

The beneficial role of Probiotics in monogastric animal nutrition and health

Abstract

The practice of probiotics in farm animals' health and production has increased significantly over the last 15 years. Probiotics are defined as live microorganisms that can confer a health benefit for the host when administered in appropriate and regular quantities. The isolation and identification of microorganisms is the first step in the selection of potential probiotics from gut, feces and milk of respective animals. The present molecular techniques mainly genomic and proteomic-knowledge based are employed to identify, characterize probiotics. The ability to examine fully sequenced genomes has accelerated the application of genetic approaches to elucidate the functional roles in the selection of new and specific probiotics. Identification of suitable probiotics may prove to be the next step to decrease the risk of intestinal diseases and reduce specific microbial disorders, as well as demonstrating their role in the production performance of animals, safety and wholesomeness of animals' meat evidencing consumer's protection. The mechanisms of action of probiotics include the inhibition of pathogen growth by competition for nutritional sources and adhesion sites, secretion of antimicrobial substances and toxin inactivation. Consequently, the primary interest in the application of probiotics has been in the prevention and treatment of gastrointestinal infections and antibiotic-associated animals' diarrheal diseases. In this review, the most important benefits of probiotics upon the gastrointestinal microbial ecosystem in monogastric animals (equines, pigs, veal calf and poultry) are described, as well as their implications in terms of animal nutrition and health. Additional knowledge on the possible mechanisms of action is also provided.

Keywords: probiotics, competitive exclusion, pig, poultry, veal calf, horse

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Introduction

Dense and complex microbial communities reside in the gastrointestinal tract of domestic animals, which can be composed of bacteria, protozoa, fungi, archaea, and viruses. Extensive research has been dedicated during the last 30 years to characterization of digestive microbial composition and functional diversity, which has directed to a better understanding of the major involvement of the gut microbiota to animal nutrition and health. Amongst the valuable effects, gastrointestinal tract microbial communities are aimed to digestion and fermentation of plant polymers, which is of specific significance in herbivorous animals. Moreover, the indigenous gut microbiota is accountable for the synthesis of vitamins, bioconversion of toxic compounds to non-toxic residues, stimulation of the immune system, maintenance of gut peristalsis and intestinal mucosal integrity and plays a barrier role against colonization by pathogens.¹

In this context, the possibility to use feed supplements to achieve better animal health, welfare and productivity through manipulation of the gut micro biota ecosystem has gained considerable attention in the last three decades. The main effects of these additives, pro biotics on top of others, are a facilitated resistance to pathogenic bacteria colonization and enhanced host mucosa immunity, thus causing a reduced pathogen load, an improved health status of the animals^{2,3} and a reduced risk of food-borne pathogens in foods.

What is a probiotics?

Over the years, probiotic in the world has been used in a number of different ways. The use of probiotics in farm animals dates back 75 years, but in the 1960s, for the first time it was demonstrated that

Lactobacillus was able to significantly stimulate growth on pigs.⁴ Chow⁵ reported the concept that food could be provided as medicine.⁵ This concept was first conceived thousands of years ago by Hippocrates (Greek philosopher and father of medicine), who once wrote: 'Let food be thy medicine, and medicine be thy food'. Nonetheless, during current times, the notion of food having medicinal value has been reborn as 'functional foods'.⁵ In the general term "functional foods", probiotics have quickly gained attention in the field of self-care and complementary medicine.⁶ Modern consumers in the world are progressively paying more attention to their personal health in order to preventing and/or curing illness through daily foods. Microbes have been used in food and alcoholic fermentations since many years, but most recently have undergone scientific scrutiny to examine their possible health benefits. The word "probiotic" comes from the Greek words "pro" and "biotic," meaning "for the life",⁷ and has been defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".⁸ Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host.⁹ Probiotics are not necessary to be viable, as nonviable forms have also been proved to provide health effects.¹⁰ It is thought to reduce potentially harmful bacteria from the intestine and to improve microbial balances in intestine and exert positive health effects on the host.⁸⁻¹¹ Today, the term "probiotic" refers to "live microorganisms which, administered in adequate amounts, confer a beneficial physiological effect on the host," according to the Food and Agriculture Organization and World Health Organization.¹²

The protective flora naturally present in the gut is very stable; however, it can be influenced by some dietary and environmental

factors such as hygiene conditions, antibiotic therapy and stress factors.⁸ In the wild state, young animal picks up its gut flora mainly from its mother by direct or indirect routes. However, modern methods of animal rearing often restrict the access that the infant has to the mother and prevents it acquiring the full complement of characteristic microbes. But, probiotic and competitive exclusion microorganisms would protect the newborn young animal against infection (Figure 1). Competitive exclusion cultures are composed of a mixture of non-pathogenic bacteria (probiotic culture) typically found in the gastrointestinal tract of the animal which are administered only in a single dose to the neonatal animal, such as the day-old chick or a newborn piglet, demonstrating the impact of the gut microbiota on gut function and disease resistance^{13,14} and also that they can help to reestablish the gastrointestinal tract flora after antimicrobial treatment.¹⁵ Because of the susceptibility of newborn animals to infection, this practice is also of commercial importance. By using this model, a number of probiotics^{13,16–19} have been shown to prevent colonization and shedding of *Salmonella* and *Campylobacter* at farm level to control food borne diseases.

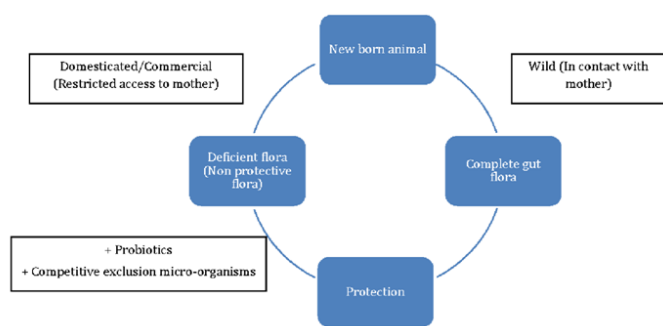


Figure 1 Schematic representation of the concept of probiotic, modified from.^{8,20–23}

The microbial species recently being used in probiotics mixtures are many and varied. The major microbial groups such as *Bacteroides*, *Clostridium*, *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Enterobacteriaceae*, *Streptococcus*, *Fusobacterium*, *Peptostreptococcus* and *Propionibacterium* are used in monogastric animals (such as pig, chicken, rabbit and man). On the other hand, in polygastric animals, (such as cow, sheep and lamb), the rumen is the most vital microbial ecosystem with the majority of fiber-degrading groups belonging to *Fibrobacter*, *Ruminococcus*, *Butyrivibrio* and *Bacteroides* together with major groups such as *Prevotella*, *Selenomonas*, *Streptococcus*, *Lactobacillus* and *Megasphaera*. Moreover, the rumen maintains some anaerobic fungi, ciliate protozoa and a large number of methanogens to keep normal rumen microenvironment.²⁴ A list of the probiotic species used in studies or in livestock feeding and health is shown in Table 1. Mammals' age, diet, health and pathological status might be influenced by the percentage of individual/various microbial groups.^{25,26} Normally, herbivores, carnivores and omnivores are respectively characterized by a high, low and intermediate number of bacterial phyla.²⁷

To ensure successful application, probiotics must have important characteristics such as resistance to gastrointestinal conditions (gastric acid and bile), ability to adhere to the gastrointestinal mucosa and competitive exclusion of pathogens.⁵² The expected health-promoting characteristics and safety criteria of probiotics are shown in Table 2.

Historical use of probiotics

The Bible and the sacred books of Hinduism mentioning about

the origin of cultured dairy products dates back to the dawn of civilization. Climatic conditions sure effect for the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, leben and dahi.⁵⁶ Many of these products are still extensively consumed, had frequently been used therapeutically before the existence of bacteria was recognized.⁵⁷ The basic concept of “probiotics” appeared long time ago when they were consumed, either as natural components of food, or as fermented foods. The interest in intestinal microbiology and dietary use of probiotics began in the late 1800s and early 1900s. The growing interest was also motivated by Escherich's isolation of *Escherichia coli* in the late 1800s, as well as active research on the benefits of feeding lactic acid bacteria and lactose near the turn of the 20th century.⁵⁸ The Nobel laureate Elie Metchnikoff noticed the high life expectancy of Bulgarians who consumed yogurt. In 1907, he reported that the indigenous bacteria were harmful and that ingestion of lactic acid bacteria in yogurt had a positive influence on health.^{59,60} He named the microorganism significant for the fermentation *Bacillus bulgaricus*, later classified as *Lactobacillus bulgaricus* that was used for the prevention of human scours and gastrointestinal diseases as early as the 1920s. At the same period, a French pediatrician, Henry Tissier, studied that infants suffering with diarrhea had a small number of Y shaped gram-positive bacteria in their stools, while healthy infant's stools contain a dominant number of these “bifid” bacteria. He even recommended, in a report to the Biology Society, to make children presenting with diarrhea drinking “1 to 2 Bordeaux glasses of a pure culture of *Bacillus acidi paralactici*, or even better of a symbiosis of this species with *Bacillus bifidus*, to accelerate the building up of a preventing flora”.

The word probiotic was first mentioned by Lilly & Stillwell in 1965, as opposite to the word antibiotic, to qualify “a microbial substance able to stimulate the growth of another microorganism”. Subsequently, the concept of microbial origin was introduced, redefining probiotics as “living micro-organisms with beneficial effects on the host, by modifying the equilibrium of its gut microbiota”. During the following decades until the 1960s and 1970s, there was little implication in probiotics when they were rediscovered for mammalian nutrition mainly human and animal. The primary potent probiotic products for animal nutrition that accomplished to the exact requirements for feed additives did not emerge on the European market until the mid-1980s.⁵³ Over a century, Eastern Europeans and Asians used probiotics invariably containing lactic acid bacteria and *Bifidobacteria* as a natural remedy to prevent and treat infections of the urogenital, intestinal tracts and skin in humans and animals as yogurt and drinks. The results of numerous *in vitro* and *in vivo* studies, in animal models and clinical trials have proven the potential of these probiotics to prevent many diseases and disturbances of the intestinal microbiota that could increase susceptibility to infection.^{59,60}

Global probiotics market

Interest and demand for probiotics are increased all over the world, driven by promising new products in the market and by consumers looking for safe therapeutic and preventive health benefits. The major factors driving the growth of the global probiotic market include growing health consciousness of the population and the availability of probiotics in the form of dietary supplements. In 1999, the Agri-food trade in Europe boasted a 48% increase in turnover with probiotic products.⁶¹ Indeed, the global use of probiotic ingredients, supplements and food is seen as a rapidly expanding market. Global sales of probiotic ingredients, supplements and foods amounted to

USD 21.6billion in 2010 and USD 24.23billion in 2011. Based on a new market statement available by Transparency Market Research, “Probiotics Market (Dietary Supplements, Animal Feed, Foods & Beverages): Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast”, the global market of probiotic is anticipated to achieve USD 31.1billion by 2015 as well as a compound annual growth rate (CAGR) of 7.6% over the previous 5year period.

It will be predictable to reach USD 44.9billion in 2018. Asia-Pacific and Europe lead the global probiotics market owing to its demand, whereas Asia-Pacific is also expected to be the most promising market in the near future.⁶² Asia-Pacific is the probiotics market leader, with an anticipated to reach CAGR of 7.0% from 2013 to 2018. In Asia-Pacific, China and Japan lead the market income for probiotics. Also India and other regions are showing significant growth.

Table 1 Some microbial species of potential use as livestock probiotics with their benefits

Microorganisms	Animals	Reference	Common Benefits
Pig			
<i>E. faecalis</i>		28	Improve colostrum quality, milk quality and quantity
<i>E. faecium</i>		29	
<i>Bacillus cereus</i>		30	Increase litter size and vitality
<i>B. subtilis</i>		31	Increase piglet weight
<i>B. licheniformis</i>		31	Reduce risk of diarrhea
<i>L. johnsonii</i>		32	Improve feed efficiency, diet digestibility and meat quality
<i>L. reuteri</i>		31,33	
<i>L. acidophilus</i>		33	Limit constipation
<i>S. cerevisiae</i>		34	Decrease stress
Poultry			
<i>L. animalis</i>		35	
<i>L. fermentum</i>		35	
<i>L. salivarius</i>		36,37	Increase body weight gain
<i>L. acidophilus</i>		38	Reduce mortality
<i>S. faecium</i>		38	Increase carcass quality decreasing contamination
<i>L. reuteri</i>		39	Increase bone quality
<i>E. faecium</i>		19	
<i>S. cerevisiae</i>		40	
<i>Bacillus</i> sp.		40	
Veal Calf			
<i>S. cerevisiae</i>		41	
<i>L. acidophilus</i>		42	Promote weight gain and optimal maturation of rumen microbiota limiting acidosis
<i>B. pseudolongum</i>		42	Increase feed efficiency, milk yield, quality and digestive safety at weaning
<i>L. animalis</i>		43	Reduce risk of pathogen colonization and limit shedding of human pathogens
<i>L. paracasei</i>		43	
Horse			
<i>Lactobacillus pentosus</i>		44	
<i>L. rhamnosus</i>		45	Improve diet digestibility, milk quality and quantity
<i>L. acidophilus</i>		46	Limit diarrhea
<i>L. plantarum</i>		47	Avoid hindgut disorders (acidosis, colic)
<i>L. casei</i>		48	Limit stress
<i>S. boulardii</i>		49	(Transportation, race etc.)
<i>S. cerevisiae</i>		50,51	

Table 2 Expected characteristics of ideal probiotics^{6,53-55}

1. To have a confirmed favorable effect on the host demonstrating ability to exert at least one scientifically-supported health-promoting property.
2. To be non-pathogenic, non-toxic and free of significant adverse side effects on targeted species.
3. To have a precise taxonomic recognition.
4. To be a normal inhabitant and modify intestinal microbiota of the targeted species.
5. To be able to survive, colonize and being metabolically active in the targeted site, which implies:
1) Resistance to gastric juice and bile
2) Persistence in the gastrointestinal tract
3) Adhesion to gastrointestinal tract epithelium or mucus
4) Competition with the resident microbiota

Table Continued..

6.To secrete molecules that are:

- a) Freely diffusible, membrane permeable being intercepted by specific receptors at the cell surface.
- b) Metabolic and pure signaling molecules which stimulate concurrently precise structural, metabolic and signaling activities.
- c) Bacteria/bacteria and microbe/host cross talk (Quorum Sensing including toxin antitoxin systems controlling programmed apoptosis and cell death).

7.To be genetically stable.

8.To be amenable and stable during industrial processing, storage and delivery.

9.To be viable at high populations.

10.To be able to restore pre-antibiotic baseline flora.

11.To be able to deliver therapeutics.

12.To be able to enhance animal performance decreasing carcass contamination and ammonia and urea excretion in animals.

Europe market is alternative for probiotic products, with consumer consciousness levels much greater than in North America; European probiotic demand is predictable to rise at a CAGR of about 6.7% from 2013 to 2018. In European market, Germany and the UK are the most demandable markets, with projected CAGR of over 6% each from 2013 to 2018. The North American probiotics markets and emerging countries such as Brazil also show increase potential for demand growth. Based on the application, the probiotics market is classified into food and beverages, dietary supplements and animal feed that are further divided into segments. Probiotic foods & beverages are the dominant segments the global market and are expected to grow at a CAGR of 6.8% from 2013 to 2018. Probiotic demand for food & beverage segment is estimated to reach USD 37.9 billion in 2018. Following food and beverages, the market for dietary supplements and animal feed are also witnessing significant growth, growing at a CAGR of 6.8% from 2013 to 2018. With respect to application segments, probiotics are widely used in dairy, non-dairy, cereals, baked products, fermented meat products, dry foods and others. Dairy products are the largest application market for probiotic foods. Probiotic demand for dairy products is estimated to reach USD 32.2 billion in 2018, growing at a CAGR of 6.8% from 2013 to 2018. Probiotics have also emerged as a critical part of the animal feed industry with a demand for estimated to cross USD 3 billion by 2018.⁶²

Fundamental criteria for selection of ideal probiotics in animal industry

Functional, safety and technological characteristics have to be taken into account in the selection process of probiotic microorganisms. There are many microorganisms to be considered as potential probiotics, but only a few numbers of microorganisms are able to satisfy the necessary criteria. The probiotic bacteria must fulfill the following functional aspects, such as to be normal inhabitant of the gut, and to be able to persist in the gastrointestinal tract, surviving the digestive stresses, immunomodulation and the competition against other micro-organisms; finally, to have antagonistic and antimutagenic properties.⁶³⁻⁶⁷ Moreover, probiotics must reach the site of their main activity in the digestive tract unharmed to be efficacious. In the main target species this is the small intestine for monogastric animals together with the crop for poultry and the rumen for ruminants. Since factors such as pH, the transit time of the digesta and the concentration of active substances in the feed can influence the growth of probiotics,^{66,67} their growth or germination in the digestive tract must be evaluated in feeding trials using diets which are relevant under practical conditions. This can be measured indirectly via

performance parameters but, better, directly by counting the living probiotic microorganisms in the various intestinal segments.

Safety aspects include specifications such as origin (healthy respective animals' gastrointestinal tract), absence of any pathogenicity, nondigestive upsets, and transmissible antibiotic resistance gene. Probiotics, specifically *Bifidobacteria* and *Lactobacilli*, have a long history of safety with their use in fermented food and milk. Moreover, these bacteria especially *Lactobacilli*, are regularly encountered in nature: plants, animals, and in humans as a commensal microbiota. *Lactobacilli*, *Bifidobacteria*, *Lactococci*, and yeasts are classified in the category of organisms Generally Regarded as Safe (GRAS). On the other hand, a few cases of infections have been occurred in immunocompromised patients.⁶⁸ Not all the probiotics used belong to this GRAS category such as *Enterobacteria* or *Enterococci*, but some strains of *Enterobacteria* or *Enterococci* are used as probiotics.⁶⁹

Satisfactory technological criteria are also important for selection of probiotics in food and feed production along with safety and functional criteria. The food and feed manufacture, distribution, and storage reduced viability of probiotic bacteria. Lactic acid bacteria do not form spores as natural residents of the intestine. They are therefore, in ordinary dried form, unprotected against the chemical and physical stresses, for example during pelleting. It is hence necessary either to use them only for feed types which place little technical stress on the microorganisms (for example in milk replacers) or to protect them specifically against mechanical and heat impacts during feed manufacturing, transport and storage. Lactic acid bacteria may be give a protective coating using special technological procedures such as microencapsulation or microspherizing, thus ensuring that these non-spore bacteria are able to reach the site of action intact and become active. The stability of the coating is determined by the quality of the process.⁶ On the other hand, for *Bacillus* spores is their natural stable form that allows them to survive in their original habitat, the soil, protected from extreme heat, cold and mechanical strain, without any loss in their vital potential. Various cell walls protect the nucleus from external stresses. This natural protection enables the *Bacillus* products to withstand massive strains during feed production and storage, such as high temperatures, pressure, shear forces or oxidation impacts.⁷⁰⁻⁷² Therefore, *Bacillus* spores are suitable for all types of feeds. In addition, their vitality is not compromised by low pH values in the stomachs of monogastric animals. Spore quality and stability of the *Bacillus* products and their ability to germinate are influenced by the fermentation conditions during production. In case of yeast culture, yeasts are living fungi and are made dormant by drying. Since their

external surface is more stable and less permeable in this state, yeasts survive many processes of feed production and storage undamaged. Then the presence of sufficient moisture and warmth in the digestive tract allows them to regain their metabolic activity. Also sensory characteristics such as unpleasant flavors or textures are important criteria to select probiotics.⁷³

The proposed tentative ways for selection of probiotics as biocontrol agents in the animal industry are illustrated in Figure 2.

There are many *in vitro* assays established for the pre-selection of probiotic strains.⁷⁴⁻⁷⁶ *In vitro* assays could give idea to the selection of the competitiveness of the most promising strains which was evaluated *in vivo* for monitoring of their persistence in animal model.³⁶ Furthermore, potential probiotics must exert its beneficial effects (e.g. enhanced nutrition, stimulation/suppression of immune responses and resistance to antibiotics) in the respective animal. Lastly, the probiotic must be viable under normal storage conditions as well as technologically suitable for industrial processes (e.g. lyophilized).

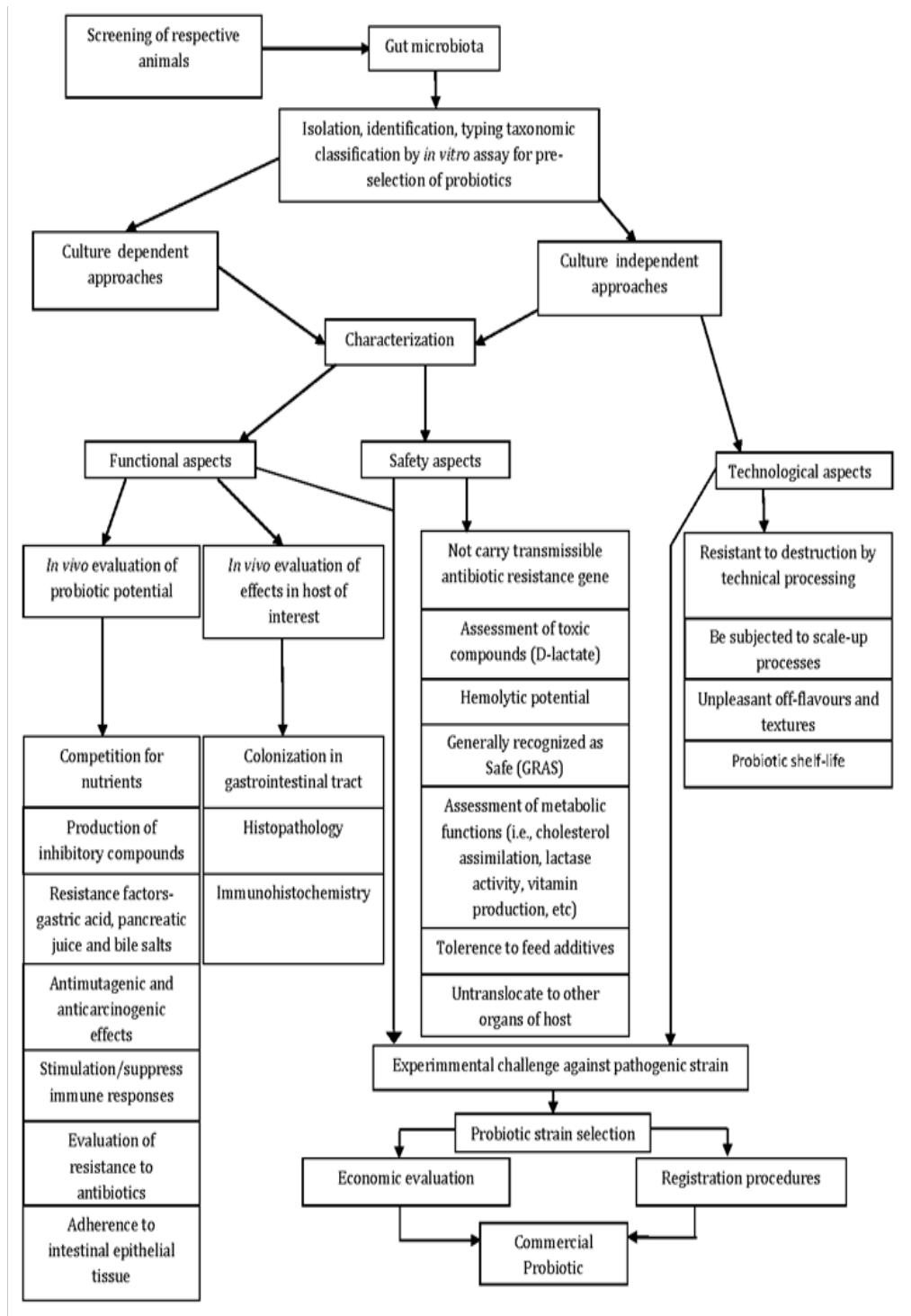


Figure 2 Diagram for selection of probiotics in the animal industry, modified from.^{36,74-83}

Selected mechanisms of action of probiotics on mammals

The mode of action of probiotics is not always well understood due to different probiotics strains may have various functions and survivability throughout the gut affecting the mammalian host in different ways.⁸⁴

Effects of probiotics can be classified in:

- A. Interaction between probiotic-microbe-gut epithelium:** Adhesion to mucosal epithelial cells, stimulation of mucus secretion, prevention of adhesion of pathogens as probiotics blocking intestinal receptors, thereby excluding pathogens, enterotoxins and hampering proliferation of pathogens, competition with pathogens for important nutrients, secretion of antimicrobial and antitoxin substances that affects establishment and/or replication of pathogens in the gastrointestinal tract.^{84,85}
- B. Interaction between probiotic-immune system:** Immune-modulation by innate as well as systemic ways, enhancing and reinforcing gut integrity and gut barrier function, eventually decreasing secretory and inflammatory molecules against microbial infection.⁸⁴ The general mechanisms by which probiotics may have an effect can be divided into various categories: adhesion activity to gut mucosal epithelium, antitoxic effect, modulation of immune system, production of antimicrobial substances and competitive exclusion between probiotics and pathogenic bacteria.

The advances on the knowledge of these mechanisms of action are detailed below.

Adhesion activity to gut mucosal epithelium: Adhesion property to the intestinal mucosa is the main selection criteria for new and existing probiotics strains for colonization, leading to the interaction between probiotics strains and host that is also related to the ability of strains to modulate the immune system.^{86–92} For many authors assess the adhesive ability of probiotics and good correlations have been established in various intestinal mucosa models system.^{93–99} Intestinal epithelial cells and mucus interact with lactic acid bacteria by showing different surface determinants from lactic acid bacteria. The principal component of mucous is mucin (complex glycoprotein mixture) that is secreted from intestinal epithelial cells. Mucin inhibits the adhesion of pathogenic bacteria.^{89,100} Anti-adhesiveness property by probiotics might be due to degradation of carbohydrate receptors by glycoprotein mixture, establishing a biofilm, production of receptor analogues and the induction of biosurfactants. Moreover, mucous gel is also composed of lipids, free proteins, immunoglobulins and salts.¹⁰¹

The specific interaction indicated a possible interaction association between the surface proteins of probiotic bacteria and the competitive exclusion of pathogens from the mucus.^{102–104} The most prominent mucus-targeting adhesin is a mucus-binding protein. Mucus-binding protein of *Lactobacillus reuteri* are either anchored to the membrane through a lipid moiety or embedded in the cell wall.^{105–109} Another kind of protein like mucous adhesion-promoting protein A from *L. Reuteri* and *L. Fermentum* mediate binding of these probiotics with mucus, specifically MUC2 and MUC3 mucins from *L. Plantarum*, inhibit the adherence of enteropathogenic *E. Coli* by enhanced mucous layers and glycocalyx overlying the intestinal epithelium as well as the occupation of microbial binding sites leading to protection against invasion by pathogens.^{102,110–112} Also adhesion of pathogenic *Salmonella*, *Clostridium* and *E. Coli* strains to pig intestinal mucus might be de-

creased in the presence of MUC3 from probiotic *Bifidobacterium lactis* Bb12 and/or *Lactobacillus rhamnosus* LGG.^{113,114} Furthermore, our results on the adhesivity of *B. Coagulans* using the INT407 intestinal cell line⁸¹ and other authors previously¹¹⁵ have indicated its ability to strongly adhere to intestinal epithelium.

In contrast, other *in vivo* data suggested transient colonization, *B. Coagulans* being lost one week after administration.¹¹⁶ Therefore, daily administration of *B. Coagulans* may be a prerequisite for efficacy. Moreover, a substantial population of growing vegetative cells in the gastrointestinal tract is not a prerequisite for the mode of action of Bacillus.¹¹⁷ The combination of probiotics and VSL3 showed to increase the synthesis of cell surface mucins and to modify mucin gene expression in a method dependent on the adhesion of bacterial cells to the intestinal epithelium. Moreover, some probiotics cause qualitative changes in intestinal mucins that help the prevention of pathogen binding.^{111,118} Many authors established that all probiotic strains and combinations tested showed capabilities to inhibit, displace and compete with pathogens,^{91,96,114} but it is important to take into account the high specificity of these processes to be tested thoroughly by means of molecular approaches for acid tolerance, bile resistance and adhesion in mucus, cell lines, cells plus mucus in order to characterize the properties of the strains. Finally, human and animal clinical trials will be the definitive tool in order to select the best strain combinations functionality to prevent or treat infection by a specific pathogen.

Antitoxic effect: Bacterial toxins may be the most important bacterial virulence factors to produce diarrhea in mammals. Certain probiotics are able to protect the host against toxins inducing diarrhea by the inhibition of their expression in pathogens. For example, *Bifidobacterium breve* Yakult and *Bifidobacterium pseudocatenulatum* DSM20439 inhibit expression of Shiga toxin in *E. Coli* O157:H7 strains *in vitro* and in mice model.¹¹⁹ This indicated that the high concentration of acetic acid produced by strain Yakult is responsible for the inhibition of Shiga toxin expression.¹²⁰ Probiotic *Clostridium butyricum* strain MIYAIRI protected gnotobiotic mice from enterohaemorrhagic *E. Coli* O157: H7 Shiga toxin-induced infection throughout production of butyric and lactic acid. Butyric acid, in particular, decreases also viability of enterohaemorrhagic *E. Coli* after neutralization (pH7).¹²¹ Furthermore, various probiotic *Lactobacilli* strains reduce Shiga toxin 2A expression via production of sub-bactericidal concentrations of organic acids for enterohaemorrhagic *E. Coli* O157:H7.¹²² Both in the murine ileal loop model and in cell culture assays it was found that the probiotic yeast *Saccharomyces cerevisiae* (*S. boulardii*) shows effective protection against *Clostridium difficile* toxin A (TcdA).¹¹⁹ This protective interference occurred between *S. Boulardii* with the TcdA-induced inflammatory signal cascade activating Erk1/2 and JNK/SAPK pathways.^{123,124} Additionally, *S. boulardii* is able to induce a specific anti-toxin A IgA immune response to destroy this toxin and secretes a protease that can hydrolyze TcdA and TcdB to inhibit their binding to their respective intestinal brush border receptors.^{125–127}

Beside bacterial toxins, probiotics *Lactobacillus rhamnosus* GG and *L. Rhamnosus* strain LC-705 are able to bind mycotoxins including aflatoxins. In rat model, *L. Rhamnosus* strain GG was able to modulate intestinal absorption and eventually increase fecal excretion of aflatoxin resulting in lowered toxicity expressed a sliver injury.^{128,129} In cell culture assay, *L. Rhamnosus* GG decreased aflatoxin B1 uptake minimizing both membrane and DNA damage.¹³⁰ Finally, designed probiotics carrying the receptor for the heat-labile enterotoxin of enterotoxigenic *E. Coli* or the receptor for cholera toxin showed good protection after enterotoxigenic *E. Coli* or *Vibrio cholerae* challenge in animal models. For some constructs formaldehyde-killed bacteria mediated also efficient protection as long as the frequency of applica-

tion was increased.¹³¹

Modulation of immune system: Metabolites, cell wall components and DNA of probiotics can stimulate the immune system through interaction with epithelial and dendritic cells and with monocytes/macrophages and lymphocytes either by innate or adaptive immune responses mechanism that might be local and limited to stimulation of gut immunity (stimulation of secretory IgA production for example) or systemic immunity.^{119,132–134} Peptidoglycan fragments or DNA (showed an anti-inflammatory reaction against DNA from pathogenic bacteria but mechanism of action is yet unknown) derived from probiotics¹³⁵ as well as immune modulatory effects might be even achieved with dead bacteria in mammalian body system. Mainly host cell receptors in gut epithelial and gut-associated immune cells recognize probiotics products by adhesion phenomena; generally the gut-associated immune system identifies intestinal microorganisms by pattern recognition receptors such as Toll-like receptors. Activation of the Toll-like receptors results trigger a signaling cascade lead to immune modulation (pro- and anti-inflammatory cytokine expression).¹³⁶ Signaling cascades in immune cells or in epithelial cells that are stimulated by release of soluble factors, subsequently affect immune cells. Mainly gut M cells and dendritic cells play an important role to direct and indirect contact with probiotics to internalization of probiotics in the gut, and then present it to naïve T cells. This usually results to T cell activation and differentiation which eventually produced secretory IgA in gut environment through plasma cells and also increased expression of IL-10 in dendritic cells, indicating the important role of dendritic cells in immune modulation by probiotics, for example *L. Rhamnosus*.^{137,138}

In healthy mammals, *Lactobacillus rhamnosus GG* (LGG) triggers the synthesis of the anti-inflammatory interleukin IL-10 which subsequently decrease the release of proinflammatory IFN- γ , IL-6 and TNF- α from CD4+ T cells pre-stimulated with intestinal bacteria.¹³⁹ Teichoic acid, a component of the gram-positive cell wall of *L. Plantarum* is involved in the anti-inflammatory activity through the involvement of Toll-like receptors-2 which highlighting the importance of Toll-like receptors for this probiotic actions.¹⁴⁰ In our trials, the use of *Pediococcus acidilactici* in piglet significantly lowered intraepithelial T lymphocytes in the ileum (P=0.013) and numerically the number of CD8+intraepithelial T lymphocytes in the cecum.¹⁴¹ These observations are likely to be in relation with the presence of catarrhal enteritis in controls, in both ileum and cecum. It is conceivable that the intestinal mucosal barrier of *P. Acidilactici* treated piglets may be more efficient in contrasting the adhesion of bacterial pathogens than control piglets, at least in ileum. Our observation appears in ideal agreement with the results by Piva, Meola and Panciroli,¹⁴² who showed in an *in vitro* trial that Pediocin A did not display influences upon pig cecal microflora. It is conceivable that *P. Acidilactici* exerts in piglets its protective effects with regards to competition with potential pathogens, above all in the ileum (and only to a limited extent in cecum), possibly via the intervention of Toll-like receptors, which are essential for the control of intestinal host-microbial interactions and protection from the adhesion of microbial pathogens.^{141,143}

Zonula occludens protein 1 and Zonula occludens protein 2 in the gut epithelium are two important proteins for preservation and maintenance of tight-junction function. Probiotics are able to protect the integrity of the mucosal gut barrier function against damages by the action of enteropathogenic *Escherichia coli* in a Toll-like receptor-independent way. Probiotics are able to restore disrupted epithelial barrier by changing protein kinase C signaling resulting in an amplification of expression and redistribution of zonula occludens protein

1 and zonula occludens protein 2 in cell culture and in mouse model.¹⁴⁴ In broad spectrum, cellular signal transduction modulated by a number of probiotics that are capable to alter population of cytokine production. They can either block degradation of the inhibitor I κ B or by interfering with proteasome function, which is, in turn activated through a peroxisome proliferator.^{145,146} Intestinal epithelial cell survival and growth maintain by probiotic *Lactobacillus rhamnosus GG*, which secrete two soluble proteins (designated p40 and p75). These proteins lead to inhibit TNF- α mediated apoptosis by activation of the anti-apoptotic factor Akt and protein kinase B and by inactivating the pro-apoptotic p38 mitogen-activating protein kinase signaling pathway in epithelial cells.^{147,148} Lastly, probiotic strains could induce release of small proteins/peptides like defensins/cryptidins from Paneth cells that reinforce and stabilize gut barrier function.^{149,150}

Antimicrobial substances: Probiotics may be able to act through the production of antimicrobial substances.^{119,151}

Overall, lactic acid bacteria can be divided into two groups on the basis of production of the antimicrobial metabolites:

- Low molecular mass compounds (≤ 1000 Da) such as organic acids (acetic acid, lactic acid), nitrogen oxide, hydrogen peroxide and antimicrobial peptides (lantibiotics, lanthionin, heat stable non-lantibiotics and cyclic antimicrobial peptides) and reuterin which have a broad spectrum of action, and
- Antimicrobial proteins, termed *bacteriocins* (≥ 1000 Da) such as lactacin B, plantaricin and nisin that have a relatively narrow specificity of action against closely related organisms and other gram-positive bacteria.^{152,153}

Reuterin showed broad spectrum antimicrobial activities against gram-positive, gram-negative bacteria as well as against yeast, fungi, protozoa and viruses.¹⁵⁴ Acetic acid and lactic acid secreted in the fermentative metabolism of carbohydrates by probiotics, have a strong bacteriocidal effect against pathogens.¹⁵⁵ These organic acids are the main antimicrobial compounds that are responsible for the inhibitory activity of probiotics against pathogens.^{156–158} The organic acid enters the bacterial cell in a undissociated form which dissociates inside its cytoplasm, subsequently resulting in a reduction of the intracellular pH that inhibits the growth of gram-negative bacteria due to their sensitivity to acidic conditions or the death of the pathogen due to the intracellular accumulation of the ionized form of the organic acid.^{156,158,159} Most of the research aimed to the characterization of *bacteriocins* or bacteriocin-like compounds focused on lactic acid bacteria, mainly *Lactobacillus*, *Pediococcus* and *Enterococcus* because of the diversity of these genera species and their potential applications as natural preservatives in foods¹⁵³ and because of their activity against food borne pathogens like *Listeria*, *Clostridium*, *Salmonella*, *Shigella*, *Escherichia*, *Helicobacter*, *Campylobacter* and *Candida*.^{85,160–163} For example, Abp118 like bacteriocin derived from *Lactobacillus salivarius* strain UCC118 is able to protect from infection against *Listeria monocytogenes* in mice model.¹⁶⁴ In a study conducted by our research group, has demonstrated the capability of *B. Coagulans* to produce lactic and acetic acids in anaerobic conditions (42.27 \pm 2.18mmol/l-1 and 46.50 \pm 4.88mmol/l-1) respectively. Thus, our results suggest that *B. Coagulans* strains isolated directly from calf feces could be considered a new single species probiotic supplement which can aid the maintenance of gastrointestinal health.⁸¹

Narrow spectrum *Bacteriocins* and small antimicrobial proteins are derived from lactic acid bacteria. Specifically, lactacin B, plantaricin and nisin are produced from *L. Acidophilus*, *L. Plantarum* and

Lactococcus lactis respectively which are acting only against closely related bacteria.¹⁶⁵ The most of *bacteriocins* kill susceptible bacteria through the damages of cells by membrane permeabilization or inhibition of cell wall synthesis by interference with essential bacterial enzymes.¹⁶⁶ For instance, nisin forms a complex with lipid II (cell wall precursor of bacteria), preventing cell wall biosynthesis mainly in spore-forming bacilli. Eventually, formation of a pore occurs in the bacterial membrane due to the formation of complex aggregates and incorporates peptides.¹⁶⁷ Finally, stronger antimicrobial activity of de-conjugated bile acids is produced by probiotic bacteria. Functionally, de-conjugated bile acids show a stronger activity in comparison to bile salts synthesized by host organism. It is yet unclear and will need more investigation to clarify mechanisms by how probiotics protect themselves from their own bactericidal metabolites or if they are resistant to de-conjugated bile acids in mammalian gut at all.¹¹⁹ More research should be carried out in future on genetics, biochemistry and mechanism of action of probiotics due to the potential interest on novel approaches of these antimicrobial proteins and other antimicrobial metabolites in the field of pharmacotherapeutics developments.

Competitive exclusion between probiotics and pathogenic bacteria: Various bacterial genera colonized and are developed in the mammalian gut, producing an almost permanent exclusion environment by one species of bacteria that more vigorously competes for receptor sites mainly in the membrane of goblet cells, enteroendocrine cells and enterocytes of the intestinal tract than another species. Competitive exclusion mechanism represents a status of physical barrier to the gut mucosa by creating a special integrity systems characterized by creation of a hostile microecology, elimination of available bacterial receptor sites, production and secretion of antimicrobial substances and selective metabolites, and competitive depletion of essential nutrients, preventing intestinal pathogens from becoming established.¹⁶⁸⁻¹⁷¹ The mechanisms used by one species of bacteria to exclude or reduce the growth of another species are varied due to strains variability of probiotics.

The interaction between surface proteins and mucins due to specific adhesiveness properties may inhibit the colonization of pathogenic bacteria leading to antagonistic activity by some strains of probiotics against adhesion of gastrointestinal pathogens.⁸⁵ Growth and multiplication of a broad range of pathogens, such as *E. Coli*, *Salmonella*, *Helicobacter pylori*, *Listeria monocytogenes* and *Rotavirus* can be inhibited by *Lactobacilli* and *Bifidobacteria*.¹⁷²⁻¹⁷⁸ Some authors proposed that competitive exclusion might be based on binding to the same glycoconjugate receptor sites in a competitive manner on the epithelial surface by probiotics and pathogenic bacteria.¹⁷⁹⁻¹⁸¹ More commonly, probiotic strains can prevent the attachment of pathogenic bacteria by steric hindrance at enterocyte pathogen receptors.^{181,182} In human mucosal material *in vitro*,^{112,183} chicken¹⁸⁴ and pig mucosal material *in vivo*²¹ demonstrated the effect of probiotic bacteria on the competitive exclusion of pathogens. A strongly adhering strain, *L. Rhamnosus* is able of preventing the internalization of enterohemorrhagic *E. Coli* in a human intestinal cell line.¹¹² More recently, glyceraldehyde-3-phosphate dehydrogenase has an adhesion property that is expressed on the cell surface of *L. Plantarum* LA 318. Glyceraldehyde-3-phosphate dehydrogenase of *Lactobacilli* is capable to bind with human colonic mucins and enables to adhesion with *Lactobacillus* to compete with pathogens for a given binding site.¹⁸⁵

Application of probiotics in livestock

Probiotics for pigs: Environmental stresses, mainly management

methods, diet, etc. can affect swine production causing discrepancy in the intestinal diversity leading to a risk factor for pathogen infections.¹⁸⁶ Weaning and post-weaning periods are the most stressful conditions in commercial porcine production resulting in transient drop in feed intake, inhibition of growth performance, negative influence on the immune function and the intestinal microbiota equilibrium finally leading to increased susceptibility to gut disorders, infections and diarrhea in the pigs.¹⁸⁷ The increased level of probiotics use started after the full ban on in-feed antibiotics and the drastic reduction in the levels of incorporation of copper and zinc by the European Union.¹⁸⁸ The majority of the researches showed a health beneficial effect of probiotic applied in piglets; increasing the number of intestinal beneficial bacteria, reducing the load of pathogenic bacteria, increased high activities of IgM, IgA against pathogens than control, enhanced defensive tools towards pathogenic invasion and increased villi morphology and function. Yeasts (*Saccharomyces boulardii*) and bacteria (*Lactobacillus* spp., *Enterococcus* spp., *Pediococcus* spp., *Bifidobacterium* spp., *Bacillus* spp.) are the most common probiotics use for monogastric animals to targeting the hindgut (caecum, colon) which harbors an abundant and very diverse quantity of microbial population mainly composed of bacteria and archaea.¹

In gestating sows, the administration of probiotics have been reported to increase performance effects on feed intake and average live weight¹⁸⁹ with at the same time a greater size and vitality of the neonatal piglets.¹⁹⁰⁻¹⁹² On the contrary, our study found that *Saccharomyces cerevisiae* spp. *Boulardii* supplementation to sows had no beneficial effects on gestating sow body weights and feed intakes but total piglets born and born alive were in a larger number than in control sows.¹⁹³ Furthermore, performance benefits of piglets have also been reported after weaning, as for example with *S. Boulardii*.¹⁹⁴ In this study, the yeast promoted a 'healthy' intestine by an early restoration of the intestinal mucosal thinning and would possibly improve the local resistance to infection. Significant longer villi and deeper crypts were found in the ileum of piglets receiving diets supplemented with the yeast *Saccharomyces cerevisiae* spp. *Boulardii*¹⁹⁵ or *Pediococcus acidilactici*.^{141,196} The benefits for intestinal IgA secretion and reduction of translocation of enterotoxinogenic *E. Coli* also observed with *S. Boulardii* or *P. Acidi* lacticified to piglets.¹⁹⁷ Strain of *Lactobacillus sobrius* provided on modulation of IgA development, mutually with a reduced ileal prevalence of enterotoxinogenic *E. Coli* has been established.¹⁹⁸

Bacillus species are also used as probiotics in some clinical trials in pigs. Inclusion of *Bacillus subtilis* strain in the feed resulted in a reduction of scours in weaned pigs challenged against K88-positive enterotoxinogenic *E. Coli* as well as the spores of *Bacillus licheniformis* and *B. Subtilis* reduces the morbidity and the mortality in weaned piglets, improves the performance parameters of the fattening pigs and improves carcass quality.^{199,200} Furthermore, a combination of *Bacillus subtilis* and *B. Licheniformis* supplementation decreased gestating sows body weights, decreased incidence of diarrhea and mortality in piglets and improved litter weight at weaning and growth rate of young piglets.³¹ Moreover, the use of a *B. Cereus* based probiotic showed not only lower prevalence of diarrhea, but also that pigs needed to eat less food to attain the same weight gain.³⁰ Amusingly, probiotic supplemented in pigs resulted in an improvement in meat color, marbling, tenderness, flavor and juiciness in Korean swine production.²⁰¹ Moreover, more sensitive to gut colonization happened from birth to post-weaning piglets by pathogenic bacteria (*E. Coli*, *Clostridium difficile*, *Clostridium perfringens*, *Salmonella*, *Listeria*), parasites (*Isoospora*, *Cryptosporidium*) or viruses (*Coronavirus*,

Rotavirus), which are responsible for growth reduction and diarrhea. During this episode, many studies have confirmed the efficacy of probiotics as well as therefore recommended to use probiotics in this period.^{198,202–204}

The adaptive immune system of pigs has been influenced by the ability of probiotics which demonstrated in several studies. Szabo et al.²⁰⁵ found that the administration of *E. Faecium* improved the course of infection in weaning piglets when challenged with *Salmonella Typhimurium*, increased the production of specific antibodies against *Salmonella*.²⁰⁵ The distribution of intestinal immune cells (granulocytes, mast cells, CD4+, CD8+, CD25+, IgA+ lymphocytes) and the mucosal expression of cytokines (IFN- γ , TNF- α , TGF- β , IL-10) by *in vitro* and *in vivo* studies on probiotic effects have been reported, but the effects of probiotics on those immune/defensive cells remain less clear.^{141,206–210} Moreover, many probiotic preparations have been tested in several separate laboratories with diverse and sometimes contradictory results. One of the most common methods used for assessment of immune function is the evaluation of lymphocyte proliferation, the principal actor in intestinal local immunity, whose anatomic counterpart was the gut-associated lymphoid tissue. More intriguing was the response to *L. Fermentum* in weaned pigs that induced an increase in the pro-inflammatory cytokines IFN- γ and TNF- α in the ileum, and an increase in the percentage of CD4+ lymphocyte subset in blood.²¹¹

In comparison to poultry, competitive exclusion culture has not been studied thoroughly in pig. Known competitive exclusion culture from porcine reduced the mortality rate and reduced shedding of enterotoxigenic *E. Coli* in neonatal pigs²¹ as well as shed significantly lower pathogen numbers in neonatal pigs after challenge with *Salmonella enteric serovar choleraesuis*.²⁰ Selected colicin-producing *E. Coli* targeting *E. Coli* K-88 isolated from environmental sources (cattle and swine feces and soil) demonstrated a significant valuable effect on performance, diarrhea in weaning piglets infected with *E. Coli* K88.⁸³ On the other hand, *Enterococcus faecium* and *E. faecalis* are routinely using in many clinical trials although they have not been proposed for QPS status by EU.²¹² In healthy piglets and sows, *E. Faecium* NCIMB 10415 decreased the pathogenic bacteria load through an interdisciplinary research study.^{192,213}

The clinical trials utilizing *Lactobacillus* strains are increasing due to their documented importance as frequent component of pig microbiota. *L. Sobrius* significantly decreased the levels of enterotoxigenic *E. Coli* in the piglets' ileum when administered directly after weaning. Moreover, *L. Sobrius* improved daily weight gain in probiotic fed animals.¹⁹⁸ *L. Rhamnosus GG* (LGG) fed in post-weaning piglets was effective in ameliorating diarrhea induced by *E. Coli* K88 via modulation of intestinal microflora, enhancement of intestinal and systemic immune response.²¹⁴

Bifidobacterium species are most widely useful as probiotics in humans, but combination with *Lactobacillus* are conducted particularly in pigs. *Lactobacilli* and *Bifidobacteria* administration reduced the incidence and severity of necrotizing enterocolitis and reduced colonization density of the potential pathogen *Clostridium perfringens* immediately after birth.²¹⁵ Probiotic preparations including *Bifidobacterium lactis* and *Lactobacillus rhamnosus* individually lowered adherence of *Salmonella*, *E. Coli* and *Clostridium* spp. to the intestinal mucosa in swine. Together the two organisms were more effective and reduced each other's adherence.¹¹³

Microorganisms reside in the gut able to influence the phenotypic

characteristics of enteric neurons of enteric nervous system. Kamm et al.²¹⁶ showed that dietary probiotics (*Saccharomyces cerevisiae* sp. *boulardii*) affect the chemical coding of swine myenteric neurons and Bar et al.,²¹⁷ demonstrated that *Escherichia coli* Nissle 1917 affects the contractile activity of human isolated smooth muscle strips. Di Giancamillo et al.²¹⁸ provided a quantitative evaluation of neuronal populations in the submucosal and myenteric plexuses of the pig ileum and cecum, and described specific changes in the neuronal and enteric glial cells in porcine treated with dietary *P. Acidilactici*, suggesting that changes in the intestinal microorganism community, like that linked to dietary probiotic administration, may conceptually support aspects related to enteric neuronal plasticity.

Probiotics for poultry: In modern broiler production practices, there are many factors that can enhanced stressors (feed changes or imbalances, transportation, processing at the hatchery and high stocking densities) during post hatching period,²¹⁹ which ultimately affect the colonization of the gastrointestinal tract by bacterial pathogens due to weaken immune system, posing a threat to birds health and food safety. *Salmonella* spp. *Campylobacter jejuni* and *Clostridium perfringens* have been show to infect chickens and hens increasing the risk of contamination through the food chain resulting in a harmful condition both for poultry and human.^{220–222} So, probiotics act as a biological alternative in the preharvest control of *Campylobacter*, *Salmonella*, and *Escherichia coli*.^{53,223,224}

Probiotics could be a possible strategy to control pathogen shedding and thus successfully demonstrates the impact of the intestinal microbiota on intestinal function and disease resistance.^{14,21,225} In poultry production, the application of probiotics is strictly associated with the concept of competitive exclusion, where 1-d-old chicks can be sheltered from succeeding *Salmonella* infections by accelerating the establishment of a complex, protective microflora.^{226,227} Many efficient commercial competitive exclusion cultures are also available on the market against *Campylobacter*¹⁴ and *Clostridium perfringens*.²²⁸ Zhang et al.²²⁹ indicated that cultures derived from free-range chickens on family poultry farms provided better competitive exclusion cultures in comparison to commercial farm chickens.²²⁹

The capability of probiotic strains to protect the growth of pathogens is believed to be important in preventing disease. Commercially lactic acid bacteria cultures have been widely used for their ability to reduce *Salmonella* infection in poultry and turkey production in many countries.^{230,231} In an all-inclusive research of 296 strains of lactic acid bacteria from the gut of 50 chicks, 77 of the strains were found to protect growth of enteropathogenic *S. Enteritidis* and *E. Coli*.³⁶ Furthermore, the probiotic cultures modulated the microbial composition and the enzymatic activities of the cecal microbiota, resultant significant probiotic effect.¹⁸⁶

Along with the control of food-borne pathogens in the poultry gut, selected probiotic cultures, mainly *Lactobacillus* spp., may also potentially increase production performance parameters; among poultry farmers, objectives (such as increasing growth rate, improving feed conversion and meat quality) are undoubtedly of primary importance. In a comprehensive in turkeys and chickens, commercial researches have established that proper administration of probiotics mixture increased performance, reduced costs of production as well as was effective in reducing abdominal fat deposition.^{232–236} Timmerman et al.²³⁷ reported that the main factors affecting the efficacy of the probiotic preparations depend on way and timing of the administration.²³⁷ Application through the feed than application in the drinking water resulted in a higher increase of average daily

gain. Furthermore, the supplementation of probiotics during early life of host is of great importance to the host because the probiotic bacteria can modulate intestinal epithelia genes expression to creating a favorable habitat for themselves.

Eggs production has been also investigated in relation to probiotic application. A combined mix culture of *Lactobacillus acidophilus*, *L. Casei*, *Bifidobacterium thermophilus* and *Enterococcus faecium* enhanced egg size and lowered feed cost in laying hens.²³⁸ Moreover, *Bifidobacterium thermophilus* and *Enterococcus faecium* improved egg production and quality.²³⁹ The use of Enterococci as probiotics is somewhat controversial in humans, but in chickens prolonged feeding with *E. Faecium* based probiotics increased egg laying intensity and feed conversion efficiency.^{240,241}

In poultry, benefits of yeast probiotic supplementation are established in broilers' production performance and increased resistance of chickens to enteric pathogens infections (*Salmonella*, *Campylobacter jejuni*, *E. coli* or *C. perfringens*).^{230,242–244} Furthermore, supplementation with yeast treatment significantly decreased the frequency of *Salmonella* colonization to lower than the pre-stress (before transport) levels, whereas non-supplemented birds had higher levels of *Salmonella* colonization.²⁴⁵ Probiotics can increase feed efficiency and productivity of laying hens^{239,246} and an improvement in egg quality (decreased yolk cholesterol level, improved shell thickness, egg weight) has also been reported.^{239,247} Invernizzi et al.,²⁴⁸ reported that selenium containing dead yeast (*Saccharomyces cerevisiae*) probiotic increased egg weight, egg shell quality with higher selenium concentration in egg and breast muscle. On the other hand, Zhang et al.²⁴⁹ conducted an experiment with male broilers to investigate the effects of *Saccharomyces cerevisiae* cell components on the meat quality and they reported that meat tenderness could be improved by the whole yeast or *Saccharomyces cerevisiae* extract.²⁴⁹

Probiotics for veal calves and horses: The most significant beneficial effects of probiotics have been established when probiotics included in the animals diet during specifically stressful periods for the gut microbiota and the animal. Due to undeveloped rumen in young pre-ruminants, lactic acid bacteria probiotic (*Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Propionibacterium* spp.) or *Bacillus* spores generally target the small intestine due to stabilize the gut microbes and limit the risk of enteric pathogen colonization.

Several *in vivo* trials have shown positive results with regard to animal performance, improved weight gain and rumen development in young calves with various type of bacterial and yeast strains supplementation.²⁵⁰ Calves fed milk fermented with either mixed lactic acid bacteria, or *L. Acidophilus* or *S. Cerevisiae* NCD49 obtained reduce incidence of diarrhea.⁴¹ Moreover, viable *E. Coli* strain Nissle 1917 administered to calf had a clear favorable effect on the prophylaxis and treatment of neonatal calf diarrhea.²⁵¹ Our lab reported that an investigation was carried out into the recovery from calf feces of *Bacillus coagulans* spores added to the feed as probiotic.⁸¹ Throughout the trial the fecal spore counts were significantly higher in the treated group than in the controls. In addition, the recovered cells were found to maintain their functionality aspects of acid production, survival in artificial gastric juice and in the presence of bile, and attachment to human intestinal epithelial cells *in vitro*. The results further elucidate the fate of spore formers administered to calves, and this will help in the development of new species-specific nutritional strategies. Improved weight gain and rumen development, improvement of lactic acid bacteria/*E. Coli* ratio and fecal score in young calves have been reported with several products.^{43,250,252,253} On

the other hand, from the first days after birth, live yeast administered to calf has been helped to favor microbial colonization and the set-up of fermentative capacities in the rumen.²⁵⁴ In young calves, incorporating live yeasts into the grain reduced the number of days with diarrhea.²⁵²

In horse, probiotic effects to the digestive compartment mainly caecum-colon. Fiber digestibility increased in the horse colon and modulated the balance of hindgut bacterial communities through supplementation with live yeast, consequently decreased risk of lactic acidosis.^{255,256} Agazzi et al.⁵¹ also reported that the administration of *S. Cerevisiae* to mature horses fed high-fiber diet increased apparent nutrient digestion rate.⁵¹ The apparent digestion rates of dry matter and organic matter were significantly improved in treated horses as compared with control subjects, but the most relevant difference among experimental groups was evidenced by a positive effect of the of live yeast over the fibrous fractions such as neutral detergent fiber and acid detergent fiber.

Conclusions

Most probiotic strains marketed today were originally selected for their superiority in a variety of easily measurable phenotypes, and not necessarily for their unique ability to confer defined health benefits. Unfortunately, the currently available *in vitro* tests are not accurate enough to predict the potential use and functionality of probiotic strains *in vivo*. Better knowledge of the structure and activities of the gut microbiota, functional interactions between gut microbes and interrelationships between microbes and host cells represent a fundamental aspect of future lactic acid bacteria probiotic research. The combination of verified molecular *in vitro* assays and *in vivo* animal models, through the monitoring of identified biomarkers with functional and comparative genomics and proteomics based approaches, should enable selection of the most appropriate probiotic strain for a particular health benefit or improvement of strain processing and/or administration regimes that strengthen the established health effect. At least the implementation of both *in vitro* and *in vivo* techniques will increase the knowledge of such an attractive perspective as the co-immobilization of probiotics microorganism with prebiotics, antioxidants, peptides or immune-enhancing compounds.

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Conflict of interest

Author declares that there is no conflict of interest.

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