

ANSWERING REVIEWERS

April 21st, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 9993-Review.doc).

Title: Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications

Author: Alejandra de Moreno de LeBlanc, Jean Guy LeBlanc

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9993

The manuscript has been improved according to the reviewers' suggestions (we highlighted the changes made to the manuscript according to the peer-reviewers' comments, using the track change system from Word).

1 Format has been modified according to the suggestions (authors' affiliations were corrected, the abstract was modified and now has more than 200 words, a summary of less than 100 words was added in Core tip section, and the figures were pasted as the original picture (ppt format) so that they can be edited easily).

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewed by 02536288

Thank you for good, interesting article. It will be better to add information about efficacy of *Saccharomyces boulardii* in treatment of irritable bowel syndrome, diarrhea and other disorders of gastro-intestinal tract

Thank you for your appreciation of our work and for your suggestion. We added information about *S. boulardii* in the revised version of the manuscript, maintaining the criteria to focus on articles where the beneficial effect was related with change in the gut microbiota. We also added two recent articles that report the effect of *S. boulardii* in human trials of irritable bowel syndrome to show the potential of this probiotic for IBD patients.

(2) Reviewed by 00061686

In general, some reviews cited do not appear full appropriate. Although this is the choice of

authors, some major recent reviews in the field of gut microbiota, dysbiosis, intestinal inflammation in relation with an increased intestinal cancer risk deserve to replace those that are cited and which have a minor interest. The Reviewer encourages authors to make an effort to update.

Thank you for your suggestion, we added information about other studies in which microbiota and dysbiosis were related with the increased risk of colon cancer. However, in the field of probiotics, there are no recent articles that include the study of the microbiota in the effect of probiotics against cancer.

In Page 1, authors say : ? Author contribution: A. de Moreno de LeBlanc and J.G. LeBlanc contributed equally to this work. Both authors performed the research and wrote the paper. The manuscript is a review not a full-length paper containing data from a work.

This sentence was modified by: Both authors performed the bibliographic search and wrote the paper

2. Correct Reference 1 as : "FAO/WHO. 2001. Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, Cordoba, Argentina, 1 to 4 October 2001. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf

The reference was corrected in the reference list.

3. Reference 10 is an auto-citation. Replace by an appropriate independent review.

We replaced the reference by a more appropriate one according to the reviewer's suggestion.

4. For: "Inflammatory bowel diseases (IBD), such as Crohn's disease (CD), ulcerative colitis (UC) or irritable bowel syndrome (IBS) can arise from the disruption of immune tolerance to the gut commensal microbiota, leading to chronic intestinal inflammation and mucosal damage in genetically predisposed hosts." add Reviews References.

A reference was added for this sentence (Fava and Danese, 2011).

5. Mixing in the same Paragraph IBD and *Clostridium difficile* infection is highly confusing. The mechanisms are different. Please reorganize.

We know that IBD and *Clostridium difficile* infection are two different pathologies but the sentence is about a case where fecal microbial transplantation was used as a therapy for a *Clostridium difficile* infection in a patient with quiescent ulcerative colitis. This treatment, that could be effective for the infection developed a flare of ulcerative colitis, indicating the need to be cautious in the use of this procedure in patients with IBD. The objective of this sentence was to demonstrate that for IBD, the results obtained with FMT are still somewhat controversial.

6. The fecal microbial transplantation is presented as too positive. This therapeutic intervention may involve risks currently underestimated. This begins to be raised. A major risk is related to the presence of viruses in the intestinal microbiota of the donor that are not sought before action and may represent a health risk for the recipient host after transplantation. This is a true question despite therapeutic successes have been reported.

The reviewer appreciation is correct, we include fecal microbial transplantation as a possibility to use against intestinal microbial dysbiosis because there are many studies that have shown the benefits of this therapy; however, as we wrote, controlled trials of FMT in specific disorders and complemented by animal models of fecal transplantation, in which variables can be controlled and manipulated, are needed before FMT can be more widely accepted and applied clinically. According to the observation of the reviewer we added a sentence about the health risk that can be associated to FMT.

7. The effectiveness of probiotic strains to treat IBD should be mitigated. According to a US Food and Drug Administration (FDA) working definition, probiotics are classified as “live biotherapeutics”: “live microorganisms with an intended therapeutic effect in humans”. Guidelines for the clinical use of probiotic strains were published after a Yale University Workshop in 2005, and updated in 2007. The advice is graded as "A", "B", "C", or no category. Classified in the "A-grade" advice are probiotic Lactobacillus strains used to treat acute childhood diarrhea and C. difficile-associated diarrhea. Probiotic Lactobacillus strains used to treat chronic disorders of the gut including IBD are classified as “B-grade” advice because there have been some negative studies. 8. It highly difficult to understand and accept that the administration of a single probiotic strain can modulate the composition of all the intestinal microbiota. The variation of certain species has been effectively observed in animal models. Authors have generally extrapolated the effect for all the species of the microbiota. Effects in human are generally not convincing. The Reviewer remains extremely suspicious on this domain.

The reviewer’s observation is true, and we know that there are many studies in animal models but not many human clinical trials that show the effectiveness of probiotics in IBD, that is the reason for which we included in the review a recent reference from Sinagra et al (2013) to mention that the translation of the potential use of probiotic for IBD patients remains uncertain. There are many articles that show the benefits attributed to certain probiotics in IBD and that explain the possible mechanisms of action; however, we only focused in articles that showed the beneficial effect of probiotic in IBD, which was associated to beneficial modifications in the gut microbiota. We understand the point of view of the reviewer, and we know and wrote in the review that more human clinical trial are needed before the medical community can accept the probiotic as alternative (with other therapies) for the IBD treatment or to improve the quality of life of patients with chronic IBD. We personally have worked with probiotics during many years using animal models and now understand some mechanisms by which probiotics can exert their beneficial effect in the host. The modulation of the intestinal microbiota is one of the most important mechanisms reported, which is also associated to other benefits, such as the

modulation of the gut immune response. Since we are not currently performing human clinical trials, in this review we searched for the most recent reports of cases where certain probiotic/s can exert benefits on the human host, focusing (according to the aim of the review) in the modification of the gut microbiota.

9. The objective of a review is to summarize the reports describing a same effect. This is not the case. The review describes study by study the results that are published. A drafting effort is required.

The review was structured to show the importance of the balance of the intestinal microbiota in health. In the first part, after generalizations and definitions, we showed the relation of microbial dysbiosis with the development of diseases, both intestinal and non-intestinal. We introduced every section, described some of the most recent articles in the field and made a conclusion that covers these and other articles that were not included in this specific review.

The same structure was followed for the effect of probiotics, always focusing in the modifications of the intestinal microbiota. We detailed some recent articles that showed the benefits of probiotics at the intestinal level, and following this, we described other non-intestinal pathologies in which the beneficial effect of probiotics were also reported and associated to the beneficial modification of the intestinal microbiota.

10. The Chapter: "EFFECT OF PROBIOTIC ADMINISTRATION ON THE INTESTINAL MICROBIOTA AND DISEASE" is extremely confusing mixing effects at the intestinal and cardiovascular levels, and against obesity and liver disease. Please reorganize for clarity.

This section was reorganized according to the reviewer's suggestion and sub-titles were added to separate it. We divided it into 3 subsections: Effects of probiotics on intestinal diseases, effects on non-intestinal diseases and effects on healthy hosts.

3 References and typesetting were controlled and corrected when necessary according to the guide for authors.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.



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