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Probiosis: Concepts and Prospects

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ABSTRACT

The use of probiotics in animal and human feeding has been a subject of increasing interest both for the academia and the industry, mostly due to their potential positive effects on health and profitability. However, the knowledge on the composition of bacterial gastrointestinal communities in humans and animals, as well as its respective nutritional requirements, is far from being elucidated. Due to the ban of the use of antibiotic growth promoters in animal feeds, there has been an increasing interest on the utilization of probiotics to improve animal intestinal health under commercial settings. However, the possibility of horizontal transference of antibiotic-resistant genes between probiotic bacteria and pathogenic species has become a concern of poultry farmers and consumers around the world. Innovative ideas have emerged, such as the addition of essential oils, spices, and other plant extracts to feeds of monogastric animals to promote intestinal health. These natural compounds are considered ecologically adequate and safe for feeding purposes. This new reality will probably change the direction of research and of the use of additives in poultry production.

INTRODUCTION

Several infectious diseases were controlled or eradicated after the first third of the 20th century, when antibiotics were discovered. However, the overuse and misuse of these compounds in humans or animals have caused the emergence and dissemination of antibiotic-resistant bacteria. The increasing concern with the spread of pathogens from animals to men led the ban on the use of antibiotics in subtherapeutic doses in animal production. Nevertheless, according to experts, despite the general reduction of the use of in-feed antibiotics in poultry production, such ban could increase the incidence of intestinal infections, and consequently the therapeutic use of antibiotics (Castanon, 2007). Methods to control intestinal disorders in poultry and swine based on competitive exclusion, such probiotics, prebiotics or both, are considered safe (Edens, 1997; Anderson et al., 1999; Schneitz, 2005).

Probiotics are necessarily live microorganisms that, when administered to animals in adequate amounts, benefit the host's health, while prebiotics are indigestible feed components that affect the host by selectively stimulating the proliferation or the activity of desirable bacterial populations, such as Bacillus spp (Heyman & Ménard, 2002; Conly & Johnston, 2004; Saad, 2006).

In nature, the genus Bacillus can be isolated from the intestine of penaeid shrimp (Rengpipat et al., 2000), bivalve mollusks (Sugita et al., 1981) and marine fish (Sugita et al., 1998). It was demonstrated that some species of this genus have an inhibitory effect on pathogens (Rengpipat et al., 1998; Balcazar Rojas-Luna, 2007).

Lactobacillus reuteri culture is sold as a product that allegedly promotes the competitive exclusion, but it seems to be relatively inefficient against enteropathogens (Schneitz, 2005). In broiler chickens, the concept of competitive exclusion initially aimed at reducing Salmonella infection, but it was later expanded to include other enteropathogens (Eimeria, Escherichia coli, Clostridium perfringens, Listeria, and Campylobacter) both in animals and humans (Huang et al., 2002; Dalloul et al., 2003; Rastall, 2004; Nava et al., 2007; Schneitz, 2005; De Vrese & Marteau, 2007). Competitive exclusion has also proven to be efficient in other poultry species, such as turkeys, quails and pheasants (Homma & Shinohara, 2004; Schneitz, 2005). However, it seems that most of these additives, individually or combined, are more efficient in fighting Salmonella than other potentially harmful bacteria, such as E. coli or Clostridium (Blankenship et al., 1993; Kubena et al., 2001; Chambers & Lu, 2002).

Although the term "probiotic" is accepted in Brazil to designate feed additives containing live microorganism cultures, it is not considered adequate by the US and EU legislation, as it conveys a mistaken concept with a commercial appeal. Probiotics are named by the Food and Drug Administration of the US government (FDA, 2009) and the Association of American Feed Control Officials (AAFCO, 1999) as direct-fed microbials (DFM), whereas for the ECC committee, it is simply called microorganisms. Therefore, in this article, these three terms will be indistinctively used. It must be mentioned that the FDA does not allow companies that sell DFM products to make therapeutic allegations, including the establishment of viable bacterial colonies in the intestine, enhancement of animal growth or feed intake, etc. (Alliance Animal Health).

Probiotics in dairy beverages are traditionally considered healthy because of their bacterial content. However, there are few scientific studies on their effects, particularly on healthy populations (Del Campo & Baquero, 2005). This subject remains controversial, even when well-known species, such as Lactobacillus and Bifidobacterium, are taken into account (Zhou et al., 2005). According to Abbot (2004), most of the studies on the efficacy of probiotics are poorly controlled and generate contradictory results in humans. Moreover, their effects on the stimulation of the immune system are poorly documented. Briefly, the role of intestinal bacteria seems to be teaching the immune system how to differentiate between invading bacteria and non-hostile challenges. Some clinical assays indicate that several "dietary bacteria" may aid in the recovery from diarrheas (Guandalini et al., 2000; Cremonini, et al., 2002; D'Souza et al., 2002; Abbot, 2004) and several inflammatory intestinal diseases (Rembacken et al., 1999; Abbot, 2004). More recently, experiments aiming at developing molecular immunoassay systems for probiotics, based on food immunology, have been performed (Kitazawa et al., 2008).

Incertitudes as to the intestinal microbiota

Volatile fatty acids production in the ceca and the occupation of the mucosa surface binding sites are the main modes of action of most probiotics (Lan et al., 2005; Donalson et al., 2008). According to Schneitz (2005), the results of studies performed with probiotics suggest that, initially, the protection is predominantly physical, rather than a phenomenon involving the synthesis of volatile fatty acids or other metabolites. In addition to probiotics, other substances that may potentially interact with the intestinal microbiota have been studied as alternatives to replace antibiotics, including enzymes (Jackson et al., 2004; McReynolds et al., 2004; Rosin et al., 2007), prebiotics (Patterson & Burkholder, 2003), and organic acids (Ricke, 2003). These additives have been tested for their modulating effects on the gastrointestinal microbiota, favoring the growth of beneficial bacteria. However, none of these alternatives has provided yet an overall solution that is efficient under different circumstances, such as the case of antibiotics.

No conclusive results have been yet achieved, as several authors have shown that the intestinal ecosystem is much more diverse than it was previously believed (Zhu et al., 2002; Józefiak et al., 2004). Moreover, microbiologists found that the traditional techniques used for microorganism isolation and culture are not able to individually isolate determined microorganisms (Abbot, 2004). In fact, most bacteria cannot be easily isolated from their habitats by the conventional methods commonly used in laboratories, because bacterial communities have specialized members that play different roles and provide vital substrates for other bacteria living in the same community. There is still relatively few information on the in-vivo activity of these organisms (Gabriel et al., 2006). Zhu et al. (2002) suggested that only 10 to 60% of the cecal bacteria can be cultivated using anaerobic techniques. Therefore, the development of strategies for the manipulation of the gastrointestinal microbiota is hindered by the lack of practical analytical instruments that monitor intestinal microorganisms as a whole. Consequently, the lack of knowledge on the role of different bacterial species and their growth requirements prevent the possibility of objectively manipulating the intestinal microbiota. Moreover, such prebiotics may to make some genera stronger. An example is Clostridium spp, which generates potentially carcinogenic byproducts, including nitrosamins and cresol, (Blaut & Clavel, 2007) - that may not be relevant for production animals, but that affect breeders and companion animals.

In this context, scientists have adapted techniques that were previously developed to study marine and soil bacteria to answer such questions (Abbot, 2004). Moreover, modern approaches to analyze the structure of bacterial communities using microbial DNA (Zhu et al., 2002; Zhu & Joerger, 2003) have shown that 90% of the gastrointestinal microbiota of broilers consists of unknown species. The genera of more than half of the 640 different bacteria found are still unknown (Zhu et al., 2002; Apajalahti et al., 2004; Gabriel et al., 2006). Even in humans, the best estimates consider that the complete description of the intestinal flora is far from being elucidated (Kullberg, 2008). Efforts to classify and understand the intestinal microbiota will open new research frontiers.

Substrates and bacterial metabolites

Intestinal microbiota species and their frequency and metabolic activity are influenced by many factors, including environment, diet, presence of aflatoxins, as well as additive type and dosage (Gibson, 1999; Kubena et al., 2001; Smirnov et al., 2005; Blaut & Clavel, 2007; Mountzouris et al., 2007). Bacterial metabolites may be useful or harmful to the host (Rastall, 2004; Gabriel et al., 2006). Experiments performed in animals and in humans suggest that the intestinal microbiota affects the production of colonocyte substrates, such as butyrate (from fiber) and vitamins. The butyrate produced in the colon regulates the differentiation of mucosal cells and reduces apoptosis, which, in turn, controls inflammation and prevents cancer development (Tsukahara et al., 2006; Peña, 2007). Although the inhibition of cancer cells does not have a practical interest for the production of animals with a short life cycle, butyrate is important, because it promotes mucus release and the absorption of water and minerals, and consequently, influences intestinal health (Holtug et al., 1992; Shimotoyodome et al., 2000; Tsukahara et al., 2003; Tsukahara et al., 2006).

Intestinal bacteria synthesize B, E, and K vitamins, but it is believed that only folic acid is available to animals (Coates, 1980[1] apud Gabriel et al., 2006;). The requirements of some vitamins, such as pantothenic acid, are higher in the presence of the intestinal microflora because bacterial metabolites need to be detoxified. Studies have shown that B-complex vitamins are little absorbed in vitro by the intestine of conventional broilers as compared to germ-free birds (with no intestinal microorganisms); however, these results were not confirmed in vivo. The microflora also has a negative impact on the absorption of fat-soluble vitamins, which requires bile salts (Gabriel et al., 2006).

Intestinal bacteria may impair lipid digestion and alter the digestion of proteins and carbohydrates (Gabriel et al., 2006). The maintenance of this microbial population requires a significant volume of substrates. Bacteria in the small intestine may utilize between 10 and 20% of carbohydrates and amino acids that could be used by the host (Apajalathi et al., 2004). This partially explains the effectiveness of antibiotic growth promoters, which eliminate part of the intestinal microbial population.

Probiotics may be as efficient as antibiotics provided their growth requirements are supplied. For instance, Bacillus spp are transient and do not colonize the intestinal tract. They are vegetative cells, and therefore, they must be regularly supplied to animals. Different bacterial species present different substrate preferences and growth requirements, and the chemical composition and structure of the digesta have a strong influence on the distribution of species in the gastrointestinal microbiota. The knowledge on the nutritional preferences of bacteria may allow shifting the composition of the intestinal microbial community to increase the number of beneficial bacteria. Several studies indicate that oligosaccharides are specific substrates for growing beneficial bacteria (Roberfroid, 2000; Donalson et al., 2008).

Since the concept of prebiotics was introduced, it has attracted scientific and commercial interest. The main characteristic of prebiotics is the selective stimulation of intestinal bacteria growth, contributing to the hosts' health and welfare. This requires prebiotics to be resistant to gastric acidity, bile salts, and hydrolysis by the host's enzymes (Roberfroid, 2007). Moreover, since most bacteria are located in the distal portion of the ileum and large intestine, the compounds required for their growth need to escape absorption by the host (Apajalahti et al., 2004).

Symbiotics, products that contain both probiotics and specific prebiotics that promote the growth of probiotic strains, are considered a good solution in many cases

[1] COATES, M.E. (1980) The gut microbiota and growth. In: Growth in animals, (T.L.J. Lawrence ed), pp. 175-188, London: Butterworths.

(Apajalahti et al., 2004; Cheng et al., 2005; Bomba et al., 2006). In this sense, some scientists are considering the possibility of combining these benefits using genetic engineering (Steidler, 2003; Abbot, 2004). There are evidences that some genetically-modified bacteria may be efficient vehicles for drugs, vitamins, or vaccines, such as genetically-modified Lactococcus lactis that may secrete medicines to fight intestinal disorders (Seegers, 2002; Steidler et al., 2000; Steidler, 2003; Charng et al., 2006).

Inhabitants of the cecum

Microbial populations of different sizes and complexity are present throughout the digestive tract. The cecal microbiota is the most important fermentative population in non-ruminant animals. The ceca contain the largest amount of bacteria in the gastrointestinal tract of broilers (Barnes et al., 1972, Barnes et al., 1973; Barnes, 1979), and most are obligatory anaerobes. The characterization of the cecal microbiota in poultry started in the beginning of the 1970s (Barnes et al., 1972). It was recently reported that only 10 to 60% of the cecal bacteria can be cultivated using anaerobic techniques (Zhu et al., 2002). As previously discussed, the impossibility of culturing many microorganisms in the laboratory has limited the advance on the knowledge on these populations.

Fecal sampling is the most significant method to study the effect of prebiotics on the microbiota in vitro because it ensures that a representative variety of bacterial species is exposed in the test material (Lifschitz et al., 1990; Flickinger et al., 2002;). Studies on changes of selected genera or species may, therefore, allow establishing whether fermentation is indeed selective. The use of excreta may provide a precise representation of events at the distal colon (Roberfroid 2007). The general problem with the use of excreta samples is the identification of the genus and species of each bacteria present. This has been traditionally accomplished by culture in different selective agars, followed by morphologic and biochemical tests.

Additives and the intestinal microbiota: a new perspective

During several decades, studies on selection and dissemination of the antibiotic resistance were mainly concentrated in clinically-relevant species (Roe & Pillai, 2003). However, many researchers have recently suggested that commensal bacteria, including lactic bacteria (Mathur & Singh, 2005), may also act as reservoirs of the genes responsible for antibiotic resistance similar to those found in humans pathogens. Genes of resistance to tetracyclin, erythromycin, and vancomycin were detected and characterized in Lactococcus lacti, Enterococcus, and, recently, in Lactobacillus species isolated from meat and dairy fermented products (Aslim & Beyatli, 2004; Mathur & Singh, 2005).

Several initiatives have been put forward by several organizations around the world to deal with biosafety concerns as to probiotic microorganisms. A large number of probiotic products is being systematically analyzed as to their biosafety, as well as to any possible direct toxic effect (Abbot, 2004).

Innovative ideas emerge every day. Groups around the world have invested in research on new antibiotics and on different modes of action to fight pathogenic bacteria in humans (Bexfield et al., 2008; Rasko et al., 2008; Nigam et al., 2006; Tang et al., 2009; Van Den Berg et al., 2008; Bexfield et al., 2004); however, the possibility of applying such solutions in animals is very remote, and therefore, livestock production relies on the improvement of health conditions to obtain better results (Tabes, 2008).

Low-technology solutions occasionally attract some interest. A reasonable number of vegetable compounds can be used either in synergy or as a replacement to antibiotics in non-ruminant animals (Bomba et al., 2006). Studies that classify the bactericidal activity of essential oils are still lacking. An increasing number of research studies have validated the utilization of these compounds in broilers, fulfilling the expectation of the most passionate ecologists. It is not difficult to imagine that such concerns will also influence markets that increasingly demand the so-called "natural" products, which nomenclature is often more ideological than technical.

FINAL CONSIDERATIONS

The common problem in the evaluation of the utilization of probiotic and prebiotic products in diets is that several factors influence their physiologic and microbiologic effects on the host, including the age of animals to which there are supplied, feedstuffs included in the formulation of feeds, in addition to the fermentative characteristics and structural differences among products. Moreover, many obstacles still prevent the full understanding of their in-vivo effects. This is why the experiments on the effects of dietary supplementation of probiotics, individually or combined to other additives, have yielded different results.

Some studies report non-significant trends of performance improvement, in spite of the significant health effects under challenge conditions. However, it is important to stress that in several experiments on the efficacy of these additives challenges are not controlled or it was not possible to determine if a challenge was indeed present. Errors in the design and performance of experiments and improper facilities, or merely careless management, may cause cross-contamination of experimental units belonging to different treatments, and this lack of control may increase the experimental error or confuse observations, making their results unreliable.

Experimental studies developed in Brazil between 1995 and 2005 were carefully reviewed to evaluate the effect of probiotics on broiler performance (Faria Filho et al., 2005). Results demonstrated that individual comparisons of commercial probiotics added to feeds as growth promoters showed trends of improvement, but no statistical significance for most evaluated parameters. However, when the analysis was performed considering data of all experiments, there were beneficial effects of the utilization of probiotics. Those authors argued that this was due to a higher number of observations, and consequently, lower confidence interval.

The optimum levels of inclusion of several of these commercial products still need to be determined, including under laboratory conditions. In the field, this is more difficult because previous health status, diet composition, and environmental factors should be taken into account. To date, the amount of these products that eventually reach the posterior segment of the intestinal tract in a viable form is not known.

The current control of foodborne health challenges posed by poultry products and the concern with the potential horizontal transference of antibiotic-resistance genes between probiotic microorganisms and pathogenic bacteria may change the focus of research and the use of "live dietary microorganisms" in animals.

Plant extracts, essential oils, and other phytogenic products have been increasingly evaluated as alternatives to the promotion of intestinal health and may prove to be safer to human health. Such studies are being performed worldwide, but bactericidal activity of these plant ingredients still needs to be further elucidated. Their effects on the gastrointestinal microbiota need to be determined in order to avoid the mistakes that occurred with antibiotics, and perhaps will give a new direction for the utilization of additives in non-ruminants diets in a near future. It must be stressed that the mode of action of these additives needs to be elucidated to explain why they have positive effects under some circumstances and not in others.

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