Probiotics as a Dietary Additive for Pigs: A Review

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Abstract: Probiotics (Direct-Fed Microbial: DFM) have been established for use as a feed additive; researchers have observed many beneficial effects of such by improving the intestinal microbial balance in livestock. The functions of probiotics within a Gastrointestinal Tract (GIT) are suggested to include the following: competing with pathogenic bacteria for nutrients in the gut; competing with pathogens for binding sites on the intestinal epithelium; producing compounds that are toxic to pathogens, and stimulating the immune system. The applications of probiotics provide a potential alternative strategy to antibiotic use in livestock. It is suggested that probiotics should be used as a feed additive in livestock.

Key words: Probiotics, feed additive, pigs, livestock, immune system, microbial balance

INTRODUCTION

There are hundreds of bacterial strains that inhabit both human and animal Gastrointestinal Tracts (GIT). The majority of these bacteria are strict anaerobes and can be divided into two classes. Harmful or toxic bacteria i.e., Salmonella species and Escherichia coli (E. coli) are bacteria that colonize within the digestive tract and produce toxic waste products which lead to gas or bloating, diarrhea, constipation, ulcers or more serious events like food poisoning (Madsen, 2001). Probiotics refer to the good or friendly bacteria that have positive benefits for the body by regulating the gut microflora balance of the host (Fuller, 1989). Presently, there is increasing interest concerning the use of probiotics in the livestock industry (Wang et al., 2009a, b; Meng et al., 2010).

During the past several decades, antibiotics have been widely used in livestock diets due to their therapeutic effects, specifically in inhibiting diseases (Solomons, 1978). However, since prolonged antibiotic use results in antimicrobial resistance, a number of countries have recently restricted the subtherapeutic use of antibiotics (Tronsid, 1997). The application of probiotics provides a potential alternative strategy to the traditional practice of subtherapeutic antibiotic use. However, there is still a need to clarify the effectiveness of probiotics in pigs and the underlying mechanisms through which they function. Several studies related to probiotics were performed on livestock and beneficial effects including growth enhancement and disease prevention were observed.

THE ROLE OF INTESTINAL BACTERIA IN PIGS

It has been confirmed that the indigenous bacteria of the intestinal tract play an integral role in the health and nutrition of animals (Conway and Macfarlane, 1995). The estimated number of bacteria species and populations are over 400 and 10^4, respectively (Holzapfel et al., 1998). Abrams et al. (1963) suggested that gastrointestinal microflora is important for the normal development of gut morphology and functioning. Exhibiting an optimum microbial balance generally indicates both good health and nutrition within an animal (Rybka and Kailasapathy, 1995). A comparison of conventional and germ-free animals suggested that the latter had reduced intestinal motility (Abrams and Bishop, 1967) and a relatively weak immune system (Umesaki et al., 1993). Those characteristics were quickly restored after the addition of a normal microflora to the gastrointestinal tracts of germ-free animals.

Besides beneficial intestinal bacteria there are also various pathogenic microbes such as E. coli which have been implicated in reducing the rates of growth and health statuses of pigs (Gaggia et al., 2010). Relevant mechanisms for these reductions are toxin production, utilization of essential nutrients to the host and the suppression of microbes that synthesize vitamins or other host growth factors (Falaki et al., 2010). In addition, enterotoxigenic E. coli colonize in the small intestine and produce enterotoxins to stimulate the epithelial cells to

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secrete fluid into the lumen of the gut, thereby causing diarrhea (Gaastra and de Graaf, 1982). Francis et al. (1998) reported that enterotoxigenic \textit{E. coli} strains that express K88 fimbrial antigens are a major cause of diarrhea and death of neonatal and weaned pigs. A recent study showed a positive effect of probiotic supplementation of \textit{E. coli} infected weaning pigs with \textit{L. sobrius} not only on pathogen levels but also on performance (Konstantinov et al., 2008).

**SPECIES OF PROBIOTICS**

Many strains of bacteria have been used as probiotics, the most commonly used species being lactic acid bacteria such as \textit{Lactobacillus}, \textit{Streptococcus} and \textit{Bifidobacteria} (Durne et al., 2001). Commercial species of probiotics are usually isolated from the intestinal microflora of the intended consumer (for example human, chicken or pig) and selected on the basis of criteria such as resistance to stomach acids and bile salts, ability to colonize in the intestine or antagonism of potentially pathogenic micro-organisms (Verdenelli et al., 2009). Lactic acid bacteria are found in large numbers in the gut of healthy animals and do not appear to affect them adversely. According to the words of the FDA (Chen et al., 2006), lactic acid bacteria were Generally Regarded as Safe (GRAS). Lactic acid bacteria used as probiotics have included: \textit{L. acidophilus}, \textit{L. casei}, \textit{L. delbrueckii} subsp. \textit{bulgaricus}, \textit{L. brevis}, \textit{L. celiobiosus}, \textit{L. curvatus}, \textit{L. fermentum}, \textit{L. lactis}, \textit{L. plantarum}, \textit{L. reuteri}, \textit{S. cremoris}, \textit{S. salivarius} subsp. \textit{thermophilus}, \textit{E. faecium}, \textit{S. diacetylactis}, \textit{S. intermedius}, \textit{B. bifidum}, \textit{B. adolescentis}, \textit{B. animalis}, \textit{B. infantis}, \textit{B. animalis}, \textit{B. longum}, \textit{B. infantis}, \textit{B. longum} and \textit{B. thermophilum} (\textit{L. lactobacilli}, \textit{S. Streptococci}, \textit{B. Bifidobacteria}) (Sekhon and Jainath, 2010).

Species other than lactic acid bacteria which are currently being used in probiotic preparations include \textit{Bacillus} species and yeasts (\textit{Saccharomyces cerevisiae} and \textit{Aspergillus oryzae}). \textit{Bacillus} species are mostly soil organisms, some of which are used for the production of antibiotic substances and are not normal components of the indigenous microflora (Jonsson and Conway, 1992). \textit{Bacillus} products could compete with other intestinal microflora for nutrients (Fretre, 1992) or might produce an antibacterial substance (Hentges, 1992) if the products were continually fed. \textit{Lactobacillus} and \textit{Bifidobacterium} species have been used most extensively in humans whereas species of \textit{Bacillus}, \textit{Enterococcus} and \textit{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 \textit{Lactobacillus} to livestock (Pascual et al., 1999; Gusils et al., 1999; Jin et al., 2000; Tellez et al., 2001). Yeasts naturally occur on plant material and can be found among the enteric microflora in animals (Mathew et al., 1998). Enzymes, vitamins and other nutrients contained within yeast have been proposed to produce beneficial performance responses in pigs (Kornegay and Risley, 1996).

**MECHANISMS OF ACTION MODES OF PROBIOTICS**

Probiotics function in several ways and the involved mechanisms may diverge due to the different types of probiotics. Generally, the action mode of probiotics are suggested to be as follows:

**Competing with pathogenic bacteria for nutrients in the gut:** Probiotics may compete for nutrients and absorption sites with pathogenic bacteria (Malago and Koninkx, 2011). The gut is such a rich source of nutrients that it may seem unlikely that microorganisms could not find sufficient food for growth. However, it should be noted be that the environment only has to be deficient in one essential nutrient in order to inhibit microbial growth. In addition, the ability to rapidly utilize an energy source may reduce the log phase of bacterial growth and make it impossible for the organism to resist the flushing effect exerted by peristalsis (Wilson and Perini, 1988).

**Competing with pathogens for binding sites on the intestinal epithelium:** Colonization by a bacterial species is defined by the presence of a bacterial population in the gastrointestinal tract which is stable in size and occurrence over time without the need for periodic reintroduction of the bacteria by repeated oral doses or other means (Mackie et al., 1999). The mechanism of colonization is suggested to be associated with certain species within the microflora which can influence the expression of glycoconjugates on epithelial cells that may serve as receptors for the adhesion of bacteria (Umesaki et al., 1997). It is commonly supported that most intestinal pathogens must adhere to the intestinal epithelium if they are to colonize in the intestine and produce diseases (Walker, 2000). Consequently, some bacterial strains have been chosen as probiotics for their ability to adhere to the gut epithelium and thus compete with pathogens for adhesion receptors (Savage, 1969). So called competitive exclusion is defined by the ability of normal microflora to protect against the harmful establishment of pathogens (Jeffrey, 1999). However, attempts to select an individual microorganism or a
specific mixture of microorganisms with the specificity of normal microflora to resist pathogen invasion have not been successful until recently. An expected effect of the addition of probiotics to the gastrointestinal tract is an increase in normal microflora colonization with inhibition of the adhesion of harmful pathogens on the intestinal epithelium.

**Producing compounds that are toxic to pathogens:** Probiotic bacteria produce a variety of substances that are inhibitory to both gram-positive and gram-negative bacteria in the gut (Corcionivoschi et al., 2010). These include organic acids, antioxidants and bacteriocins (Reid, 2001). These compounds may reduce not only the number of viable pathogenic organisms but may also affect bacterial metabolism and toxin production (Murali et al., 2010). Bacteriocins produced by lactic acid bacteria have been reported to be able to permeate the outer membrane of gram-negative bacteria and subsequently induce the inactivation of gram-negative bacteria in conjunction with other enhancing antimicrobial environmental factors such as low temperatures, organic acids and detergents (Alakomi et al., 2000).

**Stimulating the immune system:** Analysis and research into the ability of probiotics to influence the immune system of animals and humans is a recent development in this field. Probiotics provide defence to the cells by inducing antinflammatory cytokines and reducing proinflammatory cytokines from enterocytes and intestinal immune cells recruited to sites of inflammation by probiotics (O’Harra et al., 2006; Walsh et al., 2008; Wang et al., 2009b). Some probiotic strains such as Lactobacillus have proven to be capable of acting as immunomodulators by enhancing macrophage activity (Perdigon et al., 1986), increasing the local antibody levels (Yasui et al., 1989), inducing the production of interferon (De Simone et al., 1986) and activating killer cells (Kato et al., 1984). However, it is difficult to completely conclude that probiotics contribute significantly to the immune system of the host. The main reason behind this caveat is that probiotics differ from antibiotics in that they are not intended to eradicate invasive pathogens in the gastrointestinal tract. Therefore, such observed improvements or positive effects are always somewhat compromised due to the animals immune system status and the various applied situations.

**THE EFFECTS OF PROBIOTICS IN PIGS**

**Growth performance:** In the livestock industry, the use of probiotics aims to improve intestinal health which can then lead to better general health and productivity among animals. Supplementation of Bacillus species has also resulted in improved growth rates and feed efficiency in piglets (Kyriakis et al., 1999) and grower pigs (Sauci et al., 1995). Davis et al. (2008) reported that the addition of 0.05% of DFM (based on B. licheniformis and B. subtilis; 1.47×10⁶ CFU) improved Average Daily Gain (ADG) and reduced mortality rates of growing and finishing pigs. Alexopoulos et al. (2004) demonstrated that 0.04% probiotic (B. licheniformis and B. subtilis) supplementation of the feed improved sow feed intake and decreased sow weight loss during the sucking period in commercial farms. Probiotic (non-pathogenic E. coli; 50 mL of 9×10⁸ CFU mL⁻¹ per day) fed with a low protein (17%) diet increased performance of weanling pigs (Blancari et al., 2010). Malloa et al. (2010) demonstrated that the inclusion of E. faecium (10⁶ CFU g⁻¹) significantly improved growth (392 vs. 443 g day⁻¹) and feed conversion ratio (1.74 vs. 1.60 g feed g⁻¹ gain) of weanling pigs (28 days of age). Giang et al. (2010a, b) reported that piglets fed probiotic complexes diets (E. faecium, 3×10⁸ CFU kg⁻¹; L. acidophilus, 4×10⁵ CFU kg⁻¹ and L. plantarum, 2×10⁵ CFU kg⁻¹) had higher feed intake, daily gain and better feed conversion during the 1st 2 weeks after weaning. Giang et al. (2010a) also reported that lactic acid bacteria complexes, comprising combinations of Enterococcus faecium GH2 (3×10⁸ CFU g⁻¹), Lactobacillus acidophilus C3 (4×10⁶ CFU g⁻¹), Pediococcus pentosaceus D7 (3×10⁶ CFU g⁻¹), L. plantarum 1K8 (2×10⁶ CFU g⁻¹) and L. plantarum 3K2 (7×10⁶ CFU g⁻¹), increased (p<0.05) daily feed intake and weight gain and improved feed conversion ratio. Ross et al. (2010) demonstrated that feed efficiency was significantly greater in pigs supplemented with 3 mL of a mixed probiotic culture (L. amylovorus and E. faecium, 10⁶ CFU mL⁻¹) than pigs fed a control diet. Veizaga-Delac et al. (2010) demonstrated that 0.001% probiotic (L. plantarum, 5×10⁵ CFU kg⁻¹, L. fermentum, 5×10⁵ CFU kg⁻¹ and E. faecium, 5×10⁶ CFU kg⁻¹) supplementation increased body weight and ADG. Some reports have indicated that feeding lactic acid bacteria by Lactobacilli improves performance in suckling pigs 0 (Abe et al., 1995), weanling pigs (Jaisak et al., 1992), grower pigs (Baird, 1977) and finishing pigs (Jong et al., 2002; Jonsson and Conway, 1992). Live yeast supplementation to the diet of pigs has resulted in demonstrable improvements in growth rate (Mathew et al., 1998) and reductions in the quantity of pathogenic bacteria (Anderson et al., 1999). Ko and Yang (2008) investigated the effect of green tea probiotics on performance of finishing pigs. They reported that supplementation of 0.5% green tea probiotic has a positive effect compared to 0.036% antibiotic (chlorotetracycline).
Fermented diets may be an alternative to the prophylactic use of antimicrobial growth promoters in pig diets (Scholten et al., 1999). Feeding a fermented diet minimizes the time available for the gastrointestinal microflora to decarboxylate free amino acids present in the diet which has shown to improve performance in pigs (Scholten, 2001; Pedersen et al., 2002; Pedersen, 2006). Kyriakakis et al. (1999) demonstrated that fermented liquid diets with probiotics improved performance in pigs.

**Digestibility:** Regarding nutrient digestibility as influenced by probiotics, positive effects on performance were observed by Hong et al. (2002). Probiotics possess a high fermentative activity and stimulate digestion (Ouwehand et al., 2002). Lactobacilli are known to produce lactic acid and proteolytic enzymes which can enhance nutrient digestion in the gastrointestinal tract (Yu et al., 2008). Lactobacilli can colonize and adhere to the gastrointestinal tract epithelium forming a protective membrane against pathogenic microorganisms while at the same time modulate immunity with stimulating epithelial lymphocytes (Yu et al., 2008). Yu et al. (2008) demonstrated that L. fermentum (5.8×10^7 CFU g^-1) maximized the crude protein digestibility among the different concentrations of L. fermentum in the control diet. Meng et al. (2010) reported that pigs fed probiotics (mixture of spray-dried spore-forming Bacillus subtilis and C. butyricum endospores) showed greater crude protein and energy digestibility compared to those in non-probiotic treatments in growing pigs. Giang et al. (2010b) demonstrated that supplementation with lactic acid bacteria complexes (Enterococcus faecium 6H2, 3×10^8 CFU g^-1, Lactobacillus acidophilus C3, 4×10^6 CFU g^-1, Pediodioscoccus pentosaceus D7, 5×10^6 CFU g^-1, L. plantarum 1K8, 2×10^6 CFU g^-1 and L. plantarum 3K2, 7×10^6 CFU g^-1) diets increased (p<0.05) the ileal apparent digestibility of crude protein, crude fibre and organic matter and the total tract apparent digestibility of crude protein and crude fibre in the first 2 weeks after pigs weaning.

**Immunity:** Probiotics stimulation of the immune system in pigs was observed by several authors (Takahashi et al., 1998; Franscisco et al., 1995). Oelschläger (2010) reported that probiotics can influence the immune system by products like metabolites, cell wall components and DNA. Obviously, immune modulatory effects might be even achieved with dead probiotic bacteria or just probiotics derived components like peptidoglycan fragments or DNA. Wang et al. (2009a, b) demonstrated that the feeding of L. fermentum induced an increase in the pro-inflammatory cytokines and the percentage of CD41 lymphocyte subset in blood. Taras investigated the effects of long-term application of E. faecium on performance, health characteristics of sows and offspring. They reported that probiotic supplementation reduced overall pre-weaning mortality (16.2 vs. 22.3%) and the rate of piglets with post-weaning diarrhea (21 vs. 38%). Reduction of diarrhea by probiotics was studied frequently (Table 1) because diarrhea is the main problem of piglets during the 1st weeks after weaning with utmost importance for production (Simon et al., 2001).

The efficacy of probiotics under different conditions may be due to the probiotic preparation itself or various other factors. These factors may include the low survival rate of strains, varying stabilities of strains, low probiotic doses, frequency/intensity of administration, interactions with some medicines (antibiotics and antimicrobials), health and nutritional status of the animal and the effect of age, stress, genetics and type differences of animals (Bomba et al., 2002). Research points to the fact that probiotics are most effective in animals during microflora development or when microflora stability is impaired (Stavric and Kornegay, 1995). Therefore, it is also suggested that the effects of probiotics appear to be more consistent and positive in piglets rather than in growing finishing pigs (William, 2000).

*E. longum* and other lactic acid bacteria have been found to increase the total amount of intestinal IgA (Takahashi et al., 1998; Viitini et al., 2000). Likewise, *L. casei* has been reported to have immunoadjuvant activity (Perdigon et al., 2003) and *L. plantarum* was

<table>
<thead>
<tr>
<th>Probiotic</th>
<th>Age</th>
<th>Diarrhea-frequency</th>
<th>Statistical significance</th>
<th>Literature</th>
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<tbody>
<tr>
<td><em>B. cereus</em></td>
<td>Day 1-56</td>
<td>Lowered</td>
<td>+</td>
<td>Kyriakakis et al. (1999)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Day 1-85</td>
<td>Lowered</td>
<td>+</td>
<td>Iben and Lebetseder (1989)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Day 1-21</td>
<td>Lowered</td>
<td>+</td>
<td>Zani et al. (1998)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Day 24-66</td>
<td>No effect</td>
<td>-</td>
<td>Eidelshager et al. (1992)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Till 25 kg live weight</td>
<td>No effect</td>
<td>-</td>
<td>Kirchgesner et al. (1993)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>2 weeks after weaning</td>
<td>Lowered</td>
<td>+</td>
<td>Jadassus</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>Day 1-70</td>
<td>Lowered</td>
<td>+</td>
<td>Manner and Spieler (1997)</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>4 weeks after weaning</td>
<td>Lowered</td>
<td>+</td>
<td>Eigene Ergebnisse</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>8 days prior and after weaning</td>
<td>Lowered</td>
<td>+</td>
<td>Schunn et al. (1999)</td>
</tr>
<tr>
<td><em>P. acidilactici</em></td>
<td>Day 5-28</td>
<td>Lowered</td>
<td>+</td>
<td>Durst et al. (1998)</td>
</tr>
<tr>
<td><em>P. acidilactici</em></td>
<td>Day 5-28</td>
<td>Lowered</td>
<td>+</td>
<td>Durst et al. (1998)</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
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shown to increase antibody production against \textit{E. coli} (Herias \textit{et al.}, 1999). Scharek \textit{et al.} (2007) demonstrated that the population of intraepithelial CD8\(^+\) T cells was significantly enhanced in the piglets fed probiotic (\textit{B. cereus} var. toyoi) and the numbers of \(\gamma\delta\) T cells (gamma delta T cells) tended to be higher in the intestinal epithelium at the time of weaning (day 28). \textit{Lamina propria} lymphocytes were also influenced by supplementation with probiotic. \textit{E. faecium} supplementation enhanced the course of infection in weaning pigs challenged with \textit{Salmonella} serovar typhimurium and increased production of specific antibodies against Salmonella (Szabo \textit{et al.}, 2009).

\section*{CONCLUSION}

It is evident that the complex microbial flora presented in the Gastrointestinal Tract (GIT) of all warm-blooded animals is effective in providing resistance to disease. It should also be noted that probiotics are not a single entity. Different probiotics contain different microorganisms which may behave differently. Even different strains of the same species may have different metabolic activities which in turn affect the immune system and growth results of hosts when they are used as probiotics. Therefore, it is necessary to evaluate the efficacy of different probiotic preparations as well as optimal supplementation strategies.

\section*{RECOMMENDATIONS}

It is suggested that more attention should be paid to researching the application of probiotics as there is a significant amount of potential for the application of probiotics in the livestock feed industry. It was the intent of this review to further elucidate the effects of utilizing different probiotic preparations on pigs and to determine a corresponding optimal feeding strategy for these cultures.

\section*{REFERENCES}


