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Effect of Probiotic Administration on the Immune Response: A Systematic Review of Experimental Models in Rats

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ABSTRACT

The probiotic influence on the immune system, especially under pathogenic challenge conditions, still remains controversial. To address this, a systematic review of current studies concerning the efficacy of probiotics on the immune response of rats subjected to experimental challenges was conducted. The survey was conducted using PubMed, ISI Web of Science and Scielo databases. Only studies which tested probiotics in vivo *in rats were included. The experimental design, methodological quality, and results of the articles were analyzed. In total 21 articles were selected for this study. The most commonly used microorganisms in the experiments were those of the genus* Lactobacillus*, which was reported in 12 articles. The second most often used genus was* Bifidobacterium *(*B. animalis *and* B.longum*). In general, the probiotics use against experimental pathogenic challenges was successful: 86% of the selected articles reported a beneficial effect on the immune response associated with the use of probiotics.*

Key words: immunity, probiotics, rats, dietary supplements

INTRODUCTION

It is well known that the nutrition, through a series of complex interactions, is able to improve the health status of the animals. In animal production, several substances have been used as growth promoters, including probiotics, which are live microorganisms that improve the microbial balance in the gastrointestinal tract, thereby increasing the efficiency with which the nutrients are used. In other areas, probiotics have been used for preventive purposes, to inhibit the proliferation of microorganisms that cause gastrointestinal disturbances (Chaucheyras-Durand et al. 2008; Vanderpool et al. 2008; Mountzouris et al. 2009; Chaucheyras-Durand and Durant 2010; Maragkoudakis et al. 2010).

By definition, probiotics are microorganisms that are regulated as dietary supplements, when ingested in sufficient quantities, have beneficial effects on the health of the host (FAO 2002; Budiño et al. 2005; Siró et al. 2008; Tsubura et al. 2009). Most probiotics contain bacteria of the genus *Lactobacillus* and *Bifidobacterium* (Brizuela et al. 2001; Peran et al. 2006; Zeng et al. 2009; Bloise et al. 2010; de Roock et al. 2010). However, certain bacteria of the genus *Enterococcus* (Maragkoudakis et al. 2010), *Leuconostoc* and *Streptococcus* (Zanini et al. 2007) and yeasts, such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Baptista

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et al. 2005; Generoso et al. 2010) can be considered to be probiotic microorganisms.

Numerous studies have demonstrated the effectiveness of these microorganisms at improving the intestinal health of the animals and, thereby, their metabolic and physiological status Brizuela et al. 2001). Besides the direct effect of probiotics on the adherence of pathogenic bacteria in the intestinal epithelium, several studies have also correlated probiotic administration with the positive effects on the immune response in animals (Borchers et al. 2009; Amit-Romach et al. 2010; Generoso et al. 2010; Fink 2010) and humans (Nomoto 2005; Salminen et al. 2005; Lomax and Calder 2009). Other benefits identified in *in vitro* studies include significant inhibition of infection by *L. monocytogenes* (Corr et al. 2007); strong induction of IL-12 and TNF-α in monocytes and cultured human peripheral blood mononuclear cells (PBMC) (Fink 2010); and inhibition of the growth of *C. albicans* (Verdenelli et al. 2009), among others. However, no consensus exists in the literature on the preventive or therapeutic use of probiotics to improve the immune system's ability to defend against different infectious agents.

Animal models, such as rats, are often used to simulate the physiological and pathological mechanisms *in vivo*. Results are then extrapolated to other species, which cannot be directly investigated, due to ethical, financial and/or facilities management issues, or simply because of a lack of physical space (Fagundes and Taha 2004; DaMatta 2010). Thus, detailed studies on a single species are necessary for comparative analysis. Therefore, the objective of the present study was to conduct a systematic review of the efficacy of probiotics on the immune response in rats.

MATERIALS AND METHODS

Research strategy

An electronic search of the PubMed database (http://www.ncbi.nlm.nih.gov) was conducted in October 2010, using the following keywords: immunity, probiotics, rats. To confirm the findings and obtain supplementary studies, a similar strategy was employed for the ISI Web of Science database (http://apps.isiknowledge.com) and Scielo database (http://www.scielo.org/php/index.php), using the same keywords (also in Portuguese and Spanish, when applicable).

Study Selection

For the present review, only in vivo studies using probiotics and rats were selected. Studies conducted on mice, rabbits, guinea pigs, or other types of animal models were excluded.

No restrictions were made for the type of probiotic used in the study, administration form, or administration period against an experimental challenge (for prevention and treatment). Additionally, no date, lunguage or number of animals were restricted as selection criteria.

Data extraction and Quality criteria

Two researchers conducted article searches separately, and independently verified the compliance of the selected papers with the inclusion criteria. In the cases of divergence between the papers, all the criteria were reviewed and discussed. Table 1 displays the data related to the experimental design of the retrieved articles.

After study selection, quality analysis was conducted and scores were assigned to specific scientific criteria as described in Table 2. Selection criteria were defined to evaluate both the protective effects of probiotics in relation to the immune system and the methodological quality of the selected articles.

However, not all the parameters used were scored on the quality scale (such as animal strain, type of microorganism used and evaluated technique, among others), but were taken into consideration as they were relevant to the subsequent discussion. The scientific criteria used were adapted from other systematic reviews (Noli and Auxilia 2005; Negre et al. 2009; Pereira et al. 2010). The parameters were classified as either adequate (score: 2) or unclear/partially adequate (score: 1). The following parameters were scored:

- Sample number: Studies with sample groups containing ≥ 6 animals received a score of 2 and studies with less than 6 animals per group received a score of 1.
- Randomization: Studies reporting nonrandomized experiments or studies for which the degree of randomization was not clearly described in the text received a score of 1, while studies using randomized experimental designs received a score of 2.
- Control group: Studies that included a control group received a score of 2, while studies that did not include a control group or did not

clearly mention a control group in the text received a score of 1.

- Blind evaluation: Studies which included blind assessments in their experimental design received a score of 2, while studies whose experimental designs did not include blind assessments, or for which blind assessments were not clearly reported in the text received a score of 1.
- Interference factors: Studies that did not evaluate interference factors received a score of 1, while studies which considered additional factors, such as stress, hormonal evaluation, and variations between the males and females received a score of 2.
- Pathogenic challenge: Studies which did not include an experimental challenge received a score of 1, while studies which subjected the animals to an experimental challenge received a score of 2.

The maximum total score was 12 points.

Table 1 - Summary of the Selected Studies.

1	2	3	4	5	6	7	8	9	10	11	12	13
A	Wistar	*Lactobacillus helveticus and Streptococcus thermophilus* 10^6 CFU	Y	Pre- and post-operative period	30	Y	U	Y	U	Laparotomy with colon anastomosis	Intestine (colon)	Evaluation of the IGA, total protein, albumin and globulin; analysis of DNA content by the method Gyles and Meyers.
B	Wistar	*Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium lactis* 10^9 CFU	Y	Before the challenge and throughout the experiment	6	Y	U	Y	N	Induction of colitis	Intestine (colon and lower end of the ileum)	Evaluation of colonized tissues by real-time PCR. Morphology of the colon and damaged tissue were histologically evaluated.
C	Lewis, Wistar and Balb/c	*Lactobacillus casei* 10^9 CFU	N	Before the challenge and throughout the experiment	16	Y	U	Y	U	EAE induction	Ears (epidermis) or central nervous system	Isolation and proliferation of lymph nodes, IL-4 and IFN-γ by ELISA, cytokine through standard curves of recombinant IL-4 or IFN-γ, analysis of gene expression in liver and thymus tissue, as well as frozen MLN; analysis of the amount of RNA by spectrophotometry and RNA integrity by gel electrophoresis; microarray analysis
D	Wistar and Lewis	*Lactobacillus kefiranofaciens* 6 x 10^{11} CFU	N	Throughout the experiment	5	N	U	Y	Y	N	-	ELISA and blood cell count
E	Sprague Dawley	*Lactobacillus sp.* 10^9 CFU	U	Before the challenge and 9, 3 and 10 days after challenge	3	Y	U	Y	N	Cecum perforation for polymicrobial infection	Cecum	Intestine histology; counting bacterial colonies; Backlight analysis; serum TNF analysis by ELISA.
F	Wistar	*Lactobacillus casei* $2x10^9$ CFU.	U	Before and after the challenge	6	Y	U	Y	N	*Listeria monocytogenes* (sensitization caused by oral infection)	Gastro-intestinal tract and visceral organs	Bacteriological analysis; liver and spleen histological analysis; ALT levels and concentration of total serum bile acids by a Beckman Synchron CX7, cell-mediated immunity measured using the DTH assay
G	Wistar	Lactobacillus $2x10^9$ CFU	N	After the challenge	4	Y	U	Y	U	*Listeria monocytogenes infection.*	Spleen and liver	Liver and spleen bacteriological analysis and measurement of *L. monocytogenes* specific DTH.
H	Sprague Dawley	*Bifidobacterium longum* 1×10^{10} CFU	N	From birth until the end of the experiment	10	U	U	Y	U	N	-	RNA concentration by spectrophotometry, reverse transcription, RT-PCR, cytokine and immunoglobulin (by ELISA)

(Cont. …)

(Cont. Table 1)

1	2	3	4	5	6	7	8	9	10	11	12	13
I	Lewis and Balb/c	*Lactobacillus casei* 2 - 4x10^8 CFU or 1 - 2x10^9 CFU	N	Before the challenge and throughout the experiment	8	Y	U	Y	Y	Allergy Induction	Lymphocytes in the lungs and ovalbumin-specific cytokines in the spleen	Specific ovalbumin IgE and IgG1 titres in sera were determined by ELISA. Th1 and Th2 cytokines were measured in the supernatants of spleen cells that were cultured with ovalbumin; IL-4, IL-5, IL-10, IL-13 and IFN-γ analysis.
J	Lewis and Balb/c	*Bifidobacterium animalis* 1x10^9 CFU	N	Before the challenge and throughout the experiment	16	Y	U	Y	Y	OVA (respiratory allergy) or EAE	Lung or central nervous system	OVA-specific antibodies; Cytokine; IgE ova-specific (by ELISA)
K	Albino rats	*Lactobacillus sp.* 10^8 CFU	N	Throughout the experiment	6	Y	U	Y	Y	Induction of diarrhea (castor oil used as a laxative)	Gastro-intestinal tract	Protein levels were determined by the method of Buiret; blood cell count
L	Sprague Dawley	*Lactobacillus casei, Lactobacillus bulgaricus, Streptococus thermophilus, Lactobacillus acidophilus, Lactobacillus plantarum and Bifidobacterium infantis* 2x10^7 CFU or 4x10^8 CFU	Y	From the second day of the experiment until sacrifice	1	U	U	Y	U	Cryptosporidiosis	Small intestine (cecum)	Estimated amount of parasites in the mucosa of the cecum by Ziehl-Neelsen staining and *C. parvum* by real-time PCR, histological analysis of the cecum; IFN-γ, IL-10 and TNF-α
M	U	*Lactobacillus casei* 5x10^{10} CFU or 1x10^{11} CFU	N	Before the challenge and throughout the experiment	10	Y	U	Y	N	Infection with *E. coli*	U	IgA (ELISA), cytotoxicity of NK cells, macrophages, TNF-α, IL-6 and IL-12
O	Fisher and Balb/c	*Lactobacillus paracasei* 5 x 10^8 CFU	N	After the challenge	5	U	U	Y	N	Air bags (injection of sterile air)	Back of the animal	PMN accumulation and phagocytic activity of these cells, IFN-γ, TNF-α and IL-10 (ELISA), histopathology; immunohistochemistry
P	Wistar	*Lactobacillus brevis, Lactobacillus plantarum, Streptococcus faecalism and Bifidobacte-rium brevis* 6 x 10^8 CFU	Y	For 3 days before sacrifice	10	Y	U	Y	Y	Indomethacin	Gastro-intestinal tract	Percentage of damaged area (macroscopically); Histology of the gastric mucosa, ileum and colon, immunohistochemistry of lymphocytes B (CD 20) and T (CD 4 +)
Q	U	*Lactobacillus plantarum and Lactobacillus murines* CFU (UC)	N	During the challenge	U	U	U	Y	N	EAE	Central nervous system	Cytokines and DNA
R	Sprague Dawley	*Lactobacillus acidophilus, L.. helveticus and Bifidobacterium* CFU (UC)	Y	Throughout the experiment	U	Y	U	Y	U	Azinomethane (colon carcinoma)	Colon	Analysis of the proliferation rate of the mucosa, mesenteric lymph nodes were removed from rats for analysis of intestinal immune system markers, ACF determination; tumor detection
S	Wistar	*Lactobacillus paracasei* 3 x 10^7 CFU	N	During the challenge	7	Y	U	Y	U	Ischemia and reperfusion	Liver	Hepatic microcirculation, liver histology, Western blotting analysis, plasma assessment; bacteriological evaluation in the small intestine

(Cont. …)

(Cont. Table 1)

1	2	3	4	5	6	7	8	9	10	11	12	13
T	Fischer	*Lactobacillus rhamnosus and Bifidobacterium lactis* $5x10^8$ CFU or $5.5x10^8$ CFU	Y	U	32	Y	U	Y	U	Azinomethane (colon carcinoma)	Colon	Immunofluorescence of lymphocyte subpopulations through the spleen and MLN, flow cytometry analysis; IL-10 and IFN-γ by ELISA
V	Lewis	*Lactobacillus casei* $2x10^{10}$ CFU	N	After induction and during the whole experiment	U	Y	Y	Y	Y	Induction of rheumatoid arthritis (collagen type II)	Ankle (foot)	Histopathological analysis of the hind paws; cytokines by RT-PCR, IgG (ELISA), TNF-α, IL-10 and Foxp3 by FACS Calibur Flow Cytometer
X	Sprague Dawley	*Lactobacillus acidophilus* 2.5 x 10^8 CFU	N	After the challenge	7	U	U	Y	N	ICV cannulations	Brain tissue	Histopathology; immunohistochemistry; mRNA and cDNA (RT-PCR), positive colonies were confirmed by DNA sequencing, Western-blotting of the intestines and retroperitoneal adipose tissue.

A: Aguilar-Nascimento et al. 2006; B: Amit-Romach et al. 2010; C: Baken et al. 2006; D: Beaulieu et al. 2007; E: Bu et al. 2006; F: de Waard et al. 2002 a; G: de Waard et al. 2002 b; H: Dong et al. 2010; I: Ezendam and van Loveren 2008; J: Ezendam et al. 2008; K: Flore et al. 2010; L: Guitard et al. 2006; M: Ishida-Fujii et al. 2007; O: Kourelis 2010; P: Laudanno et al. 2008; Q: Maassen and Claassen 2008; R: Marotta et al. 2003; S: Nardone et al. 2010; T: Roller et al. 2004; V: So et al. 2008; X: Sousa et al. 2008; CFU: colony forming unit; U: Unclear; Y: YES; N: NO; IgA: immunoglobulin A; IgE: immunoglobulin E; IgG1: immunoglobulin G; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; mRNA: Messenger ribonucleic acid; ELISA: Enzyme Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction; RT-PCR: reverse transcription polymerase chain reaction; EAE: experimental autoimmune encephalomyelitis; IL-4: interleukin-4; IL-5: interleukin-5; IL-6: interleukin-6; IL-10: interleukin-10; IL-12: interleukin-12; IL-13: interleukin-13; ICV: intracerebroventricular; MLN: mesenteric lymph nodes; PMN: polymorphonuclear leukocyte; ACF: aberrant crypt foci ALT: alanine aminotransferase; DTH: delayed-type hypersensitivity ; Th1: T helper cell type 1; Th2: T helper cell type 2; IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-α; OVA: ovalbumin; NK: natural killer; 1: author and year of publication; 2: lineage; 3: microorganisms used; 4: association of microorganisms; 5: period of probiotic administration; 6: number of animals per experimental group°; 7: randomization; 8: blind assessments; 9: control group: 10: interference factors°°, 11: pathogenic challenge; 12: tissue where the challenge was induced; 13: technical evaluated. °Studies in which the "n" experimental varied, was considered the smallest n; °°Stress, hormone assessment, gender, etc.

Table 2 - Evaluation criteria and scores for the selected articles.

Author	Mean number of animals per group*	Type of assay**	Control group***	Blind assessmentd+	Interference fators++	Pathogenic challenge+++	Total
Ezendam and van Loveren 2008	2	2	2	1	2	2	11
Ezendam et al. 2008	2	2	2	1	2	2	11
Laudanno et al. 2008	2	2	2	1	2	2	11
So et al. 2008	1	2	2	2	2	2	11
Aguilar-Nascimento et al. 2006	2	2	2	1	1	2	10
Amit-Romach et al. 2010	2	2	2	1	1	2	10
Baken et al. 2006	2	2	2	1	1	2	10
Flore et al. 2010	2	2	2	1	1	2	10
Bu et al. 2006	2	2	2	1	1	2	10
Ishida-Fujii et al. 2007	2	2	2	1	1	2	10
Roller et al. 2004	2	2	2	1	1	2	10
de Waard et al. 2002a	2	2	2	1	1	2	10
Beaulieu et al. 2007	2	1	2	1	2	1	9
Marotta et al. 2003	1	2	2	1	1	2	9
Nardone et al. 2010	2	1	2	1	1	2	9
Sousa et al. 2008	2	1	2	1	1	2	9
de Waard et al. 2002b	1	2	2	1	1	2	9
Kourelis 2010	1	1	2	1	1	2	8
Guitard et al. 2006	1	1	2	1	1	2	8
Maassen and Claassen 2008	1	1	2	1	1	2	8
Dong et al. 2010	2	1	2	1	1	1	8

* Scores for the sample number were 1 (less than 6 animals/group) and 2 (6 or more animals/group)

** Nonrandomized experiments or when randomization was not described clearly in the text (score 1) and randomized experiments (score 2)

*** Studies without control groups or those which did not clearly mention a control group in the text (score 1) and studies with a control group (score 2)

+ Experiments without blind assessments or those in which blind assessments were not clearly reported in the text (score 1) and experiments with blind assessments (score 2)

++ Studies that did not evaluate interference factors (score 1) and studies which evaluated additional factors such as: stress, hormonal evaluation, variations between males and females (score 2)

+++ Studies in which animals were not subjected to experimental challenge (score 1), and studies in which animals were subjected to an experimental challenge (score 2).

RESULTS

An initial search of the PubMed database retrieved 24 articles. Of these, three were excluded because were conducted in humans or mice; one evaluated the isolated action of prebiotics; three others were also excluded because they were literature reviews. Thus, of the initial 24 articles retrieved, 18 were selected for this study.

A search of the ISI Web of Science database also retrieved 24 articles, eight of which were duplicates of articles retrieved from PubMed. Of the 16 remaining articles, five were excluded because they were studies on humans, sows and piglets, prebiotics, or were performed *in vitro*; two others were excluded because they did not evaluate probiotics, and six more were excluded because they were literature reviews. Therefore, three additional papers were selected from this search. A search of the database Scielo did not identify additional articles. Thus, in total 21 articles met the inclusion and exclusion criteria, and were selected for this review. Table 1 presents a summary of the selected studies.

The following rat lineages were used in the studies included in this review: Wistar, Lewis, Sprague Dawley and Fischer. The combinations of two distinct lineages of rats were also used, as well as the combinations of rats with Balb-C mice. In three studies, the authors did not report the rat lineages used.

Bacteria from the genus *Lactobacillus* were the most commonly used microorganisms in the selected studies, and were reported in 57% of the papers. The second most common genus was *Bifidobacterium* (*B. animalis* and *B. longum*). The combinations of microorganisms were used in 38% of the papers, such as *Lactobacillus helveticus* + *Streptococcus thermophilus* or *Streptococcus thermophilus* + *Lactobacillus acidophilus* + *Bifidobacterium lactis*, among others.

In 48% of the articles, probiotics were administered in the feeding, while in 38% of the studies, probiotics were administered by gavage. Other forms of administration, such as water or castor oil were also mentioned. There was a large variability in the amount of colony forming units (CFU) used among the surveys, and no consensus technique emerged, even among the studies dealing with the same species of bacilli. The duration of probiotic administration (e.g. before, during or after experimental challenge) also varied considerably: probiotics were administered both before and after the challenge in 33% of the studies; only during the challenge in 14%; and only after the challenge in 19%. In 19% of the studies, probiotics were administered throughout the study period, independent of the timing of the experimental challenge. Other studies administered probiotics only a few days before the animals were killed, in the pre- and post-operatory period.

Of the 21 selected articles, only one reported having conducted a blind evaluation (So et al. 2008), while 16 articles reported randomization of the sample. The number of animals per group ranged from 1 to 32, although three papers did not report the number of animals used per experimental group.

A total of 90% of the articles induced a pathogenic challenge: 38% introduced an intestinal challenge (e.g. colitis or tumors, among others); 9.5% introduced encephalomyelitis; 9.5% induced liver injury; 4.8% induced respiratory allergies; 4.8 % induced arthritis; 4.8% challenged the animals with *Escherichia coli*; 4.8% induced ischemia and infusion; 4.8% induced intracerebroventricular cannulation; and 4.8% were challenged by the introduction of air pockets into the back of the animals. In 4.8% of the papers, both encephalomyelitis and respiratory allergy were induced simultaneously (Ezendam et al. 2008).

With respect to the interference factors 24% of the articles separated male and female groups, while 5% used the models of stress. However, the vast majority (71% of the articles) did not report any interference factor. In the work of Laudanno et al. (2008), both the sexes (male/female) and stress were evaluated. All the studies used control groups.

DISCUSSION

Literature reviews are useful to the scientific community in general, and can provide significant insight into a particular research field, since they enable a more complete view of current results. In addition, they can suggest the best protocols to be employed and/or future directions for research (Snodgrass 2006). The present literature review on the efficacy of probiotics at improving the immune response in rats focused on targeting which therapeutic protocols were associated with the best (or more promising) results in this species, and

could be used as a guide to future studies attempting to reproduce these experiments in other species.

Research using animal models are important, especially given the limitations of investigating certain diseases directly in humans, which often involves the ethical issues and/or risks related to the disease under study. Diseases which can be induced in animal models have the potential to reveal the pathological mechanisms that can be extrapolated to humans, increasing the understanding of human disease. Thus, the use of animal models can help overcoming numerous research limitations and often provides causal relationships more quickly. For these reasons, experimentation on animal systems often represents the first step in many research projects (Taha and Fagundes 2004; DaMatta 2010).

According to Nomoto (2005), excessive use of the antibiotics can induce an imbalance in the intestinal microbiota, encouraging the emergence of antibiotic-resistant bacterial infections, and, at the same time, reducing the possible activation of the immune system prior to infection. Because of this problem, interest in the use of probiotics as a complement to antibiotics has been growing.

Although it is known that probiotics have different properties and functions, the mechanisms by which individual probiotics act in a host are not fully understood. As described in the literature, probiotics are assumed to act via several mechanisms, including: a) competitive exclusion, where probiotics compete with the pathogens for fixation sites and nutrients, thereby temporarily preventing the pathogenic action; b) production of antimicrobial substances, such as bacteriocins, hydrogen peroxide and volatile organic acids; c) induction of direct changes in the immune response, through immune stimulation of residing cells in the enteric tract, which then initiate activation of macrophages, increasing phagocytosis; and d) modulation of enzyme activity by changing the microbial metabolism (Audisio et al. 2000; de Vrese et al. 2001; Ogawa et al. 2001; Cross 2002; Puupponen-Pimia et al. 2002; Hamilton-Miller 2004; Boirivant and Strober 2007; Gillor et al. 2008; Borchers et al. 2009; Ng et al. 2009; Rijkers et al. 2010; Yan and Polk 2010).

Of the 21 articles selected, 86% reported the beneficial effects from the administration of probiotics on the immune response in rats. Two studies, one conducted by Baken et al. (2006) and one by Guitard et al. (2006) reported unsatisfactory results from the use of probiotics, suggesting that further studies were necessary. Baken et al. (2006) induced autoimmune encephalomyelitis, the same challenge experiment used by Maassen et al. (2008), who concluded that probiotics could suppress this disease. Ezendam et al. (2008) observed a significant reduction in the duration of clinical symptoms, and an improvement in weight gain versus the control group. Guitard et al. (2006) investigated the effect of probiotic administration on the development and progression of an experimental parasite infection (cryptosporidiosis) in lactating rats. Although the rats administered probiotics tended to display faster parasite clearance than the controls, no significant effect was observed in terms of weight gain, parasite burden, mucosal damage or cytokine kinetics in the mucosa during the course of the infection. Overall, these authors found that daily administration of probiotic mixtures containing *Lactobacillus casei* was not able to eradicate the parasite in their experimental model. However, differences in probiotic strains and dosages could justify the discrepancies between these studies.

The animals underwent intestinal challenge in 48% of the assessed studies, and all responded positively to the use of probiotics, with the exception of Guitard et al. (2006). The immunostimulant effect associated with probiotic administration could be related to the ability of these microorganisms to interact with Payer's patches and intestinal epithelial cells, thereby activating the mucosal immunity by stimulating the plasma cells, IgA secretion and migration of intestinal T cells (Park et al. 2002; de Vrese et al. 2005).

Of the articles investigating induced respiratory allergies (n=4 articles), only two reported a positive response. However, probiotics were found to increase the phagocytic activity of alveolar macrophages, suggesting that they could act systemically by inducing the secretion of mediators which could then stimulate the adaptive immune system (Cross 2002).

Significant differences were observed with respect to the doses of probiotics used. However, no differences in results were noted between the highest (Beaulieu et al. 2007) and lowest administered doses (Aguilar-Nascimento *et al*. 2006): both showed positive immune responses. The immune response to the use of probiotics was

also not dependent on the time of administration: positive responses were noted when probiotics were administered before pathogen challenge, during the challenge, or both before and after the challenge.

However, no trend was found among the studies analyzed regarding the type of microorganism used for the treatment, preventing the establishment of a general protocol. *Lactobacillus* were used against several different types of pathogenic challenges, including encephalomyelitis (Baken *et al*. 2006; Maassen and Claassen 2008); colitis (Amit-Romach et al. 2010); laparotomy with colon anastomosis (Aguilar-Nascimento et al. 2006); and E. coli infection (Ishida-Fujii et al. 2007), among others. With the exception of Baken et al. (2006), all of these articles reported satisfactory results associated with the probiotic administration. However, numerous other microorganisms were also used in the analyzed studies, both alone and in combination. This variation probably stemmed from the fact that the objective of the research was to generally stimulate the immune response of the animals, not to evaluate the specific infections. No relationship was identified between the type of probiotic, pathogenic challenge and the effectiveness of probiotic administration.

Only one of the papers analyzed was conducted by blind assessment. However, 71% of the articles included a randomized experimental design (the remaining 29% did not clearly state if the study was randomized or not). Use of blind assessments and randomized evaluations improved the reliability of scientific works, by preventing study investigators from knowing which treatment was administered and in the case of randomized trials, distribution was done randomly (Snodgrass 2006; Taylor and Yildirim 2011).

CONCLUSION

In the studies assessed in this review, the administration of probiotics has been shown to be associated with a positive induction of the immune response in the presence of a wide range of experimental pathogenic challenges. Therefore, further studies should be encouraged in this field in order to develop new protocols with respect to the microorganism type, dosage and the timing of probiotic administration for specific illnesses.

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