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## The Benefits of Probiotics in Human and Animal Nutrition

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### 1. Introduction

At birth, the gastrointestinal tract of any animals is sterile, and it is rapidly colonized by bacteria from the mother and the environment. This colonization by the gut microbiota plays an important role in intestinal tract maturation of newborn (in terms of anatomy, digestive physiology, and immunology) (Hooper 2004). After this colonization, considering healthy human individuals, the gastrointestinal tract harbors 10 or more times as many microbes than there are eukaryotic cells ( $10^{14}$  viable cells for indigenous microbiota/ $10^{13}$  body cells). These microorganisms, altogether weighing approximately 1.5 kg, can be considered as a complementary major organ, responsible for three main functions: colonization resistance, immunomodulation, and nutritional contribution (Hayashi et al., 2002; Zoetendal et al., 2011). Colonization resistance inhibits the installation of exogenous microorganisms as well as the uncontrolled multiplication of microorganisms belonging to the indigenous microbiota. Immunomodulation maintains the immune system under a watchful state, which permits a faster but adequate response in the case of infectious aggression. Nutritional contribution furnishes complementary sources of vitamins, enzymes, and energy substrates (volatile fatty acids).

Unfortunately, several factors can disturb both the initial colonization and posterior maintenance of the gut microbiota, leading to a microbial ecosystem with beneficial functions transitorily or irreversibly less efficient. As examples, the type of delivery (cesarean or natural) or the reduction of mother-child contacts (premature baby in an incubator or in an intensive care unit) interfere with the supply of microorganisms necessary for post-natal colonization. Additionally, the alimentation (breast- or formula-fed) and the ingestion of antibacterial drugs may be other factors that modify the normal sequence of colonization (Harmsen et al., 2000; Bonnemaïson et al., 2003; Westerbeek et al., 2006; Chen et al., 2007). Once installed, the beneficial functions of the microbiota are very powerful but also fragile and can be disturbed by ingestion of drugs (especially antibiotics), drastic changes in diet or stress. In view of what was presented above, the importance of a correct initial colonization and a subsequent preservation of the gut microbiota is evident to obtain optimal functions from this microbial ecosystem. When disturbances of the indigenous microbiota functions are forecasted or installed, you should think about the possibility of compensating failures of these functions. In this sense, probiotics can be considered as

biotherapeutics to be used in microbial ecosystems during the installation phase (colonization of the newborn), or with installed (treatment) or forecasted disturbances (prophylaxis).

Probiotics have been defined in a joint meeting of the Food and Agriculture Organization/World Health Organization as "live microorganisms which when administered in adequate amounts confer a benefit to host health" (WHO / FAO, 2002). The objective of its use is to install, enhance or compensate the functions of the indigenous microbiota inhabiting the digestive tract or other body surfaces. The suggestion of using fermented food to obtain some benefits for the health is not new. It was mentioned in the Persian version of the Old Testament (Genesis 18:8) that "Abraham attributed his longevity to the consumption of sour milk." Later in 76 BC Pliny, a Roman historian, recommended the use of fermented milk products for the treatment of gastroenteritis (Schrezenmeier & de Vries, 2001). However, a scientific approach, recognizing the beneficial role of certain microorganisms has been only applied in the first decades of the twentieth century with the suggestion of the use of *Lactobacillus* (Elie Metchnikoff attributing the longevity of Bulgarian populations to the yogurt consumption in 1907), *Bifidobacterium* (Henri Tissier observing a higher presence of bifidobacteria in the feces of healthy breast-fed children in 1906) and *Saccharomyces boulardii* (Henri Boulard noting the use of a tropical fruit colonized by this yeast to treat diarrhea by local populations in Eastern Orient during a cholera outbreak in 1920) to prevent or treat gastrointestinal disorders (Shortt, 1999). The probiotics most often used at the moment are bacteria producing lactic acid (*Lactobacillus*, *Bifidobacterium*) and yeasts (*Saccharomyces boulardii*).

Many health benefits have been related to human and animal intake of probiotic. Several studies have supplied clinical evidences of the benefits generated by probiotics, as for example in diarrhea treatment (Billooet et al., 2006; De Vrese & Marteau, 2007), lactose intolerance (He et al., 2008), irritable bowel syndrome (De Vrese et al., 2001; Nagala & Routray, 2011), allergies (Jain et al., 2010), cancer (Chen et al., 2009) and hypercholesterolemia (Baroutkoub, 2010). According to recent meta-analysis based on well conducted clinical trials with probiotics, a clear protective effect was evident, which did not vary significantly between products containing *S. boulardii*, *Lactobacillus rhamnosus* GG, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *Bifidobacterium longum*, *B. bifidum* var. *infantis* and *B. animalis* var. *lactis* (Sazawal et al., 2006).

Probiotics have also received special attention by animal nutrition researchers who search for alternatives to the use of traditional growth promoters (antibiotics). Therefore, the use of probiotics is seen more and more as an alternative to the use of antibiotics in animal production, and many scientific works show the beneficial effects of supplementation with probiotic strains in diets fed to chicken, swine, cattle and fish (Veizaj-Delia, 2010; Soleimani et al., 2010; Ignatova, 2009; Aly et al., 2008). Therefore, this chapter will approach action mechanisms and the effects on health of probiotics for human and animal use.

### 1.1 Mechanisms of action

The potential mechanisms by which probiotic agents might exert their protective effect include: antagonism by the production of substances that inhibit or kill the pathogen (Servin, 2004); competition with the pathogen for adhesion sites or nutritional sources

(Servin & Coconnier, 2003; Momose et al., 2008); immunomodulation of the host (Ezendam et al, 2006); and inactivation of microbial toxin (Brandão et al., 1998). Other mechanisms by which probiotics may exert protection is through a recuperation of mucosal barrier function when disturbed (Penna et al., 2008), trapping pathogens on their surface (Martins et al, 2010; Martins et al, 2011) and stimulating mucus production (Caballero-Franco et al., 2007).

According to De Vrese & Marteau (2007), mechanism and efficiency of probiotic effect depend mainly on the interactions between probiotic microorganisms and microbiota of the host or with immunocompetent cell of the intestinal mucous.

Although they had not been completely elucidated, the classical mechanisms of action of bacteria used as probiotics are described as: i) competition for bound sites: also known as "competitive exclusion", where bacteria of probiotics are linked to the bound site in the intestinal mucosa, making a physical barrier, impeding the bound by pathogenic bacteria; ii) production of anti-bacterial substances: bacteria of probiotics synthesize compounds as for example bacteriocins, hydrogen peroxide, which has antibacterial action, mainly in relation to pathogenic bacteria, in addition to the production of organic acid which reduced pH in the gastrointestinal tract, preventing growth of many pathogens and development of certain species of *Lactobacillus*; iii) competition for nutrients: shortage of available nutrients which can be used by pathogenic bacteria is a limiting factor for their maintenance; iv) stimulus to the immune system: some bacteria of the probiotics are directly linked to the stimuli of immune response by increasing antibodies production, activation of macrophages, T cells proliferation and interferon production (Fuller, 1992; Jin et al., 1997).

Action mechanism of yeasts still needs studies for their evidencing. A probable mechanism of action of yeast is related to total (*in vitro*) or partial inhibition of pathogenic microorganisms. Dead yeasts contain in their walls important quantities of polysaccharides and proteins able to act positively in the immune system and on nutrient absorption. Moreover, yeasts produce nutritive metabolites in the digestive tract which increase animal performance, in addition of having minerals (Mn, Co, Zn) and vitamins (A, B<sub>12</sub>, D<sub>3</sub>) which improve action of beneficial microorganisms (Hill et al., 2006).

Although some mechanisms had been suggested on the action of probiotics, they are not completely clarified, but it is known that they inhibit growth of pathogenic microorganism by producing antimicrobial compounds; they compete with pathogens for adhesion sites and nutrients; and they model immune system of the host (Oelschlaeger, 2010). In the present, a more complete view on the possible mechanisms of action are been studied, based mainly on the manipulation of normal microbiota.

The composition of human microbiota has about 10 - 100 trillion members and varies within the gut and among individuals (Zoetendal et al., 2011; Hayashi et al., 2002). These members belong mainly to the dominant bacteria, but there are also representatives from Archaea (Eckburg et al., 2003), Eukarya, and viruses, including bacteriophages (Breitbart et al., 2003). Intestinal microbiota plays a fundamental role in maintaining immune homeostasis which, in other words, involves minimizing the adverse health effects of intestinal microbiota, such as shifts in microbial community structure, changes in the diet of the host or overt pathogenic challenge (Hooper & Macpherson, 2010).

According to Sonnenburg, et al. (2006) some evidences show that by manipulating the microbiota with probiotics could influence the host health and probiotic bacteria could be

used as a therapeutic strategy to improve human health. The precise mechanisms influencing the crosstalk between the microbe and the host are still unclear but there are evidences suggesting that bacteria in the gut could modulate the functioning of the immune system at systemic and mucosal levels (Ng et al., 2009).

## 2. Effect of probiotics on human health

### 2.1 Probiotic selection

Requirements that a probiotic organism should meet are the following: resistance to gastric acidity, resistance to bile and pancreatic enzymes; adherence to intestinal mucosa cells; colonization capacity; keep itself alive for a long time during transportation, storage, so they can effectively colonize the host; production of antimicrobial substances against pathogenic bacteria and absence of translocation (Capriles et al., 2005).

For a microorganism be used as a probiotic, it is necessary its isolation, characterization and assessments which will prove its probiotic efficiency (Figure 1).

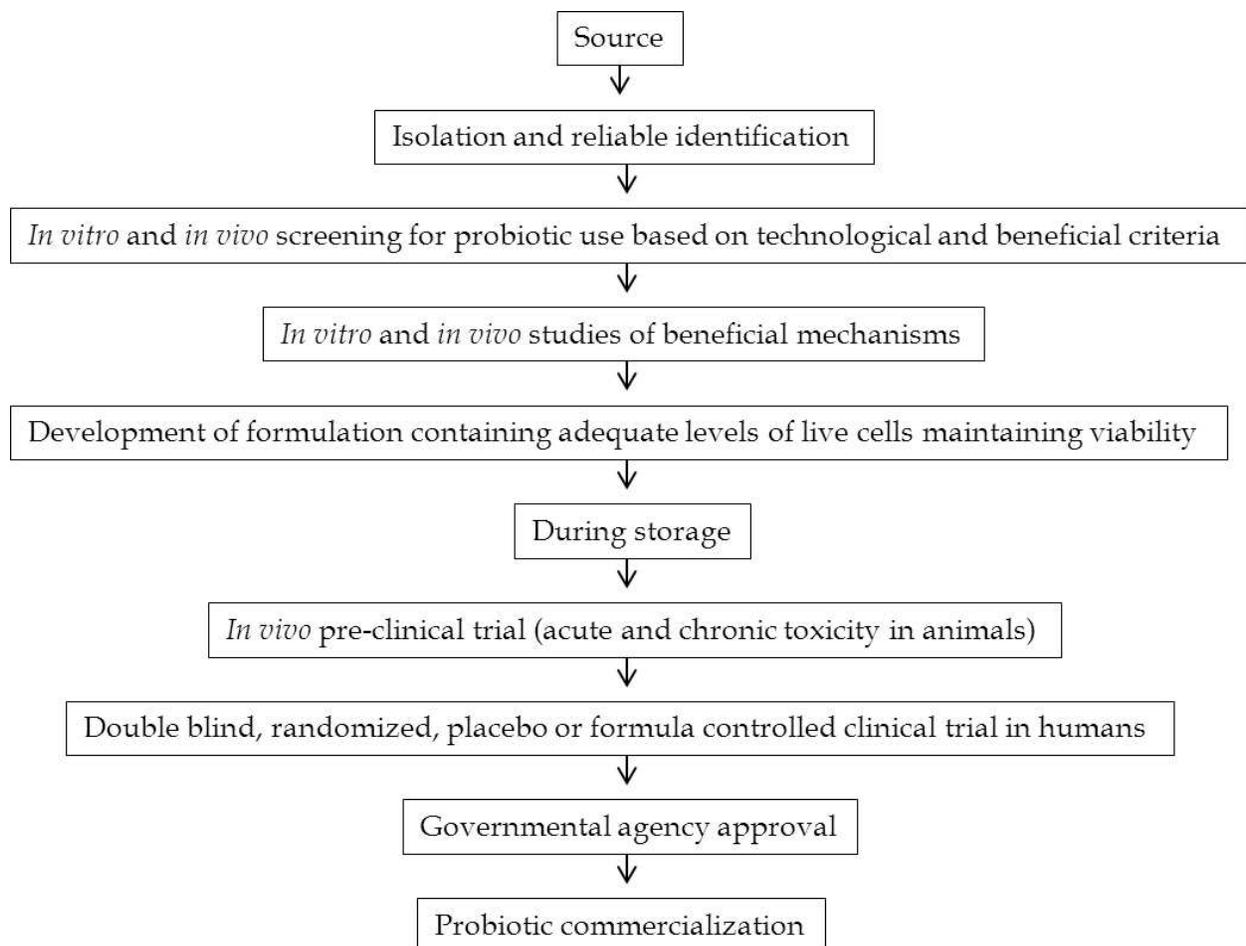


Fig. 1. Probiotic selection chart.

Firstly, a microorganism source have to be selected (for example: digestive tract of health animals or other niches such as flowers, decomposing fruits). Then, the microorganisms which are intended to work with are isolated and identified through selective culture media.

Afterwards, a new culture is prepared only with the target colonies for *in vivo* assessment (pathogen inhibition, target species pathogenicity; resistance to host conditions; among others). If there are no restriction to the use of the target species, experiments with *in vivo* supplementation at big and small scale are carried out to check if there are real benefits to the host. Finally, the probiotic which presented significant satisfactory results can be commercially produced and used.

## 2.2 Prevention or reduction of diarrhea symptoms

One of the main applications of probiotic microorganisms is at preventing or in the treatment of gastrointestinal disturbances. In a clinical trial carried out with children hospitalized for acute rotavirus diarrhea, three treatments were assessed. The first group of children received oral rehydration therapy plus placebo; the second group was submitted to oral rehydration plus *Saccharomyces boulardii* treatment and the third group received oral rehydration and a compound containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum* e *Saccharomyces boulardii*. Mean duration of diarrhea was shorter for the children who received the treatment with *Saccharomyces boulardii* strain (58 hours) and for the children who received the compound with for different microorganism strains (60 hours), when compared to the control group (84.5 hours) (Grandy et al., 2010). In Brazil, a double-blind, placebo controlled trial showed that protection against diarrhea (32.2% reduction in diarrhea during the first year of life) was obtained by oral inoculation with a single dose of plasmid-free human *Escherichia coli* EMO soon after birth (Figueiredo et al., 2001).

Treatment with antibiotics can cause an unbalance in the indigenous microbiota, increasing concentration of pathogenic microorganisms and toxin production, promoting diarrhea symptoms (Vasiljevic & Shah, 2008). A significant effect was observed in a study carried out with patients who presented diarrhea caused by antibiotics, in which intake of a probiotic drink containing *L. casei*, *L. bulgaricus* e *S. thermophilus* reduced the incidence of diarrhea (Hickson et al., 2007). In a double-blind, formula controlled trial performed in Brazil, a milk lyophilized formulation supplemented with *B. bifidum* and *S. thermophilus* was compared with another without supplementation for the prevention of antibiotic-associated diarrhea in children 6 to 36 months old. The authors observed a significant reduction of diarrhea frequency in children treated with the probiotic formula (16% of 80 patients) when compared to the control group (31% of 77 patients) (Corrêa et al., 2005). In another double-blind, placebo controlled trial also performed in Brazil, the treatment with a lyophilized preparation of *S. boulardii* in children with acute diarrhea was evaluated and a reduction in duration of rotavirus diarrhea was observed in the group treated with the probiotic yeast (Corrêa et al., 2011). Other examples of clinical trials related to prevention or shortening of diarrhea symptoms by using probiotics are summarized in Table 1.

## 2.3 Irritable bowel syndrome

Irritable bowel syndrome is a disease characterized by abdominal pain, diarrhea, constipation and mucus secretion along with feces (Vahedi et al., 2010). Although many physiopathology factors had been correlated to the cause of this disease, in the last years, researchers have considered feed intolerance and unbalance of intestinal microbiota as the main factors responsible for symptoms of the irritable bowel syndrome. Probiotics are a

Author (year)	Assessed probiotic	Results
Figueiredo et al. (2001)	<i>Escherichia coli</i> EMO	Protection against diarrhea during the first year of life was obtained by oral inoculation of <i>Escherichia coli</i> EMO soon after birth
Biloo et al. (2006)	<i>Saccharomyces boulardii</i>	The use of <i>S. boulardii</i> reduced frequency and duration of acute diarrhea in children
Giralt et al. (2007)	<i>Lactobacillus casei</i> DN-114 001	<i>L. casei</i> DN-114 001 did not reduce radiation-induced diarrhea incidence
Corrêa et al. (2005)	<i>Bifidobacterium bifidum</i> + <i>Streptococcus thermophilus</i>	Use of lyophilized milk supplemented with <i>Bifidobacterium</i> and <i>Streptococcus</i> reduced antibiotic-associated diarrhea in hospitalized infants
Beausoleil et al. (2007)	<i>Lactobacillus acidophilus</i> + <i>Lactobacillus casei</i>	Addition of <i>Lactobacillus</i> strains in the fermented milk was effective in the prevention of antibiotic-associated diarrhea

Table 1. Clinical trials on the use of probiotics in the treatment of diahea

good alternative for the treatment of this syndrome inasmuch as the use of probiotic may lead to an unbalance of intestinal microbiota, making the carrier more susceptible to the disease (Rolfe, 2000).

Nagala & Routray (2010) studied the effect of a probiotic supplement containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *B. longum* and *B. lactis*, on patients with irritable bowel syndrome. It was observed a significant improvement after two months of the treatment, with 84% of the patients showing improvement in abdominal pain, 73.9% in bloating, 88% in flatulence, 90.9% in diarrhea and 86.9% in constipation.

## 2.4 Bowel inflammatory disease

Inflammatory bowel disease involves two subtypes: ulcerative colitis and Crohn's disease. Ulcerative colitis is determined by a continuous inflammation, which starts in the rectum and it is restricted to colon, whereas inflammation from Crohn's disease can occur in any region of the gastrointestinal tract (Bousvaros et al., 2007; Mack, 2011).

The non-pathogenical strain *E. coli* Nissle 1917 showed to be efficient in the Crohn's disease maintenance therapy. This microorganism was able to adhere to intestinal epithelial cells in addition to its inhibitory effect observed against pathogenic strains isolated from patients with the disease (Boudeau et al., 2003).

A study conducted by Furrie et al. (2005) pointed the efficiency of *Bifidobacterium longum* associated to inulin-oligofructose prebiotic in the treatment of ulcerative colitis. The treatment resulted in an improvement of the full clinical appearance in patients who received this therapy.

## 2.5 Hypercholesterolemia

Saturated fat rich diets can increase serum cholesterol rates, which is one of the main risk factor for cardiovascular disease (Vasiljevic & Shah, 2008). Many studies have been carried out on the hypocholesterolemic activity of non-pathogenic bacteria through mechanism of hydrolysis of biliar salt (Pereira et al., 2003; Noriega et al., 2006; Parvez et al., 2006; Nguyen et al., 2007).

Baroutkoub et al. (2010) observed that consumption of probiotic yogurt with *Lactobacillus acidophilus* and *Bifidobacteria* cepas by people with hypercholesterolemia resulted in the reduction of total cholesterol and LDL (Low Density Lipoproteins: it is believed they are the harmful class to human beings) and in the increase of good cholesterol, HDL (High Density Lipoproteins: it is believed that they are able to absorb cholesterol crystals which are deposited in arteries/veins wall, therefore delaying arteriosclerotic process) in the blood.

Despite the great number of studies, reduction of serum cholesterol effect by probiotics is not considered an established effect, yet. Thus, new clinical trials controlled by placebo should be carried out to prove the efficiency of those microorganisms.

## 2.6 Cancer control

The fight against cancer is one of the biggest challenges faced by humanity. According to some authors, consumption of probiotic-supplemented products can prevent and even suppress tumor growth. According to Ma et al. (2010), *Bacillus polyfermenticus* was able to suppress *in vitro* and *in vivo* growth of cancer cells, suggesting that such microorganism can be used to prevent colon cancer development. Probiotic strains of *E. faecium* RM11 and *L. fermentum* RM28, isolated from fermented-milk were also shown to have antiproliferative properties against colon cancer cells, suggesting that such microorganisms can be used as an alternative to colon cancer (Thirabunyanon et al., 2009).

It was observed in volunteer subjects who received *Lactobacillus rhamnosus* LC705 and *Propionibacterium freudenreichii* a reduction of intestinal absorption of aflatoxin B1, a toxin correlated to the high liver cancer index. Therefore, probiotic supplementation can be effective in preventing development of liver cancer and other types of cancer caused by environmental factors (El-Nezami et al., 2006).

## 2.7 Allergy

Probiotics are able to reduce *in vitro* various inflammatory cytokines and intestinal permeability, which are effects considered beneficial in allergic conditions. In addition, gut microbiota of atopic patients seems quite different, with an increase in clostridia and a decrease of bifidobacteria when compared to microbiota of non-atopic individuals. Studies have been performed to evaluate the effectiveness of probiotics in food allergy, atopic eczema and rhinitis (Michail et al., 2006).

The administration of *Lactobacillus* GG in pregnant women, nursing mothers and babies in the first months of life was associated with a decrease in the occurrence of atopic eczema in children at risk of developing allergies compared to a placebo group at the end of a year of life (Kalliomäki et al., 2003). Another two controlled studies showed improvement of atopic dermatitis in children after use of *L. rhamnosus* and *L. reuteri*, and children with atopic eczema and allergy to cow's milk responded more effectively to a hydrolyzed formula supplemented with *Lactobacillus* GG (Majamaa et al., 1997; Rosenfeldt et al., 2003). These results are promising for the use of probiotics in allergies, but more studies are needed to confirm this property.

### 3. Probiotics for animal use

The use of growth promoters permit to improve animal performance. Initially, a great variety of antibiotic function substances, particularly penicillin and tetracyclines, were used to improve performance of birds, swines and cattle. The use of antibiotics as feed additive showed great benefits to animal production, mainly expressed in an improvement of weight gain and feed conversion. Antibiotics were used for many years, but they are being banned from animal production activities especially because of risks presented by resistant bacteria, which can result in problems to animal and human health. Therefore, probiotics are receiving special attention by animal nutrition researchers, who search for alternatives to the traditional use of growth promoters.

Probiotics have been incorporated through diets, with the objective to keep intestinal microbiota balance of animals, preventing digestive tract diseases, improving feed digestibility, leading to a greater use of nutrients and improving animal performance (Fuller, 1992).

Overall, effects of probiotic addition tend to be more outstanding in inadequate production conditions or in stress conditions, in which microbiota are unbalanced, especially in young animals. The most commonly highlighted factors among those previously cited are: temperature below or above thermal comfort zone; presence of pathogens; deficient sanitary conditions; management stressing conditions; change in feeding; weaning; transportation; high stock density; post-antibiotics treatment; sudden environment change. Regarding the results obtained in experiments with probiotics, those can be affected by factors as for example: type of probiotic microorganism; method and administered amount; host condition; intestinal microbiota condition; age of the animal.

#### 3.1 Probiotic in aquaculture

Probiotics in aquatic organisms can act similarly to terrestrial animals. However, the relationship between aquatic animals and cultivation environment is much more complex than that involving terrestrial animals. Because of this closer relationship between animal and cultivation environment, the traditional definition of probiotics is insufficient for aquaculture. Therefore, Verschueren et al. (2000) suggest a broader definition: "it is a microbial supplement with living microorganisms, with beneficial effects on the host, by modifying its microbial community associated with the host or its cultivation environment, by ensuring improved use of the artificial feed or its nutritional value, by

enhancing the host response towards diseases and by improving the quality of its ambient environment.”

Microorganisms in the aquatic environment are in direct contact with the outer part of the animals, as for example gills and with the supplied feed, with easy access to the digestive tract of the animal. Among those microorganism present in the aquatic environment are the potentially pathogenic ones, which are opportunists, that is, they take advantage of some stress situation of the animal (high density, deficient feeding), causing infections, which can worsen animal performance and even death. *Vibrio* sp., *Plesiomonas shigelloides*, in addition to *Aeromonas* sp. are the main agents causing death in aquaculture, and they can also cause feed infections in human beings. Thus, the objective of using probiotics by aquatic organisms is not only the direct beneficial to the animal but also its effect in the environment (Verschuere et al., 2000).

The interaction between environment and host in an aquatic environment is complex. Microorganism in the water influence host intestinal microbiota and vice-versa. Makridis et al. (2000) showed that supply of the bacteria strains through the feed and direct in the cultivation water, in turbot (*Scophthalmus maximus*) larvae incubators, promoted their maintenance in the environment, and they also promoted colonization of the digestive tract of the larvae.

Changes in the salinity, temperature, dissolved oxygen variations, alter conditions of the environment, which are propitious to different organism, with consequent changes in the dominant species, which may lead to a efficacy loss of the product. Thus, addition of a probiotic into the cultivation water must be constant, because the medium conditions undergo periodical changes. So, when choosing the pro biot to be used in aquaculture, variety of the microorganism present in the medium must be taken into account.

Intensive cultivation systems use high stock densities, among other stressing factors (for example: management), which result in low growth rates and feeding efficiency, a fragility in the immune system, making those animals susceptible to the presence of opportunist pathogens present in the cultivation environment. Thus, the effect of the probiotics on the immune system has led to a great number of studies with results beneficial to the health of aquatic organisms, although the way they act have not been clarified, yet. Gram et al. (1999) showed that the use of *Pseudomonas fluorescens* AH2 as probiotic, reduced mortality of rainbow trout (*Oncorhynchus mykiss*) juveniles exposed to *Vibrio anguillarum*. The joint administration of *Lactobacillus fructivorans* and *Lactobacillus plantarum* through live or dry feeding promoted colonization in intestine of sea bream (*Spaurus aurata*) larvae and the reduction of animal mortality during larva culture and nursery (Carnevali et al., 2004). Kumar et al. (2006) observed a greater survival in *Labeo rohita* carp fed *Bacillus subtilis*, submitted to intraperitoneal injection with *Aeromonas hydrophila*.

Regarding cultivated shrimp, bacterial illness are considered the greatest mortality cause in larvae. The administration of a bacterium mixture (*Bacillus* sp. e *Vibrio* sp.) influenced positively survival and presented protection effect against *Vibrio harveyi* and the white spot virus (Balcázar et al., 2006). In the clam *Argopecten purpuratus*, an *Alteromonas haloplanktis*, able to reduce larva mortality when submitted to challenge with *Vibrio anguillarum*, was isolated (Riquelme et al., 1996).

Probiotics can also be used to promote growth in aquatic organisms, either by a direct help in nutrient absorption or by supplying them. Lara-Flores et al. (2003) concluded that the use of *Saccharomyces cerevisiae* yeast as probiotic for Nile tilapia (*Oreochromis niloticus*) alevino as growth promoter, resulted in a greater growth and feed efficiency, suggesting that yeast is a proper growth promoter in the tilapia farming. Lin et al. (2004) used *Bacillus* sp. in the diet of shrimp *Litopenaeus vannamei* improving feed digestibility indices. Ziaei-Nejad et al. (2006) added probiotic *Bacillus* sp. in the cultivation of shrimp *Fenneropenaeus indicus* larvae and observed that in addition to the increase of survival, there was an increase in the activity of enzymes lipase, protease and amylase in the digestive tract of shrimp, which may stimulate the better use of the artificial feed.

However, addition of probiotics *Bacillus subtilis* at different doses (2.5; 5.0 and 10 g kg<sup>-1</sup> of diet) in diets for bullfrog (*Lithobates catesbeianus*) with initial weight of 3.13g did not improve weight gain, apparent feed conversion and survival when compared to control treatment (without addition of probiotic), but the immunostimulatory effect was evidenced through the increase of phagocytic capacity in the animals (França et al., 2008).

Another aspect of using probiotics in aquaculture is the improvement of the water quality in culture ponds. Reduction on nitrogen and phosphate compounds in the water used in *Litopenaeus vannamei* shrimp cultivation was observed when commercial probiotics were added into the water (Wang et al., 2005). Similarly, it was observed an improvement in the water used for cultivation of *Penaeus monodon* shrimp when *Bacillus* sp. was used as probiotic (Dalmin et al., 2001).

The conditions in which animal are submitted during cultivation can influence directly efficiency of probiotics. Thus, when they are not submitted to stressing situations, the obtained results many times do not show significant effect of probiotics on animal performance, so, more scientific studies should be conducted to know better interactions between those factors with the animals.

### 3.2 Probiotics in poultry production

The objective of using probiotics in poultry production is to improve performance in broiler chickens and to increase egg production in laying hens in addition to reduce intestinal colonization by pathogens as *Salmonella* sp. the main genus of bacteria identified in gastrointestinal tract of birds are: *Bacillus*, *Bifidobacterium*, *Clostridium*, *Enterobacter*, *Lactobacillus*, *Fusobacterium*, *Escherichia*, *Enterococcus* and *Streptococcus*.

The starting point in the use of probiotics in birds was set by Nurmi & Rantala (1973), who observed that when intestinal content of the healthy adult birds were orally administrated to birds at one day of age, it changed their sensitivity to *Salmonella* sp. in poultry production, manners of probiotic administration to birds more commonly observed are: by the feeds, by drinking water, by pulverization on the birds, inoculation via cloaca or in embryonated eggs (*in ovo*), among others, and the manner of administration has effect on intestinal colonization capacity.

Except if submitted to stress situation, bacteria which colonize gastrointestinal tract of the birds since their birth, tend to remain there for the rest of their lives. Therefore, *in ovo* inoculation is an aspect of using probiotics for birds. *In ovo* inoculation of probiotics

*Lactobacillus casei*, *Lactobacillus plantarum* and *Enterococcus faecium* at  $10^6$  UFC egg<sup>-1</sup>, at 16 days of incubation and the performance of challenge of chicks at one day of age via stomach with  $13.6 \times 10^6$  UFC mL<sup>-1</sup> of *Salmonella enteritidis*, improved performance of animals fed probiotics when compared to control treatment (Leandro et al., 2004). Moreover, in the same experiment, authors observed that from 7 to 21 days of age, *Salmonella* sp. was identified only in challenged animals which were not fed probiotic. It is suggested that probiotic avoided bacterium colonization in the gastrointestinal tract of the birds.

In chicks emerging from incubators, pH concentration and the presence of volatile fatty acids, which are one of the main protection barriers of the animal organism, are not sufficiently chemically to avoid that pathogens enter in their organism. Moreover, the small variety of the birds' intestinal microbiota in this phase is considered as a limiting factor for the digestion and for the possibility of intestinal colonization by enteric pathogens. Thus, probiotic supplementation seems to be a beneficial action for the animal performance and health of birds from commercial incubators.

An efficient immune response is related to the presence of immunomodulators in the diet, which will act by reducing immune stress and then reducing nutrient mobilization to activities which are not related with production (meat or eggs), permitting in addition to a greater survival in stress situations, the non-harmful effect on animal performance.

The use of yeast *Saccharomyces boulardii* in the diet for broiler chickens reduced the level of *Salmonella* sp. from 53.3% to 40.0% at stress conditions in transportation to slaughter (Line et al., 1998). The used of yeast *Saccharomyces cerevisiae* var. *chromium* reduced negative effects of caloric stress on broiler chickens (Guo & Liu, 1997).

The results of studies with probiotics in poultry production have been showed to be rather contradictory regarding to its efficiency. Not always are positive results observed by using probiotics. Those vary with age of the animal, type of probiotic used, viability of the microorganisms, storage conditions, level and manner of administration, in addition to the low challenge in relation to the experimental condition concerned to sanity, management and other stressful conditions. Some researchers have stated that the addition of probiotics into the diet did not improve animal performance in broiler chickens. Estrada et al. (2001) observed that the administration of *Bifidobacterium bifidum* did not alter significantly animal growth. But, according to Zulkifli et al. (2000), even by observing an increase in the feed intake, there was no reduction in feed efficiency in broiler chickens when *Lactobacillus* sp. was administered in the diet.

On the other hand, several studies have shown extremely interesting results on addition of probiotics into diets for broiler chickens. The addition of *Bacillus subtilis* into the diet increased weight gain and feed conversion (Fritts et al., 2000). The addition of *Lactobacillus* increased weight gain and improved feed conversion of supplemented animals (Kalavathi et al., 2003). The use of yeast *Saccharomyces boulardii* in *Salmonella enteritidis* infected broiler chickens improved feed efficiency by 10% when compared to control treatment, and by 12% in animals supplemented with *Bacillus cereus* var. *toyoi* (Gil de los Santos, 2004).

Concerning to carcass quality of broiler chickens, the beneficial effect of probiotic use was also observed. The addition of *Lactobacillus acidophilus* e *Streptococcus faecium* reduced plasma protein concentration, levels of total cholesterol and HDL in addition to an increase in the protein content of probiotic supplemented animals (Pietras, 2001).

### 3.3 Probiotics in swine farming

The bacteria usually found in the gastrointestinal tract of swine are: *Bacteroides rumnicola*, *B. uniformis*, *B. succinogenes*, *Butyrivibrio fibrilans*, *Clostridium perfringens*, *Escherichia coli*, *Eubacterium aerofaciens*, *Lactobacillus acidophilus*, *L. casei*, *L. fermentum*, *Peptostreptococcus productis*, *Selenomonas ruminantium*, *Streptococcus salivarius* and the yeast found are: *Saccharomyces cerevisiae* and *Candida* sp. (Russel, 1979).

By evaluating the balance between beneficial and pathogenic bacteria in the intestinal epithelium of swines in normal conditions, Robinson et al. (1984) found *Lactobacillus acidophilus* in 11.9%, *Streptococcus faecium* in 54.4% and *Escherichia coli* in less than 1%. When there were intestinal disorders, reduction of *L. acidophilus* and *S. faecium* up to 6% was observed, resulting in an increase of *E. coli* to 14%.

Regarding microbiota in the gastrointestinal tract, it is found two critical moments in the swine farming, which are birth and weaning. Piglets are born without microbiological contamination, but in a short time, gastrointestinal tract is mostly colonized by *Lactobacillus*, *Bifidobacterium* and *Bacteroides*, and less by potentially pathogenic organism as for example *Escherichia coli*, *Enterococcus*, *Clostridium* and *Staphylococcus*. After weaning, there is a drop in lactic bacteria population, so population of pathogenic organism increases (for example: *E. coli*). These pathogenic microorganisms can be adhered to the intestinal epithelium, then they multiply, unbalancing intestinal microbiota, causing post-weaning diarrhea.

Administration of *Lactobacillus* sp. as probiotic for piglets during a six-week period increased its presence in the intestine and reduced *Pseudomonas* sp. and *Clostridium perfringens*, in addition to reduce intestinal pH, although effect on weight gain and apparent feed conversion had not been observed, compared to the control group (Tereda et al., 1994). Likewise, administration of probiotic for sows from the end of gestation to the end of lactation will be able to stabilize intestinal microbiota of the female, establishing a favorable microbiota in piglets in suckling. This fact was evidenced by Alexopoulos et al. (2004).

Conditions of microbial unbalance during stress create a favorable condition for fixation of pathogenic microorganisms, leading to structural changes, as for example shortening of villi. This reduction results in a smaller absorption area, lower production of enzymes and nutrient transportation, predisposing animals to poor absorption, a possible dehydration and conditions of enteric infections. Upon this aspect, results obtained with the use of probiotics for swines are very contradictory. Pollman & Bandick (1984) reported that animals fed *Lactobacillus* based products did not present difference on small intestine morphology when challenged by *E. coli*. But, Jonsson & Henningsson (1991) did not observe probiotic effect on the size of the villus. Kritas et al. (2006) observed less incidence of diarrhea in piglets supplemented with *Bacillus subtilis* and *Bacillus licheniformis* during suckling and post-weaning. However, Utiyama et al. (2006) did not observe any benefic effect of supplementation with *Bacillus subtilis* and *Bacillus licheniformis* in diets for weaned piglets under diarrhea control when compared to the ones which were not fed probiotic.

Those differences can come from factors as for example: genetics of the animals, species of the microorganisms used in the product; the used dose; environment temperature and sanitary condition of the swine farm inasmuch as many experiments evaluate the use of probiotics in low sanitary challenge conditions. In addition to a good sanitary quality, those

conditions must provide no stress for the animals and balanced microbial community, probiotics and even antibiotics used at subclinical dose will have little or any effect on animal performance. However, it is difficult that an animal will not suffer from stress or will live in an environment free of pathogenic microorganisms in the exiting commercial production nowadays.

Regarding animal performance, Roth & Kirchgessener (1988) observed improvement in weight gain, feed intake and feed conversion when using *Bacillus toyoi* based probiotic in diets for piglets. Cristani et al. (1999) observed an improved of up to 8% in feed conversion of piglets in the nursery phase, when bacteria *Lactobacillus acidophilus* was administered in the diet. Those results can be related to enzymatic production of probiotics with improve nutrient digestion by lactase and galactosidase production, which hydrolyzes lactose, permitting its absorption. The use of a probiotic congaing *Bacillus licheniformis* and endospore of *Bacillus subtilis* increased feed intake, reduced weight loss and reduced the interval between weaning and estrus in sows (Alexopoulos et al., 2004). In the same study, it was observed in piglets from those sows, an average of 0.38 kg more than the control group.

### 3.4 Probiotics for ruminants

From the possible effects observed with the addition of probiotics for ruminants, it stands out: increase in the number of bacteria in the rumen; increase in rumen digestion of cellulose, increasing nutrient availability for production process, improving use efficiency of roughage, in addition of stimulating greater dry matter ingestion; competitive exclusion in the intestine, resulting in a reduction of bacteria which cause diarrhea; production of bacteriocine; acting as immunostimulants.

Yeasts are used in ruminant feeding with the objective of increasing dry matter digestibility, especially neutral detergent fiber and acid detergent fiber (Kamalamma et al., 1996). Yeast supplementation increases the number of bacteria in the rumen, particularly cellulolic bacteria. Growth factor supply (for example: vitamins), removal of oxygen by *Saccharomyces* (rumen content is essentially anaerobic), buffer effect and reduction in the number of protozoan are some of the factors associated to this response (Callaway & Martin, 1997).

Among the several species of bacteria present in the rumen, cellulose bacteria, essential for ruminant nutrition for cellulose digestion, stands out. Another important function of the microbiota in the rumen is the production of complex B vitamins. Fermentative activities and qualitative content of microorganisms in the rumen can vary and decrease according to the diet and stressing situations as well. In the modern agriculture, cattle are constantly submitted to stressing factors as for example frequent management in the barn, vaccinations, identification, castration, contention, artificial insemination, confinement, and so on. Therefore, it can be concluded that depression in rumen microbiota, resulting from stress, will reduce feed digestibility, vitamin synthesis, resulting in growth and milk production.

Because increase of genetic potential of the animals it becomes more and more necessary the development of diets with greater genetic and protein content jointed with adequacy of fibrous fraction, which is important in rumen health. Therefore, the inclusion of grains into the diet favors growth of bacteria as *Streptococcus bovis*, which is lactate producer, causing reduction in the rumen pH. Submitting cattle to diet with high percentage of concentrate

may result in rumen fermentation detriment. Therefore, addition of products which are able to keep or change rumen fermentation pattern, maintaining animal health, has become an important strategy in the feeding of those animals.

Ruminants have a differential in their digestive organ which confers to them a great capacity of digesting fibrous feedstuff. However, this capacity of converting fibrous feed into meat due to an inadequate feeding management, for example, can be poorly efficient. Thus, nutrition of those animals should search optimization of rumen fermentation, improving nutrient digestibility with a consequent better animal performance.

As it was previously mentioned for other species, positive effect of probiotics in animal nutrition are not always evidenced due to differences in sanitary conditions and different types of diets used as well. However, when this happens, increase in productivity parameters and improvement in the sanitary status are observed (Breul, 1998).

Krehbiel et al. (2003) observed that there was a smaller incidence of diarrhea when feeding bezeras with *Streptococcus* and *Lactobacillus acidophilus*, compared to animals which were not fed probiotic. In the work of Bechman et al., (1977), it was demonstrated that administration of *Lactobacillus acidophilus* for dairy calves improved feed conversion and reduced diarrhea incidence. Zhao et al. (1998) also observed that the possibility of reduction in the detection of *Escherichia coli* in probiotic supplemented animals.

According to Martin (1998), direct supplementation of microbial additive can improve ruminant production up to 8%. By analyzing results from several trials on confinement, Krehbiel et al. (2003) observe an increase in daily weight gain of 2.5 to 5.0% in addition to improving feed efficiency of 2% in animals supplemented with probiotics in the diet.

The use of high concentrate content diets results in increase of disturbances related to rumen fermentation, as for example bloat and acidosis. Mir & Mir (1994) observed that the addition of *Saccharomyces cerevisiae* into grain high content diets resulted in less occurrence of acute rumen acidosis in supplemented cattle compared to the control, suggesting that yeast promoted the use of lactate in the rumen. Administration of *Saccharomyces cerevisiae* for cattle submitted to a rapid fermentable diet improved daily weight gain in comparison to a diet without yeast (Agazzi et al., 2009) and improved digestion of low quality neutral detergent fiber in ruminants (Sommart et al., 1993).

Regarding to the reproductive system, it is observed that uterine pathologies during puerperal period are responsible for the reduction in reproductive efficiency in cows. Colonization by *Lactobacillus* is considered the first microbiologic barrier against pathogen infection in the genital tract (Ocaña et al., 1999). Thus, addition of those microorganisms as probiotics may improve reproductive efficiency of those animals, either by lactic acid production which reduced vaginal pH or by competition for nutrients and adhesion site in the vaginal epithelium.

### **3.5 Use of probiotics in other animals**

#### **3.5.1 Rabbits**

In rabbits, the occurrence of digestive disorders associated to feeding changes has risen mortality indices in the period close to weaning. So, the use of probiotics has been an

alternative in this phase because it favors improvement of digestive tract conditions by action on beneficial microbiota able to improve sanitary and physiologic status of the animal. Michelan et al. (2002) used probiotic Calsporin® (based upon endospores of *Bacillus subtilis*) for growing rabbits, evaluating digestibility of diets and their intestinal morphometry. The presence of probiotic did not influence nutrient digestibility neither morphometric traits of jejunum. Hollister et al. (1989) observed an improvement in apparent feed conversion, in addition to mortality reduction caused by enteritis in growing rabbits supplemented with Lacto-Sacc® (probiotic constituted by *Lactobacillus acidophilus*, *Streptococcus faecium*, *Saccharomyces cerevisiae*, and fermentation residues of *Aspergillus oryzae* and *Aspergillus niger*). Moreover, the use of Lacto-Sacc® improved crude fiber digestibility (Yamani et al., 1992) by weaned White New Zealand rabbits. However, Lambertini et al. (1990) did not observe any influence of probiotic composed of *Bacillus subtilis* on the performance of growing rabbits.

### 3.5.2 Equines

According to Frappe (1998), the use of probiotics stimulates intestinal biota growth and improves digestibility of crude fiber and crude protein. The main sources of fiber on composition of diets for equines are grass or legume hays. Legume hays normally presented greater nutritional value when compared to grass hay, however, they are more expensive and more difficult to be produced. So, the use of probiotics increases efficiency of grass hay use. But, results from the use of probiotics with this aim are not conclusive, yet. Morgan et al. (2007), evaluating addition of yeast in low and high quality Russel Bermuda grass hay diets observed an increase in crude protein and neutral detergent fiber digestibility only in the low quality diets, although neutral detergent fiber digestibility had not presented improvement. Likewise, Hill et al. (2006) observed increase in crude protein apparent digestibility for equines fed diets with high proportion of roughage:concentrate (80:20) supplemented with yeast. Increase in crude protein digestibility may be due to microbial activity in the large intestine which favored nitrogen compounds digestibility. By contrasting with those results, Moura et al. (2009) did not observe any improvement in total dry matter digestibility in foal fed grass and concentrate, yeast supplemented. Moore & Newman (1993), supplementing foals with yeast, observed maintenance of the highest values of pH in the large intestine. According to these authors, reduction in pH below 6.5 affect cellulose bacteria therefore it affects fiber digestion and help to prevent colic and laminitis.

The use of yeast in diets for mare during gestation and lactation resulted in greater contents of crude protein, sugar, total lipids and proteins in the milk, and beneficial effects were also observed in the foals of those mares (Glade, 1991).

### 3.5.3 Dogs

Kosaza (1989) reports that in cases of acute diarrhea in dogs, treatment with *Bifidobacterium pseudolongum* was positive. Swanson et al. (2002) observed that administration of *Lactobacillus acidophilus* increased digestibility of dry matter, organic matter and crude protein. However, Biourgue et al. (1998) observed no improvement regarding digestibility of dry matter, protein, lipids and energy.

#### 4. Conclusions

The use of probiotics for the prevention and / or treatment of gastrointestinal disorders have a strong theoretical justification, based on the beneficial functions of the indigenous microbiota, fundamental for maturation and health of the digestive ecosystem. However, a number of issues need to be resolved before general guidelines regarding the use of probiotics can be given. Basic research must provide more detailed data on the mechanisms of probiotic action on the molecular level, after which coordinated rigorously conducted clinical trials must be undertaken to find the probiotic strain and dosage with optimal results for each clinical situation. It is unlikely that one strain or probiotic combination will be sufficient for all purposes. At the moment, the heterogeneity of probiotic clinical trials hampers interpretation – in particular the diversity of probiotic strains, dosing regimens and forms of administration used and the varied patient groups recruited in the available studies makes interpretation difficult. Concluding, more biological and well controlled clinical trials must be carried out for a more precise understanding of both the mechanisms underlying the probiotic action and the complex gastrointestinal ecosystem with which probiotics are expected to interact.

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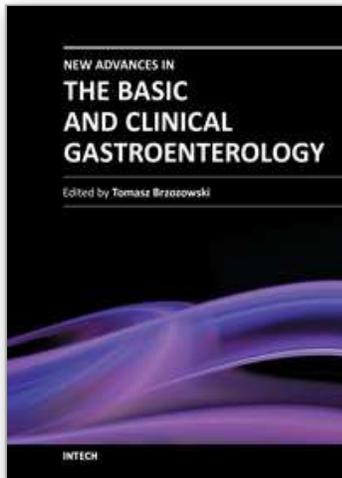
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The purpose of this book was to present the integrative, basic and clinical approaches based on recent developments in the field of gastroenterology. The most important advances in the pathophysiology and treatment of gastrointestinal disorders are discussed including; gastroesophageal reflux disease (GERD), peptic ulcer disease, irritable bowel disease (IBD), NSAIDs-induced gastroenteropathy and pancreatitis. Special focus was addressed to microbial aspects in the gut including recent achievements in the understanding of function of probiotic bacteria, their interaction with gastrointestinal epithelium and usefulness in the treatment of human disorders. We hope that this book will provide relevant new information useful to clinicians and basic scientists as well as to medical students, all looking for new advancements in the field of gastroenterology.

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