinal microbiota are in highest numbers in the lumen of the intestinal tract, where competition for nutrients and production of toxic compounds would logically be important. The intestinal microbiota also colonize the mucus layer lining epithelial cells and thus competition for binding sites, in addition to competition for nutrients and production of toxic compounds may be important. It is also most likely that the mucosally associated microorganisms are directly, or indirectly responsible for stimulating the immune system. Thus, manipulation of the microbiota in specific ecosystems influences the efficacy with which the symbiotic microbiota inhibits pathogens.

The symbiotic intestinal bacteria do not act alone to inhibit enteric pathogens, but act in concert with the intestinal epithelial barrier and the mucosal immune system. There are a number of management approaches that either enhance or suppress this alliance. However, understanding how they function is important in developing approaches to influence these defence systems.

Epithelial Tissues

The epithelial lining provides a physical barrier against entry of large molecules and microorganisms into the body proper. The morphology of the epithelial lining changes along the intestinal tract, but essentially consists of crypt regions (where cells are generated from stem cells) and apical or villi (depending upon location) regions, where cells have differentiated to perform different functions, and eventually die and are released into the lumen of the intestine. Cell turnover is rapid along the intestinal tract with cells being replaced every 3-5 days. Changes in epithelial tissue morphology alter nutrient absorption and defence against pathogen invasions. Although it is well known that pathogens that attach to epithelial cells conscript the regulatory control of that cell, new information indicates that at least some species of the symbiotic microbiota that are in close association with the epithelium also influence activities of epithelial cells (Hooper, et al, 2002, Xu and Gordon, 2003). These changes in epithelial cell metabolism enhance colonization by those bacterial species and potentially enhance protection of the epithelial surface from colonization by pathogens. Thus, it is becoming increasingly clear that there is extensive cross-talk between the intestinal microbiota and epithelial cells.

Mucosal Immune System

The mucosal immune system is comprised of lymph nodes (mesenteric lymph nodes and Peyer's Patches, located in the ileum) and diffuse lymphoid cells. The immune system has both innate and adaptive arms (Pickard et al., 2004). The innate system includes cells (macrophages, dendritic cells) that mount a non-specific response to the presence of any foreign antigen. The adaptive system has memory and once exposed to a pathogen, the adaptive system produces antibodies that are protective against subsequent infections by the same organism. Since the intestinal tract is constantly exposed to bacteria, it has to develop tolerance to the symbiotic bacteria and does so through a complex process. The mucosal immune system develops tolerance to the indigenous microbiota and food antigens, resulting in an accumulation of IgA secreting B cells, T cells, macrophages and dendritic cells in the tissues lining the intestinal tract. In essence, the mucosal epithelium elicits a mild or controlled (Th1) inflammatory response to the symbiotic microbiota and a much more dramatic inflammatory response to pathogens. This allows the mucosal epithelium to respond more rapidly to pathogen challenge; however, it is expensive, from an energetic standpoint, to maintain this primed immune system in the absence of pathogen challenge (Anderson, et al. 2000). Good management practices can minimize pathogen load.

Neuroendocrine

The mucosal epithelium is heavily innervated, with sympathetic and parasympathetic nerves influencing normal function of the intestinal system and mucosal tissues are exposed to hormonal action. When the animal is stressed, the hypothalamic-pituitary axis (HPA) responds by secretion of corticosteroids and direct neuronal stimulation of the mucosal tissues (Matteri, et al., 2000, Petrovsky, 2001). Thus, stress associated with production, including environmental, nutritional, weaning, transportation, and commingling suppress the pig's ability to resist pathogens and increases the incidence of subclinical or clinical infection. Growth of some pathogenic bacteria is stimulated by addition of the stress hormones norepinephrine and epinephrine. This has been shown by growth in serum simulating medium and by increases in gram negative (*E. coli*) bacteria in the intestinal tracts of animals stimulated to release norepinephrine (Lyte, 2004). More information is needed on the interactions between the neuroendocrine response to stressors and regulation of pathogenic and symbiotic microbiota in the intestine.

Thus, there is extensive cross-talk between the various defence systems. These systems are effective in suppressing pathogens, and under normal conditions, few pigs become sick. Pigs that do become sick effectively control the infection. However, when the pig is challenged by environmental conditions that suppress one or all of these systems and when the pathogen load is high, then it becomes much more likely that an individual pig will become infected,

the pig will become sicker and contribute to the pathogen load of pen mates and results in spread of the disease.

■ Prebiotics

In complex animal ecosystems, animals tend to be generalists or specialists in food selection. Cattle tend to be grazers, goats tend to select more succulent leaves and pigs are omnivores and eat a wide variety of foods. Although there is some overlap in food selection, they can coexist in the same area and availability or limitation of different nutrients affects each species differently. Similar principals exist in the intestinal tract where different bacterial species have different preferences and abilities to scavenge for specific nutrients. Carbohydrates are thought to be the first limiting nutrient for many bacterial species in the intestinal tract and thus the type of carbohydrates available influence the growth of bacterial species differently, selectively enriching growth of some bacterial species, or limiting growth of others. 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). Prebiotics are commonly fed to weanling pigs in Japan and are increasingly being used in Europe (Ficklinger, 2003); however, their use is just beginning to increase in North America.

Probiotic, or direct fed, microorganisms enhance one or all of the defence systems as described above for the symbiotic microbiota. Prebiotic compounds act by enriching beneficial bacterial populations, which subsequently influence the defensive capability of the animal through the above mechanisms. An advantage of prebiotics from a delivery standpoint is that one does not have to be concerned about viability of the product. The majority of prebiotic compounds are oligosaccharides (**Table 1**) with oligofructose and inulin being the primary research and commercial prebiotics.

Table 1. Prebiotic oligosaccharides studied or in use

Arabinoxylan	Isomaltose (IM)
Agarooligosaccharides (AOS)	Lactosucrose
Cyclodextrins	Lactose
Fructooligosaccharides (FOS)	Lactulose
β Galactooligosaccharides (GOS)	Mannanooligosaccharides (MOS)
Raffinose, stachyose	Oligofructose
Glucosyl sucrose (GlcS)	Sucrose thermal oligosaccharide
Isomalturose (IMT)	caramel (STOC)
Inulin	Xylooligosaccharides (XOS)

According to the definition of prebiotics, mannanoligosaccharides are not true prebiotics. Their primary mode of action is not through selective enrichment of specific bacterial populations. However, mannanoligosaccharides have also been shown to enhance pig performance (Miguel, et al., 2002). Most of the prebiotic oligosaccharides are small molecular weight compounds with linkages that are resistant to mammalian enzymes, yet are susceptible to fermentation by certain microbial populations.

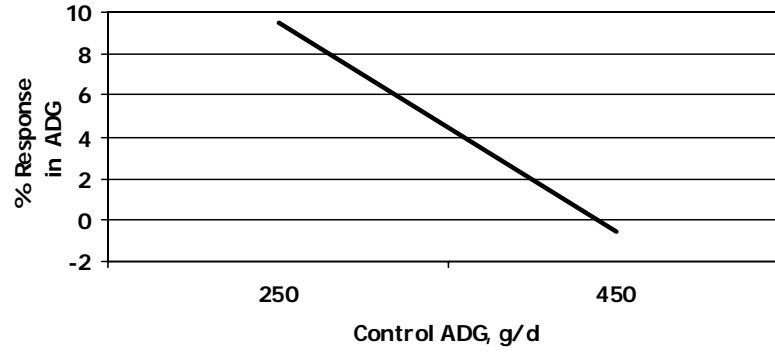
Prebiotic Effects on Host Defence Systems

Studies in rodent models and humans have shown that prebiotics alter immune function, intestinal morphology, microbial populations, intestinal pH, VFA concentrations, mineral absorption and disease resistance (Buddington, et al, 2002, Saavedra and Tschernia, 2002, Scholz-Ahrens and Schrezenmeir, 2002, Schley and Field, 2002). There is less information on the effects of prebiotics on pigs, where the emphasis has been on performance and microbial population and activity changes (Patterson and Burkholder, 2003).

Prebiotic Effects on Performance

Prebiotics are thought to give more variable responses than do growth promotant antibiotics, however many of the studies do not have growth promotant antibiotic treatments for direct comparison. Although some studies do show little response, a number of studies have shown at least trends for improvements in growth performance, decrease in variation, mortality and morbidity, or decreased medicine costs when prebiotics are fed (Patterson and Burkholder, 2003). Many of these studies do not show statistically significant responses because of low replication, low environmental challenge, level of prebiotic fed, timing of feeding the prebiotic vs. challenge or stressor level. As with growth promotant antibiotics, environmental conditions affect response to prebiotics. **Figure 1** demonstrates that as growth performance of pigs fed control diets increases (pigs are performing closer to their genetic potential, i.e. less antigenic and environmental stress), the response to Bio-Mos decreases. Similar responses to growth promotant antibiotics can be seen in **Figure 2**, where as ADG of the pigs fed control diets increases, the treatment response to the antibiotic decreases. **Figure 3** shows that gain/feed response is also lower for these same pigs when they are growing closer to their genetic potential. Thus, performance response to growth promotant antibiotics and to Bio-Mos are affected by the genetics of the pigs, stress and their environment.

Figure 1. Growth Response to BioMos



Adapted from Pettigrew, 2000.

Figure 2. Effect of Antibiotics on Average Daily Gain of Weanling Pigs ¹

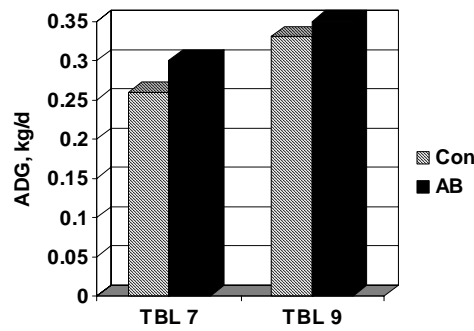
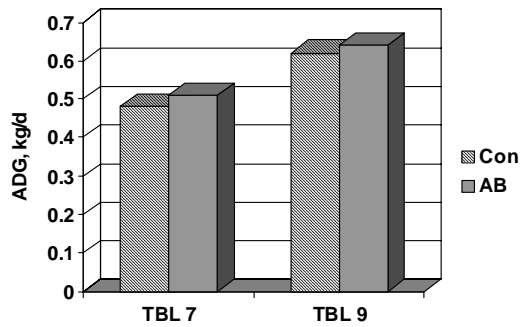


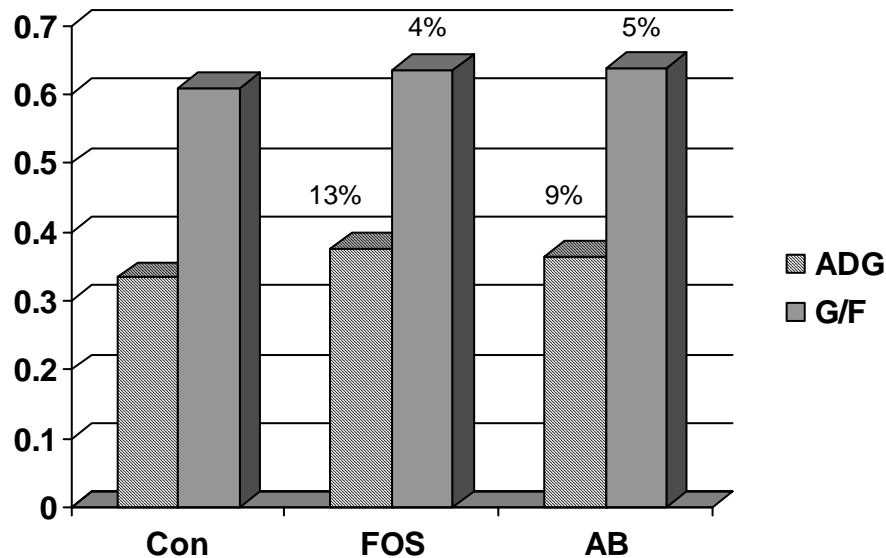
Figure 3. Effect of Antibiotics on Average Gain/Feed of Weanling Pigs ¹



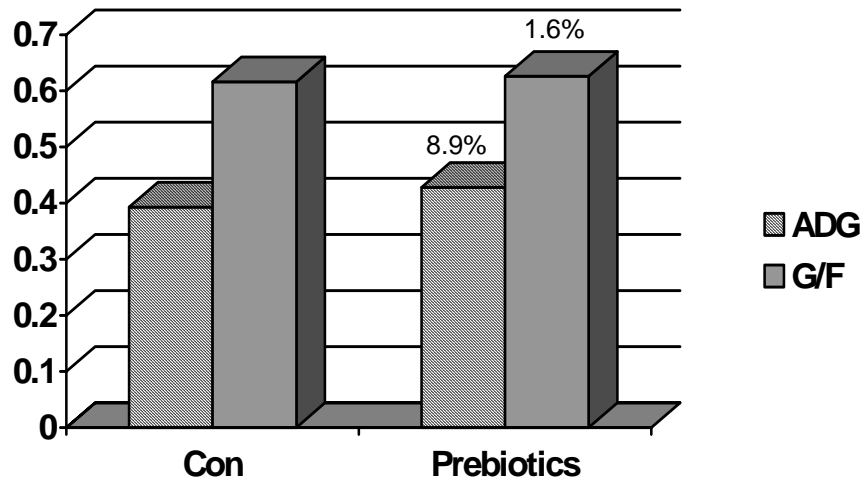
¹ Adapted from Cromwell, 2002. Table 7 was a summary of 14 experiments; Table 9 was a summary of seven experiments. Percent values are the increase over controls.

Response to growth promotant antibiotics is greater when pigs are stressed (Cromwell, 2000) and it seems logical that response to prebiotics would also be increased when pigs are stressed. In a comparison of nine experiments that contained control, FOS and growth promotant antibiotic treatments, there was a slightly greater increase in ADG for FOS treated pigs than for growth promotant antibiotic treated pigs (**Figure 4**). Gain/feed was slightly greater for the growth promotant antibiotic treated pigs. In a comparison of eighteen experiments with both control and prebiotic treatments, pigs fed the prebiotic treatments had an 8.9% increase in ADG and a 1.6% increase in G/F (**Figure 5**). In all of these comparisons, performance response was greater when ADG of the controls was closer to 0.3 than to 0.4 kg/d. This data suggests that when comparing efficacy of prebiotics, it is important to include a growth promotant antibiotic treatment as a positive control and that the growth rate of the control animals should be a good indicator of whether one should see a growth promotant response with any treatment.

Figure 4. Effects of Prebiotics and Antibiotics on ADG (kg/d) and G/F in Weanling Pigs



Data from nine experiments where FOS and growth promoting antibiotics were used in the same experiment. Per cent values are the increase over controls.

Figure 5. Effect of Prebiotics on ADG (kg/d) and G/F in Weanling Pigs

Data from 18 experiments. Per cent values are the increase over controls.

■ Conclusions

Concern over the spread of antibiotic resistance is stimulating interest in alternatives to antibiotics. At the same time, we are making breakthroughs in our understanding of the intestinal microbiota and how they interact with the intestinal epithelium and mucosal immune system to modulate animal health, performance and subsequently preharvest food safety. The symbiotic microbiota enhance pig health by inhibiting pathogen colonization and by stimulating both the epithelial lining and the mucosal immune system. We are beginning to understand how environmental stressors negatively affect some components of the symbiotic microbiota, resulting in reduced resistance to infection by pathogens. There are a number of dietary approaches that producers can undertake as alternatives to growth promotant antibiotics to enhance pig health and performance. Dietary approaches that specifically affect the symbiotic microbiota include organic acids, probiotics and prebiotics. As with growth promotant antibiotics, these dietary approaches, and their combinations, have greater efficacy in enhancing performance when animals are growing slowly due to environmental stressors and high pathogen loads. From a research standpoint, it is important to include both negative and positive (growth promotant antibiotic) treatments in experiments on efficacy of dietary alternatives to growth promotant antibiotics.

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Internet search sites for scientific articles and reviews, many of the articles may be downloaded or printed.

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