Rev. Salud Anim. Vol. 33 No. 3 (2011): 137-146

Review article

TERMINOLOGY CONCEPTS OF PROBIOTIC AND PREBIOTIC AND THEIR ROLE IN HUMAN AND ANIMAL HEALTH

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ABSTRACT: **The mode of action of specific probiotic bacteria and specific prebiotic ingredients and their effects on the intestinal microbiota modulation in humans and animals has been demonstrated in many studies. Intestine is characterized for harboring a complex and dynamic microbiota which, among others, has a function to protect the host from inflammatory disorders. A mature and balanced endogenous microbiota has also an important role in the maintenance of a desirable activity of the immune system. Deviations in gut microbiota composition, due to improper diet, radiotherapy, antibiotic treatment, stress and others, may lead to a variety of gastrointestinal disorders. To overcome an unbalanced microbiota, the consumption of specific probiotics and prebiotics has been proved to be effective. For more than three decades, many probiotic microorganisms have been characterized and evaluated. However, the term prebiotic has only recently been identified, characterized and evaluated in human intervention studies. The list of prebiotic ingredients remains limited, and yacon (*Smallantus sonchifolia*) is an Andean root to which prebiotic attributes have been inferred recently. Humans and animals could seemingly benefit from the consumption of specific prebiotics and probiotics. In this work, terminology aspects of pre- and probiotics, and their roles in human and animal health are discussed.**

*(Keywords: probiotic; **Bifidobacterium**; prebiotic; yacon; animal health; human health)*

CONCEPTO DE LA TERMINOLOGÍA DE PROBIÓTICOS Y PREBIÓTICOS Y SU ROL EN LA SALUD HUMANA Y ANIMAL

RESUMEN: **Se ha demostrado en muchos estudios el modo de acción de las bacterias probióticas específicas y de determinados ingredientes prebióticos y sus efectos en la modulación de la flora intestinal en humanos y animales. El intestino se caracteriza por hospedar una microbiota compleja y dinámica que, entre otras, tiene como función proteger al huésped de trastornos intestinales. Una microbiota endógena madura y equilibrada, también es importante para el mantenimiento de una actividad deseable del sistema inmunológico. La perturbación de la microbiota intestinal puede tener un impacto en la fisiopatología de una variedad de trastornos gastrointestinales. Para superar este desequilibrio debido a la dieta, radioterapia, terapias con antibióticos, situaciones de estrés y otros, se ha demostrado la eficacia del consumo de probióticos y prebióticos específicos. Durante más de tres décadas, se han descrito y evaluado muchos microorganismos probióticos; sin embargo, el término prebiótico sólo se ha identificado, caracterizado y evaluado recientemente en estudios de intervención humana. La lista de ingredientes prebióticos queda frenada y el yacón (*Smallantus sonchifolia*) es una raíz andina a la cual los atributos prebióticos se han indicado recientemente. Los seres humanos y animales, aparentemente se podrían beneficiar del consumo de prebióticos y probióticos especiales. En este trabajo se abordarán los aspectos de la terminología de pre y probióticos y su función en la salud humana y animal.**

*(Palabra claves: probiótico; **Bifidobacterium**; prebióticos; yacón; salud animal; salud humana)*

INTRODUCTION

Intestine is characterized for harboring a complex and dynamic microbiota which, among others, has a function to protect the host from intestinal disorders. A mature and balanced endogenous microbiota, has also an important role in the maintenance of a desirable activity of the immune system. To overcome an unbalanced microbiota, due to improper diet, radiotherapy, antibiotic treatment, stress situations and others, the consumption of specific probiotics and prebiotics has been proved to be effective. Probiotics are defined as «live microorganisms which when administered in adequate amounts confer a health benefit on the host» (1).Prebiotics are defined as «non-viable food component that confers a health benefit on the host associated to microbiota» modulation (2). Product containing probiotic (s) and prebiotic (s) is called synbiotic.

For more than three decades, many probiotic microorganisms have been described and evaluated. However, the term prebiotic has only recently been identified, characterized and evaluated in human intervention studies. The list of prebiotic ingredients remains limited. The prebiotic activities of yacon(*Smallantus sonchifolia*), originally an Andean root, have only been recently studied (3) . Humans and animals could seemingly benefit from the consumption of specific probiotic and prebiotic. Though there are the numerous functions of pre- and probiotics attributed to different health conditions in humans and animals, the most studied are related to the modulation of the intestinal microbiota.

In this work, the terminology of the main topics related to pre- and probiotics, the main probiotic organisms and prebiotic ingredients with emphasis in yacon, as well as their roles in human and animal health have been discussed.

Intestinal microbiota

Varieties of organisms such as protozoa, bacteriophage, fungi and bacteria are normal residents of the gastrointestinal tract (GIT). From these, intestinal bacteria are the most studied entity. This microbiota complies with more than 500 cultivable species (4) achieving the highest densities not found in any other studied ecosystem (5). However, studies using modern molecular techniques have indicated that more than 1000 species are present in the gut. This complex community is metabolically active and contributes to homeostasis (6). The gut microbiota composition varies with race, sex, age, diet and other factors (7). It possesses key functions such as:

i) fermentation of intestinal mucus produced by epithelial cells (8), ii) metabolism of bilirubin, cholesterol, bile acids, steroid hormones, pancreatic enzymes, fatty acids (9, 10), iii) nutritional activity such as providing the host with vitamins K and B (11) and contributing to the protein homeostasis of the host via synthesis of aminoacids, iv) immune functions, acting for example, in the stimulation of the innate and adaptative immunity, aiding the host to respond more quickly to pathogen challenge (12, 13) and v) «colonization resistance», a phenomenon characterized namely by the resisting of colonization of opportunistic pathogens that preferentially overgrew due to inflammation or use of specific medications and broad-spectrum antibiotic (14). When microbiota is disrupted, the host becomes more susceptible to infections. In addition, antibiotic therapy affects microbial homeostasis, which can be recovered in a time period as long as eight weeks after discontinuity of antibiotic therapy (15). This situation leads to the potential use of specific probiotics and prebiotics which act aiding to a faster re-establishment of the normal intestinal microbial community in the host gut. This microbial community, estimated in 10^{14} cells, varies quantitatively and qualitatively along with the different environments from the stomach, small and large intestines and outnumbers the total human eukaryotic cells (10^{13}) (16, 17).

Different habitats are encountered in the stomach, small and large intestines, leading to a qualitative and quantitative diversity of bacteria families, genera and species.

Stomach microbiota. The numbers of the adapted resident bacteria and fungi are controlled by the acidic environment found in this habitat. Among the described genera usually found in the stomach are *Actinomyces*, *Fusobacterium*, *Peptostreptococcus*, *Streptococcus*, *Staphylococcus* and *Veillonella*. The concentration of microrganisms in the stomach is low, barely reaching levels of 10^3 CFU/g of gastric content (16). As pH increases along the gastrointestinal tract (GIT), the microbial numbers increase.

Small intestine microbiota. The small intestine is a transitional zone between the stomach and the colon. The presences of bile, pancreatic juices and others, as well as the high rate of motility are among the main variables responsible for controlling microbial overgrowth in the small intestine. Facultative microorganisms predominate and their numbers are in the range of 10^4-10^8 CFU/g of the small intestine content (12). The ileum harbours a transition microbiota, the numbers of anaerobes increase and the presence of

coliforms and species of the genera *Bacteroides*, *Fusobacterium* and *Lactobacillus* is common. Bacterial concentration in the ileum reaches 10^9 CFU/g (12).

Large intestine microbiota. Anaerobes predominate in the large intestine of mammals. The proximal colon is characterized by a high concentration of substrates, a pH in the range of 5-6, and a rapid microbial growth with accumulation of short chain fatty acids (SCFA), while in the distal colon substrate is less available, pH is close to neutrality and putrefactive fermentation predominates (18). Colonic microbiota consists of large numbers of anaerobes and the presence of genera from the *Enterobacteriaceae* and species of *Enterococcus*, *Bacteroides*, *Fusobacterium*, *Clostridium* and *Bifidobacterium* is common (16,19). The densities achieved reach concentrations up to 10^{11} to 10^{13} CFU/g of colonic content (20).

The faecal content has the highest concentration of microorganisms, and bacteria comprise 33% of its dry weight (7). Species belonging to *Bacteroide*, *Clostridium* and *Bifidobacterium* genera have been found to predominate in human faeces (21), whereas in rats, the leading genera are *Staphylococcus*, *Enterococcus*, *Serratia* and *Proteus* (22).

This diversity of pathogenic and saprophytic microorganisms exists in equilibrium, and the consumption of probiotics, prebiotics and synbiotics have been considered potential alternatives to counteract its disruption.

The concept of probiotic and probiotic-like microorganism

Concerning and understanding the concepts and terminologies of functional foods including probiotic/ prebiotic/synbiotic have been materialized in a recent publication (23). For the first time conceptual diagrams depict probiotic and prebiotic from origin to labelling. With the increasing of globalization, the terminology work in specific areas is of utmost importance for economic and scientific reasons. Knowing the possibilities of a probiotic origin might help the search for new probiotic species in similar ecological niches. It is also important to differentiate a probiotic from a probiotic-like microorganism. A probiotic species is defined as «species of safe bacterium originated from the host, mainly from the intestinal tract, and/or fungus originated from plant which when administered in adequate amounts confer a health benefit». Probiotic microorganism refers to «safe bacterium originated from the host intestinal tract which when administered in adequate amounts confers a health benefit», and a probiotic-like microorganism refers to safe bacterium and/or fungus originated from other sources of the host intestinal tract which when administered in adequate amounts confer a health benefit» (23). Figure 1 indicates a conceptual relation of these terms.

The most common probiotic microorganisms are species belonging to the genera *Lactobacillus* and *Bifidobacterium* for humans and *Bacillus*,

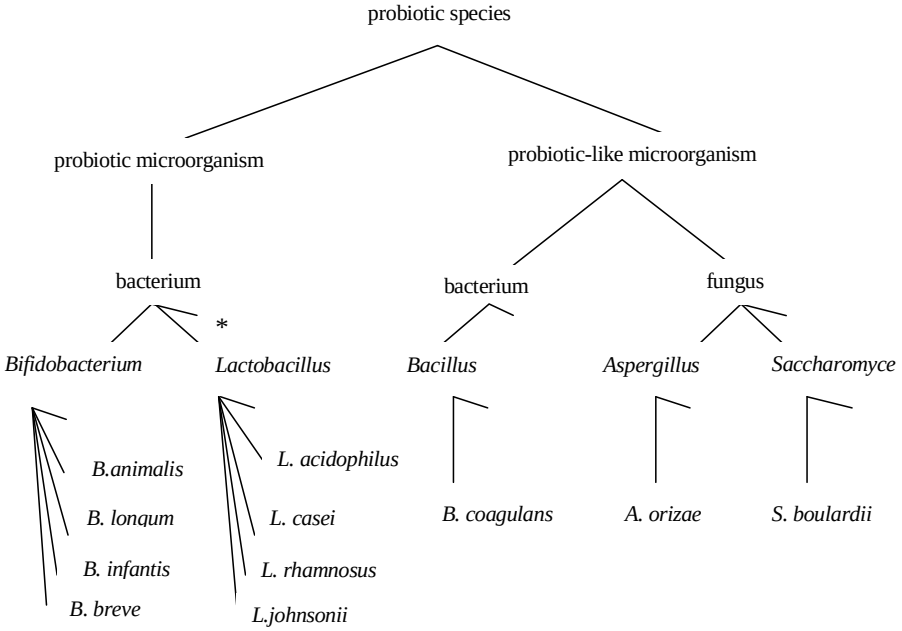

FIGURE 1. Diagram relating probiotic species, probiotic microorganism and probiotic-like microorganism. Adapted from Magalhães *et al.* (23)./ *Diagrama sobre especies de probióticos, microorganismos probióticos y microorganismos como probióticos. Adaptado de Magalhães et al. (23)* .
*Others

Enterococcus, *Lactobacillus*, *Aspergillus* and *Saccharomyces* for animals.

Probiotics are marketed in different formats (Fig. 2).

The main probiotic functions are indicated in figure 3.

The prebiotic concept

Natural defenses of human and animals could be enhanced with the inclusion of functional foods into human food and animal feed. Nowadays, the prebiotics available are mainly carbohydrate in nature. They are not metabolized at the upper intestine but rather by the microbiota in the colon. Acting as main energy source, prebiotics stimulate the growth of beneficial microorganisms such as bifibacteria; and for this reason prebiotics were firstly known as bifidogenic factors. Also other microbial groups might be stimulated as well. This stimulus is followed by accumulation of metabolites, mainly SCFA (acetate, butyrate, and propionate) responsible for the maintenance of a desirable dynamic microbiota and innate/specific immune markers important for the host health. Prebiotics currently marketed are disaccharide, oligosaccharide and resistant starch in nature (Fig 4). Inulin-type prebiotics are among the most commercialized and studied prebiotics. Inulin and fructooligosaccharides share this same nature. They are carbohydrates and, as such, are classified according to their degree of polymerization, i.e., according to the number of monosaccharide units combined. They are also classified as non- digestible, as the anomeric carbon atom (C1 or C2) of the monosaccharide unit of the oligosaccharide has a configuration that makes their osidic bonds unsusceptible to the hydrolytic human enzymes. Inulin (3-60 sugar moieties) and fructooligosaccharides (3-20 sugar moieties) are linear â 2'!1 fructans which are present in a variety of plants (24, 25).

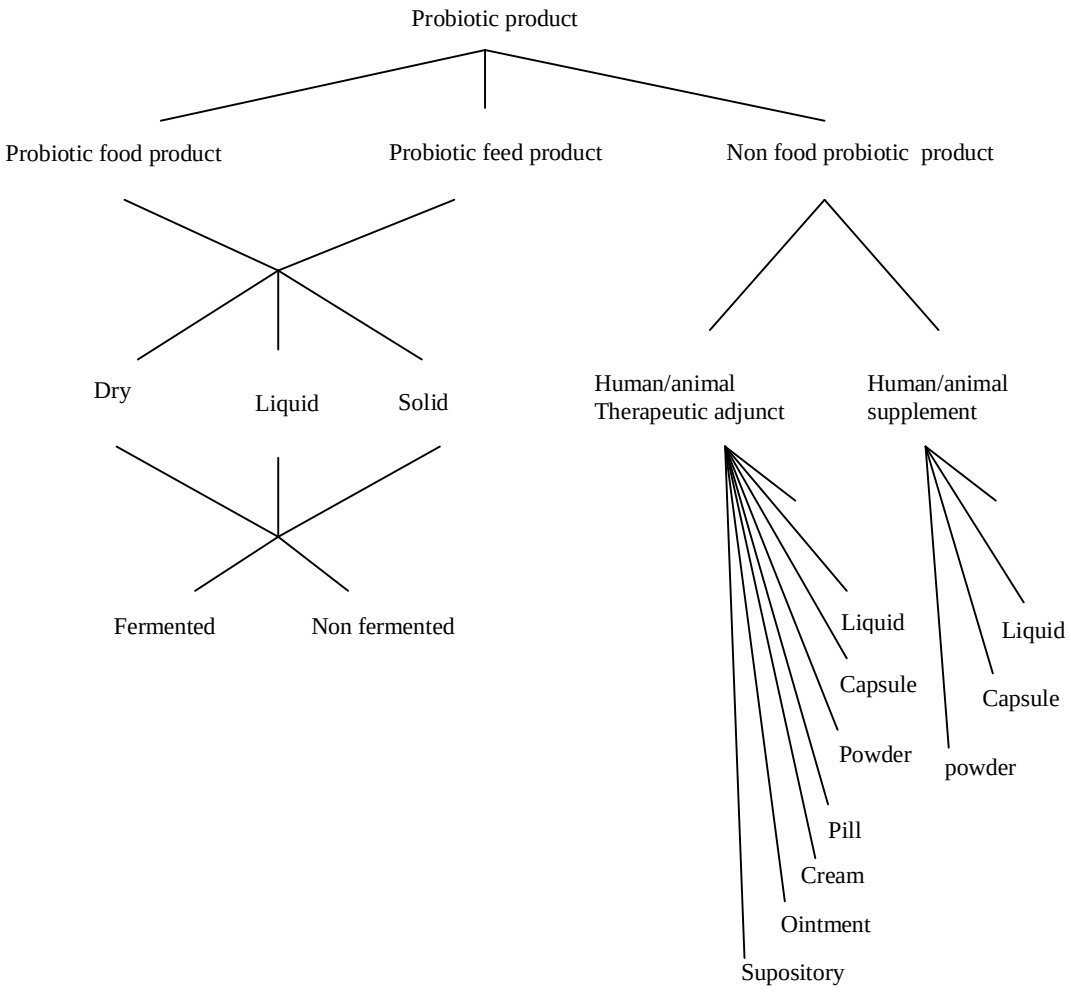

FIGURE 2. Formats of probiotic products generally marketed. Adapted from Magalhães *et al.* (23)./ *Formatos de los productos probióticos generalmente comercializados.*

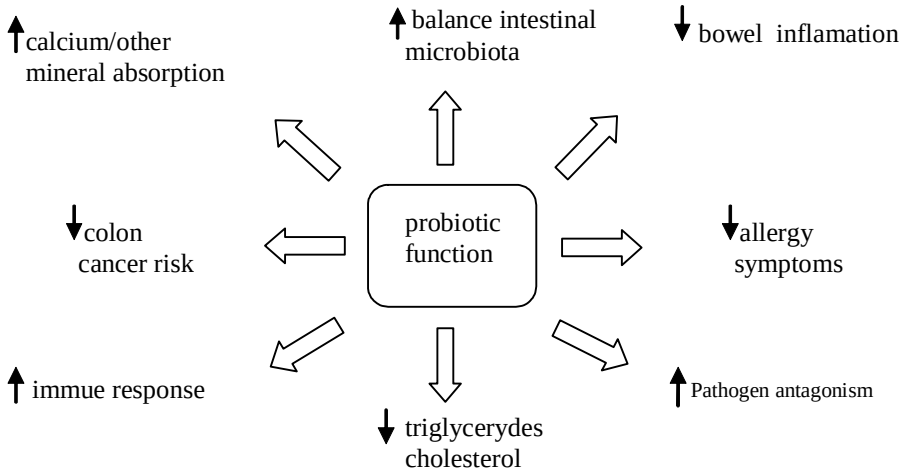

FIGURE 3. The main probiotic functions./ *Principales funciones de los probióticos.*

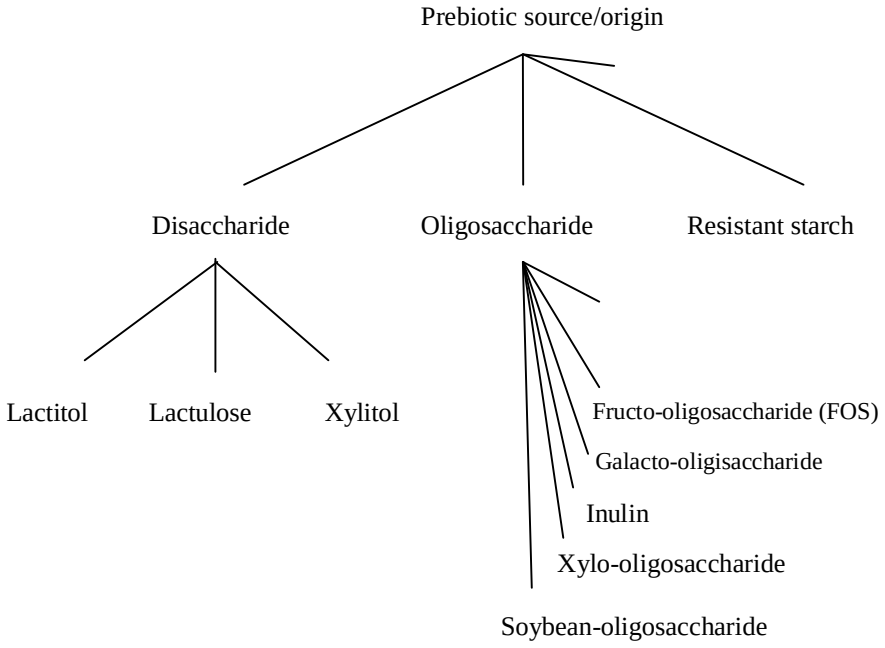

FIGURE 4. Prebiotic origin. Adapted from Magalhães *et al.* (23)./ *Origen de los probióticos. Adaptado de Magalhães et al. (23).*

Currently, many non-digestible oligosaccharides (NDOs) are commercialized as food ingredients. They are carbohydrates in which the monosaccharides unit is glucose, galactose, fructose and xylose. Thirteen commercialized types of NDO have been described: i) cyclodextrin, ii) fructooligosaccharide, iii) galactooligosaccharide, iv) gentiooligosaccharide, v) glycosilsucrose, vi) isomaltooligosaccharide, vii) lactosucrose, viii) lactulose, ix) maltooligosaccharide, x) raffinose, xi) soybeanoligosaccharide, xylooligosaccharide (26). Production processes of some NDO are indicated on Table 1.

From the currently known NDO, the inulin-type ingredients - inulin and fructooligosaccharides (FOS) - are the most commercialized and studied. These compounds have many important properties, making them suitable alternatives in the process of balancing a disturbed colon microbiota, thereby inhibiting many undesirable diseases.

TABLE 1. Production processes of some non digestible oligosaccharides (NDO)./ *Procesos de producción de algunos oligosacáridos no digeribles (NDO)*

Raw material	Method/Reaction	Main Carbohydrate	Reaction/Method	NDO
Inulin	Hydrolysis	-	-	Fructooligosaccharide
Beet	Extraction	-	-	Raffinose
	Extraction	Sucrose	Transglycosilation	Isomaltulose
Cow's milk	Extraction	Lactose	Isomerization	Lactulose
	Extraction	Lactose	Transglycosilation	Galactooligosaccharide
Starch	Hydrolysis	Soluble Starch	Transglycosilation	Cyclodextrin
	Hydrolysis	Soluble Starch	Hydrolysis	Maltooligosaccharide
	Hydrolysis + Transglycosylation	Soluble Starch	Hydrolysis	Isomaltooligosaccharide
Soybean	Extraction	Soluble Starch	Extraction	Soybean oligosaccharide
Xylan	hydrolysis	-	-	xylooligosaccharides

Adapted from Sako *et al.* (26)

A summary of the main benefits imparted to the consumption of prebiotics is indicated in Figure 5.

Many of the functions of prebiotics are part of the portfolio of probiotic functions, as the promoter of such benefits relies in the modulated microbiota (fig. 4 and 5).

Nowadays, commercial FOS are mainly originated from extraction/hydrolysis of Jerusalem artichoke and chicory. However, recently renewed interest has been directed to a rediscovered crop from the Andes, the yacon (*Smallanthus sonchifolius*), which harbors a much higher concentration of FOS. In this fruit-like root sugar FOS is stored instead of starch. Yacon is so rich in FOS that «an effective prebiotic dose can be achieved by only consuming the root in moderate amounts»(27). To date, most of the yacon studies are upon its antidiabetic effect (28). Other studies indicated that colonic transit was ameliorated in volunteers (29), positive effect was detected on calcium/magnesium balance and bone calcium retention in rats (30) and that no adverse effect on rats was detected through an oral toxicity evaluation (31). Important finding is the potential of yacon to be used as prebiotic and to control obesity and insulin resistance in humans (32). The richness of the yacon in bio-ingredients and functionality has recently been highlighted as it has been indicated as novel food candidate to the EU market (27). Therefore it is important that more clinical studies be undertaken to clarify the mechanistic effect of yacon consumption as this functional food might have an important role in public health to mitigate the effects of a number of chronic conditions affecting the growing aging population.

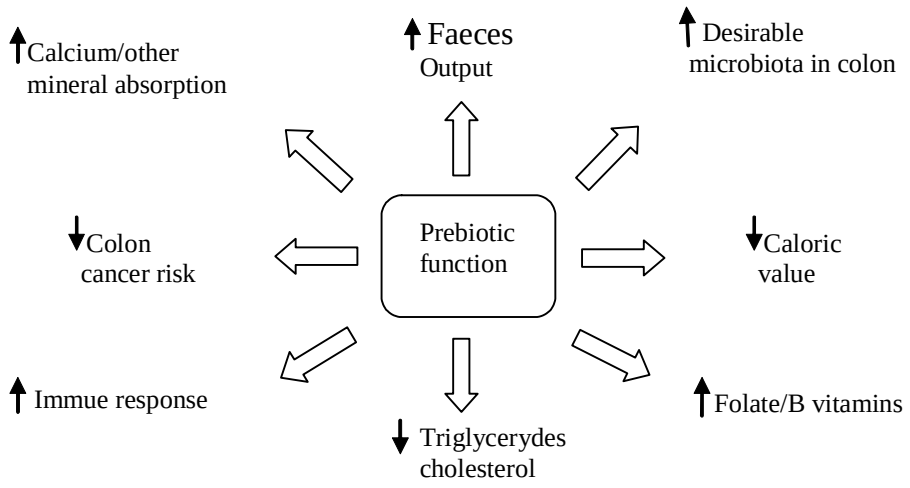

FIGURE 5. The main prebiotic functions./ *Principales funciones de los prebióticos.*

Health effect of probiotics and prebiotics

There is an increasing evidence that pro- and prebiotics can play a role in alleviating some diseases and conditions as summarized in figures 4 and 5. It is important, however, to highlight that almost all of these effects rely on the beneficial modulation of the intestinal microbiota and in the increasing importance that pro- and prebiotics have in the modulation of different types of diarrhea, as they represent «supportive therapy for conditions established by the disruption of the normally protective microbiota» (14).

In animal husbandry and management, pro- and prebiotics are emerging as important tools to fight microbial unbalance mainly during stress conditions. Reductions in pathogenic and increases in desirable intestinal bacteria can result in local and systemic immune activation, rendering animals healthier and increasing their performance.

Promising results have been found upon administration of probiotics in swine, poultry, cattle and sheep. The surge in popularity of the application of probiotic in the agriculture industry relies basically upon its potential for livestock growth promotion especially after the 2006 EU-wide ban on in-feed antibiotic growth enhancers. The physiological state of animals influences their performance (33). In swine, the weaning period has a great economic impact due to the common occurrence of intestinal disorders, and this transition time is characterized by changes in the feeding procedure and in the relation of the siblings with the sows. Furthermore, this separation at an early age (usually 21 days), coupled with an immature intestine, exacerbate animal stress. At this stage, a probiotic function such as barrier, competitively excludes pathogens. This mechanism is also promising in animals after treatment with antibiotics, mainly in the prevention of *Salmonella* infection. In previous studies, body weight was improved upon supplementation of weaned pigs with *Lactobacillus sobrius* and challenged with *E. coli* K88 (34). In another report, *E. faecium* and *E. faecalis* have been used in several clinical trials, albeit not proposed by QPS (35). Baum and colleagues found that sows´ diet supplemented with *Enterococcus faecium* protected one week post weaned piglets from diarrhea. They also reported that weaned pigs had improved growth after 4 months of feed consumption containing *Saccharomyces boulardii* (36).

The main stressors in broiler production are related to post hatching, transportation and stocking densities (37). The poultry intestinal microbiota seems to have an effect on growth, egg production and feed conversion, and probiotic use is directed to maintain the health state by enhancing the competitive exclusion (CE) of pathogens (38). The term Competitive Exclusion is also known as the Nurmi concept, and has originated from the observation that *Salmonella* was inhibited when the infected bird was fed with a suspension containing gut material from a healthy adult chicken (39). The CE is seen as a main alternative to maintain a healthy gut in poultry studies. An artificially infected hatchery experiment with *Salmonella enteriditis* (10^4 CFU) indicated that chicks receiving doses of *Lactobacillus* FM-B11 (10^{6-8} CFU) were protected from the infection (40). Natural antibodies for some antigens was increased in serum and gut of chicks following ingestion of a mix of *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecalis* (41). Probiotics are a good strategy to maintain pathogens at bay, thus protecting the gut microbiota of poultry and other production animals. Although prebiotic use in this area is quite new, conflicting results have already been reported (42). Comprehensive reviews on these subjects are indicated elsewhere (43, 17, 27).

Considering that probiotics and prebiotics are part of functional foods, it is important to remember the main features for the maintenance of such ingredients within that classification are basically twofold: i) to be consumed with the normal, everyday diet, ii) have a positive effect on target function(s) that may enhance well-being and health, and/or reduce the risk of disease.

CONCLUSION

Within the last decade, studies on the relationship of human/ animal gut microbiota and immunology have shed light to the role of probiotics and prebiotics on the health of mammals. Evidences exist pertaining the influence that pro-, pre- and synbiotics have in the microecology of the intestines of human and animals, and their role in alleviating various diseases. Microbial ecology coupled with the understanding of the human genome have «paved the path for the clarifying of the mechanisms involved with the health effects of probiotics and prebiotics» (44). Because of the large amount of evidence and diversity of the beneficial effects of these functional foods/ingredients, presently their potential could be considered underestimated. The contingent of individuals aged 65 or older is increasing globally, which constitutes a public health concern. Functional foods are now part of the modern thinking based on prevention and/or alternatives for decreasing the risk associated to chronic diseases. Furthermore, the concerning of maintaining «cleaner» feeds to curb dysbiosis in humans and animals has increased the use of pro- and prebiotics for the promotion of livestock

growth, specially after the 2006 EU-wide ban on in-feed antibiotic growth enhancers. Probiotic, prebiotic and synbiotic food, feed supplements and ingredients abound the market, albeit confirmation of the benefits and mechanisms of action still remains mostly putative. Still, they constitute important tools to help maintaining humans and animals in good health.

ACKNOWLEDGEMENTS

The authors thank CAPES, CNPq and FAPEMIG, Brazil.

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(Recibido 9-8-2011; Aceptado 23-10-2011)