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The Potential Benefits of Probiotics in Animal Production and Health

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Abstract: Probiotics are defined as microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance. The probiotics were improved feed conversion for the target species, reduced morbidity or mortality and benefits for the consumer through improved product quality. In this study, we found that a combination of probiotics with different mechanisms of action could amplify the protective range of bio-therapeutic preparations and the potentiated probiotics are more effective than their components separately. Bacterial probiotics were effective in chickens, pigs and pre-ruminant calves, whereas yeasts and fungal probiotics were given better results in adult ruminants. Probiotics were enhanced the growth of many domestic animals improved the efficacy of forage digestion and quantity and quality of milk, meat and egg. Probiotics protected animals against pathogens, enhanced immune response, reduced antibiotic use and shows high index of safety. The trend for future could be focus on basic research to identify and characterize existing probiotics strains, determine optimal doses needed for certain strain and asses their stability through processing and digestion.

Key words: Probiotics, growth, meat, milk, health, animal

INTRODUCTION

Understanding how probiotics exert their beneficial effects is the issue of debate nowadays. Foods containing probiotic microbes for human consumption have been marketed in Japan since the 1920s (Svensson, 1999). The first bacteria used were *Lactobacillus acidophilus* and *L. casei* which were components of fermented milk products. The definition of probiotic was formulated simultaneously with the use of living cultures in feed for various animals in order to substitute the application of nutritive antibiotics or chemotherapeutics. In the meantime probiotics are applied as feed supplements, pharmaceuticals, dairy products, fruit juices, chocolates and even meat products. A prebiotic is defined as non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or more of the gut-beneficial microbe groups (Gibson and Roberfroid, 1995). The most commonly used prebiotics are carbohydrate substrates with the ability to promote the components of the normal intestinal microflora which may evince a health benefit to the host. However, the synbiotics is the combined administration of specific prebiotics with probiotics to provide definite health benefits by synergistic action (Harish and Varghese, 2006). The selection of a suitable strain of a microorganism can be regarded as the primary requirement for the use as a probiotic. The composition of the probiotic preparations varies from those containing one strain of microorganism or those containing many strains. The multi-strain probiotic can act in broad spectrum and expected to be active in different species of host animals and against microbial infections (Timmerman *et al*., 2004).

The conventional use of probiotics to modulate gastrointestinal health, such as improving lactose intolerance, increasing natural resistance to infectious diseases in the gastrointestinal tract, suppressing traveler's diarrhea and reducing bloating, has been well investigated and documented (Liong, 2007). The probiotic research was applied in pets, horses and farm animals, while the majority of research was done in chickens and pigs. In clinical trials, probiotics have been reported to enhance the growth of many domestic animals including cows (Doreau and Jouany, 1998), neonatal calves and piglets (Kyriakis *et al*., 1999) and broilers (Tellez *et al*., 2001). Studies on efficacy of probiotics strains must be performed in target species/animal categories. The claims for microbial products are: improved performance and feed conversion for the target species; reduced morbidity or mortality; benefits for the consumer through improved product quality. Recombinant probiotics and the principle of alternative gene therapy represent the latest approach of using

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Genetically Modified Organisms (GMO) for biomedical applications. Probiotic treatment has no clinical side effects (Furrie *et al*., 2006). In this study, we investigated the potential benefit of probiotic strains both in animal health and production.

MICROORGANISM USED IN PROBIOTICS

The crucial event in the development of the probiotic approach to animal health was the finding that the newly hatched chicken could be protected against salmonella colonization of the gut by dosing it with a suspension of gut contents prepared from healthy adult chickens (Nurmi and Rantala, 1973). Microorganisms used in probiotics include those derived from the *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium*, *Bifidobacterium* species and *E. coli* Nissle 1917 (Kruis *et al*., 2004). Bacterial probiotics have been effective in chickens, pigs and pre-ruminant calves; whereas yeasts and fungal probiotics such as (*Saccharomyces cerevisiae*) and Amaferm (*Aspergillus oryzae*) have given better results in adult ruminants (Fuller, 1999). The combinations of probiotics strains could increase the beneficial health effects compared with individual strains, because of their synergistic adhesion effects (Collado *et al*., 2007).

PROBIOTICS CRITERIA

The potential probiotic strains were characterized as a normal inhabitant of the target species, have ability to adhere and colonize the epithelial cells of the gut and to survive and grow in the respective ecological units. The strains were genetically stable, able to produce antimicrobial substances, antagonistic toward pathogenic or cariogenic bacteria (Kullen and Klaenhammer, 1999). The strains were able to compete with normal microflora and resistance to bile and acids can exert one or more clinically documented health benefits (Parvez *et al*., 2006). Cell immobilizations, selections of acid and bile-resistant strains, oxygen-impermeable containers have been proposed to improve the viability of probiotic bacteria (Shah, 2000; Champagne *et al*., 2005). In addition, molecular tools based on 16S ribosomal DNA sequences and PCR techniques have been developed for identifying probiotics strains (Ben Amor *et al*., 2007).

PROBIOTICS MECHANISM OF ACTION

Four mechanisms have been summarized to explain the protective effects of probiotic: Antagonism through the production of antimicrobial substances (Vandenbergh, 1993); competition with the pathogen for adhesion sites or nutritional sources (Guillot, 2003) immunomodulation of the host (Isolauri *et al*., 2001) and inhibition of the production of bacterial toxins (Brandao *et al*., 1998). The first three mechanisms are ordinarily attributed to lactic acid bacteria while the last two are more specifically attributed to yeast. Probiotic bacteria exhibit host-specific and strain-specific differences in their actions, a combination of probiotics with different mechanisms of action could perhaps amplify the protective range of bio-therapeutic preparations (Lima-Filho *et al*., 2000).

POTENTIATED PROBIOTICS

Potentiated probiotics are a bio-preparations containing production strains of microorganisms and synergistically acting components of natural origin that potentiate probiotic effect on both small intestine and colon. Potentiated probiotics more effective than their components separately and their potentiated protective and simulative effects were expressed in all parts of the digestive tract (Bomba *et al*., 2002). PUFA-potentiated probiotics positively affected the adhesion of lactobacilli, pH and the level of organic acids in the digestive tract of germ-free piglets (Kastel *et al*., 2007) and fish (Ringo and Gatesoupe, 1998). Organic acids together with probiotics and specific carbohydrates are often suggested as alternatives to the use of antibiotic growth promoters (Jensen, 1998). The application of probiotics in conjunction with antibiotics prevented both the increase in the number of ampicillin-resistant bacteria and their translocation into the liver (Bomba *et al*., 2002). Some microbes have the ability to bind metal ions present in the external environment at the cell surface or to accumulate them in the cell. The addition of magnesium to the growth medium increases the viable count of *L. casei* and *L. plantarum* (Calomme *et al*., 1995). The inhibiting effect of zinc has been used successfully in the treatment of *E. coli* diarrhea in post weaning piglets (Owusu-Asiedu *et al*., 2003).

PROBIOTICS APPLICATIONS IN ANIMAL PRODUCTION

Improved growth rate: In animal nutrition microorganisms used as probiotics was linked with a proven efficacy on the gut microflora. Administration of probiotic strains separately and in combination was significantly improved feed intake, feed conversion rate, daily weight gain and total body weight in chicken, pig, sheep, goat, cattle and equine (Chiofalo *et al*., 2004; Li *et al*., 2006;

Torres-Rodriguez *et al*., 2007; Samli *et al*., 2007; Casey *et al*., 2007). Probiotic reduced leg weaknesses in broilers (Plavnik and Scott, 1980), prevented starvation sterility of young sows (Bohmer *et al*., 2006). Probiotics has a positive effect on various digestive processes, especially cellulolysis and synthesis of microbial protein (Yoon and Stern, 1995). Probiotics were stabilizers of ruminal pH and lactate, increased the absorption of some nutrients and displayed a growth-promoting effect that was comparable to avilamycin treatment (Mountzouris *et al*., 2007).

Milk production: The supplementing animals feed with probiotics have a beneficial effect on subsequent milk yields, fat and protein content (Sara *et al*., 2002; Kritas *et al*., 2006). Blood and milk parameters were significantly improved using probiotic, as shown by higher serum cholesterol and total lipids concentrations and higher milk fat and protein content at mid-suckling period in sow (Alexopoulos *et al*., 2004). *Aspergillus oryzae* and *Saccharomyces cerevisiae* have increased milk production, milk Solids-Not-Fat (SNF) and tended to increase milk protein percentages in dairy cows (Yu *et al*., 1997). This is due to the numbers of cellulolytic bacteria, fiber degradation and changes in Volatile Fat Acid (VFA) in the rumen (Martin and Nisbet, 1990).

In the traditional milk products, microbes are selected for their ability to grow and produce organic acids in milk. In the case of probiotic products, the microbes are mainly selected on the basis of their potential health-associated properties. The number of viable microbial cells that should be present in a probiotic product has been considered to be between 106 and 108 CFU mL^{-1} (Kailasapathy and Rybka, 1997). Cheese was optimized by the addition of *Bifidobacterium lactis* and *Lactobacillus acidophilus* (Gomes *et al*., 1998). Quarg produced by probiotics has shown a beneficial effect on intestinal function and promoting a good health (Milanovic *et al*., 2004).

Meat production: The demand of safe and qualitative meat on the market has considerably increased nowadays. The producers are eager to use natural and safe non-chemical forage supplements, which positively effect animal health, increase their productivity and improve quality of the production. Probiotics increased the carcass output and water holding capacity and decreased cooking loss and meat hardness (Ceslovas *et al*., 2005). Probiotics was reduced morbidity and mortality of growing rabbits during the fattening period (Paulius *et al*., 2006). Sub-therapeutic use of antibiotics in poultry feeds has become undesirable because of the residuals in meat products and development of antibiotic-resistant bacterial populations in humans. Chicken meat contaminated with *Campylobacter jejuni* can be the source of human enteritis. To decrease the risk of human infection, Campylobacter should be controlled at farm levels by orally given probiotic bacteria to prevent colonization of chicken with Campylobacter (Chaveerach *et al*., 2004).

Several probiotic strains have been utilized for fermented sausages (Hammes and Knauf, 1994), such as lactic acid-producing mainly belong to the genera *Lactobacillus*, *Pediococcus* and *Streptococcus* (Hammes and Knauf, 1994). The most important reason for applying probiotics in the production of fermented sausage is their ability to produce a consistent and controlled acidification that inhibits growth of undesirable microorganisms (Luecke and Hechelmann, 1987). With respect to microbiological safety, *Staphylococcus aureus* was responsible for food poisoning incidents in many types of food, including fermented sausages (Smith *et al*., 1983). Therefore, in the meat industry lactic acid bacteria are widely used as starter cultures for suppressing the growth of *S. aureus* in the manufacturing of fermented meat products (Marcy *et al*., 1985).

Egg production: Probiotics increased egg production and egg quality (Haddadin *et al*., 1996; Kurtoglu *et al*., 2004) and decrease egg contaminations (Van Immerseel *et al*., 2006). Probiotic was also increased egg shell weight, shell thickness and serum calcium (Panda *et al*., 2003, 2007). In addition, probiotic was significantly reduced the plasma cholesterol and triglyceride (Haddadin *et al*., 1996), confirming the important roles of Gastrointestinal Tract (GIT) microorganisms in recycling of lipids. Probiotics had no effect on chick quality or production efficiency in broilers produced by the breeder flock (O'Dea *et al*., 2006).

PROBIOTICS APPLICATIONS IN ANIMAL HEALTH

Protection against pathogens: The indigenous intestinal bacteria inhibit pathogens by competition to colonization sites and nutritional source and production of toxic or stimulation of the immune system (Paravez *et al*., 2006). These mechanisms are not mutually exclusive and inhibition may comprise one, or all of these mechanisms. The variation in efficacy of probiotics under different conditions may be attributable to the probiotic preparation itself or may be caused by external conditions. Probiotics can significantly protect mice against infection with the invasive food borne pathogen *Listeria monocytogenes* and *Salmonella typhimurium* and protect pigs against diarrhea (Corr *et al*., 2007). The protection included a ten-fold increase in survival rate, significantly

higher post-challenge food intake and weight gain and reduced pathogen translocation to visceral tissues (Shu *et al*., 2000). Probiotics have been shown to be involved in protection against a variety of pathogens in chicken including *Escherichia coli* (Chateau *et al*., 1993), Salmonella (Stern *et al*., 2001), Campylobacter (Stern *et al*., 2001), Clostridium and Eimeria (Dalloul and Lillehoj, 2005). Probiotic activity was largely inhibitory since the probiotics bacteria can reduce the level of *E. coli* O157 carriage and faecal shedding in cattle and calves (Brashears *et al*., 2003) and decreased the severity and duration of diarrhea in *Escherichia coli* O157:H7-infected infant rabbits (Ogawa *et al*., 2001; Casey *et al*., 2007). The growth of Enterohemorrhagic *Escherichia coli* EHEC O157:H7 and the production of Shiga-like toxins were inhibited by co-incubation with *Clostridium butyricum* (Takahashi *et al*., 2004). Probiotic was reduced gastric inflammation and bacterial colonization in Helicobacter pylori-infected animals (Johnson-Henry *et al*., 2005) and induced an inflammatory response in feedlot steers fed high-grain diets (Emmanuel *et al*., 2007). Probiotics tended to improve the health status and fertility of sows (Alexopoulos *et al*., 2004), reduce the adhesion of porcine enteropathogenic *E coli* and invasion of *Salmonella typhimurium* with epithelial cells *in vitro* (Kleta *et al*., 2006). The Pediococcus acidilactici based probiotic effectively enhances the resistance of birds and partially protects against the negative growth effects associated with coccidiosis (Lee *et al*., 2007). Probiotic can exhibit antibacterial activity against fish pathogenic bacteria (Sugita *et al*., 2002) and could reduce mortality of fish challenged with a virulent strain of *Aeromonas salmonicida* (Nikoskelainen *et al*., 2001). Probiotic can alter the balance of gastrointestinal microflora in healthy cats (Marshall-Jones *et al*., 2006) and were shown to be effective in preventing antibiotic associated diarrhea (Hawrelak *et al*., 2005).

Enhance the immune response: The intake of probiotics has been associated with beneficial effects on the immune system, such as improved disease resistance and reduced risk of allergies. Probiotic in the organism of a healthy animal stimulate non-specific immune response and enhance the system of the immune protection (Ceslovas *et al*., 2005). Probiotic increased intestinal IgA secretion both in sows and piglets and elevated IgG and IgM levels in turkey (Cetin *et al*., 2005). The effect of intestinal IgA secretion could be related to a more successful mucosal defense which in turn led to a lower level in systemic IgG production in piglets after weaning (Scharek *et al*., 2007). Furthermore, administration of probiotic results in beneficial systemic and immunomodulatory effects in cats (Marshall-Jones *et al*., 2006). A probiotic influence transport properties of small intestine epithelium and increased absorption of glucose could be interpreted as a positive effect for the animal (Lodemann *et al*., 2006). The probiotic that enhance immunoglobulin levels have more positive effect on growth performance, production and ability to disease resistance (Cetin *et al*., 2005). The protective effects of feeding the immunoenhancing probiotic to mice can reduce the severity of *E. coli* O157:H7 infection and this reduction may be associated with enhanced humoral and cellular immune responses (Shu and Gill, 2002). Probiotic shows an immunoregulatory on cell-mediated immunity and humoral immune response in poultry and this provides a rationale for further study to investigate the beneficial effects of probiotics in animals' food (Panda *et al*., 2007). *Clostridium butyricum* can mediate the humoral immune responses and improve the growth performance in *Miichthys miiuy* (Song *et al*., 2006). The addition of probiotic in the diet significantly increased the cutaneous basophilic hypersensitivity response (Panda *et al*., 2003).

SAFETY ASPECTS OF PROBIOTICS

The concentration of probiotics in food production varies tremendously and there are currently no national standards of identity for levels of bacteria required in yogurt and other fermented product. Theoretically probiotics may responsible for four types of side-effects systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals and Gene transfer (Marteau and Boutron-Ruault, 2002). Most of the micro-organisms used as a probiotics in animal are safe, although some have problems particularly the enterococci, which may harbour transmissible antibiotic resistance determinants and *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxin (Arturo *et al*., 2006). Particular attention for safety assessment is focused on the presence of transmissible antibiotic resistance markers and the potential for production of harmful metabolites. Thus the appealing properties of probiotics include the ability to reduce antibiotic use, the apparently high index of safety and the public's positive perception about natural or alternative therapies (Strompfoa *et al*., 2006). *In vitro* tests are critical to assess the safety of probiotic microbes, although it is useful to gain knowledge of strains and the mechanism of the probiotic effect. The main currently used *in vitro* tests for the study of probiotic strains are resistance to gastric acidity and bile acid, adhesion to gut epithelial tissue, antimicrobial activity against potentially pathogenic bacteria and ability to modulate immune responses (Collins *et al*., 1998).

FUTURE TREND IN PROBIOTICS RESEARCH

Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. The trend for future could be focus on basic research to identify and characterize existing probiotics strains, determine optimal doses needed for certain strain and asses their stability through processing and digestion. For the probiotics to represent a real and effective alternative to antibiotics and chemotherapeutics it is absolutely necessary to ensure their consistently high efficacy. It is important to search for ways to potentiate the efficacy of probiotic microorganisms in all parts of the digestive tract. The efficacy of probiotics may be enhanced by selection of more efficient strains of microorganism, gene manipulations, combination of a number of strains of microorganism and combination of probiotics and synergistically acting components. Genetic engineering techniques can be use to insert one or more antigen from a pathogen into probiotic strains with good colonizing capacity for use in immunotherapeutic applications, such as vaccination and delivery of immunoregulatory substances.

CONCLUSION

Although, the highly complex relationship of food and health is still poorly understood, recent research advances in different disciplines provide promising new approaches to improve our understanding. The demand of safe and qualitative animal product on the market has considerably increased. In this study, we conclude that probiotics have a positive effect on animal production by improving growth rate and increasing milk, meat and eggs production.

In addition, probiotics can inhibit pathogens by competition for a colonization sites or nutritional sources and production of toxic compounds, or stimulation of the immune system. In order to enhance the efficacy of probiotics, it is necessary to obtain additional knowledge on their mode of action. The efficacy of probiotics may be potentiated by several methods: The selection of more efficient strains; gene manipulation; the combination of several strains and the combination of probiotics and synergistically acting components. The adoption of logical criteria for the *in vitro* selection of probiotic bacteria can result in the isolation of strains capable of performing effectively in the gastrointestinal tract.

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