Author's response to reviews

**Title:** Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-severe protein-energy-malnutrition model in mice

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**Version:** 2 **Date:** 14 February 2011

**Author's response to reviews:** see over
To whom it may concern,

As corresponding author, I’m sending the revised version of the manuscript entitled “Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-severe protein-energy-malnutrition model in mice” for publication in BMC Gastroenterology by Maldonado Galdeano C, Novotny Núñez I, de Moreno de LeBlanc A, Carmuega E, Weill R and Perdigón G. The manuscript was corrected according with the reviewer and editorial suggestions.

In the present cover letter we address the responses (italics) for all two reviewers and the editorial giving a point-by-point response to the concerns.

We hope that the present version of the manuscript fulfill your requirements.

Sincerely,

Gabriela Perdigon

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Reviewer's report
Title: Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-sever protein-energy-malnutrition model in mice
Version: 1 Date: 22 December 2010
Reviewer: HARIOM YADAV

Reviewer's report:
Galdeano and colleagues reported the immunomodulatory effects of probiotic fermented milk in non-severe protein energy-malnourished mice model. Although the study is interesting, but before final acceptance of this article, few improvements must be made:

1. There are abundant typographical and grammatical errors all over the running text, which must be corrected carefully

The text was corrected for typographical and grammatical errors

2. Although these findings are interesting in relation with malnutrition, but probiotics and probiotic containing fermented milk products have shown health-beneficial effects in high-energy fed animal models (i.e. Yadav et al Nutrition. 2007 Jan;23(1):62-8, described this effect), and reviewer wonders how does you explain your findings in reference of high energy induced obesity vs malnourished animal models.

It is true that probiotic and probiotic fermented milks, as the reviewer remark, can exert beneficial effect in high-energy fed animal models. We are also working in that field and we can also observe that in both nutritional problems (obesity and under nutrition), there are alterations of the intestinal barrier and in the immune response. Probiotics and probiotics fermented products can modulate the immune response and the intestinal barrier in both models using experimental animals. We focused our research in these improvements. In this paper, we demonstrated the importance of probiotic fermented milk as adjuvant of the mucosal or systemic immune response in a malnutrition model. The reference Yadav et al was added in the background section (lines 56-57).

3. The gut flora has been closely correlated with obesity in recent era, I am wondering while mice fed with probiotic-fermented milk gained fat mass in comparison to control mice.

In our model, the mice fed with PFM or its supernatant gained body weight compared to malnourished mice, but they reach the body weight of the control animals, no increased the fat mass compared to the well nourished animals. This observation was now clarified in the result section (lines 234-235)

4. Recently Jain et al (J Med Food. 2009 Jun;12(3):576-83) also have reported that probiotic containing fermented milk product improved immunological parameters in normal mice, how does your findings are different than Jain et al findings, please discuss.

Our findings are not different to those reported by Jain et al. (2009). We have also a prevention of Salmonella infection in the group of mice re-nourished with PFM and this finding is related to the improvement of the immune response in these animals. We also
know that PFM administration to well nourished mice (adult and newborn) had a protective effect against Salmonella infection and this effect was related to different immune responses in the animals fed with or without probiotics (de Moreno de LeBlanc et al., 2011). We also discuss these observations and the data reported by Jain et al. in the new version of the manuscript (lines 464-475).

Minor points:
1. Please replace ‘sever’ with ‘severe’ in title
2. Abstract: In 17-21; please complete the sentence
3. Background: In 34-37; complete/correct the sentence
4. In 46; insert ‘known’ by replacing ‘know’
5. In 56; define what is ‘PFM’, or Is that PEM and PFM is the same, if it is than keep one consistent in whole text
6. Methods: In 74, replace ‘experience’ with ‘experiments’ and other places too in the text
7. In 89; correct temperature

All the minor points were corrected in the new version of the manuscript

**Reviewer's report**
**Title:** Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-sever protein-energy-malnutrition model in mice
**Version:** 1 **Date:** 26 December 2010
**Reviewer:** Federico Lara-Viloslada

**Reviewer's report:**
Dear Editor,

First of all, I would like to apologize for the delay in sending my report.
The manuscript by Maldonado Galdeano et al. deals with an interesting topic as is the effects of probiotics on immunity and the intestinal recovery after a malnutrition period.
The work done by the authors is very width containing up to three experiments in the present manuscript, which it is really valuable but it also makes more difficult the understanding of the work and the interpretation of the results.
I think that the manuscript is suitable for publication, although I would suggest some changes to make it easier to understand and to help the reader to focus on the most important information.

I would suggest the following minor essential revisions:

1. Abstract: the three different experiments should be included, I mean, the general recovery experiment, the OVA assay and also the Salmonella experiment. In addition the results should be described with more specific details rather than with general terms such as ‘function of certain immune cells’, systemic immune response’ or innate immune response’. Finally, the item conclusions contains results rather than conclusions and I would suggest to include one or two conclusions of the work.

*The abstract was improved according to the reviewer suggestion.*
2. Methodology:
- the number of mice used for each experiment should be clearly stated in each item and not only in the section of 'statistical analysis'.

*The number of mice used for each experiment was added in the corrected version of the manuscript.* (line 71, 73, 78, 9, 100-10, 11, 213, 215).

- the design of the OVA experiment is not very well described and the objective of giving the supplement diets prior and after the injection is not well justified.

*The OVA experiment was re-written and the justification of the different groups was added.*

3. Results:
- as a consequence of the huge work done by the authors there are a lot of results and they are poorly described in the text. For example, a complicated figure such as figure 4 is only accompanied with a paragraph in the text (lines 294-298). I would suggest one of two, simplify the figures or explain the results more in detail.

*The results of the figure 4 are explained in more detail in the corrected version of the manuscript.*

- Line 313: is too speculative since there are not statistically significant differences.

*The sentence was corrected and the word “significantly” was added* (line 3299 because there are significant differences between mice re-nourished with PFM and malnourished control mice. This was observed in liver, spleen and large intestine and re-nutrition with PFM diminished the translocation to spleen and the count of Salmonella in large intestine to values similar to those observed in well nourished animals. In the liver, the re-nutrition with PFM diminished significantly the translocation of Salmonella even compared to the well-nourished mice.

- Figure 2C: the number of lactobacilli is significantly higher in the malnourished mice. This result should be mentioned and discussed.

*This result is now mentioned in the result section (LINES 240-242) and also discussed in the discussion section (lines 358-365).*

4. Discussion:
- the results from the different experiments are discussed individually and they are not connected throughout the discussion. In my opinion the discussion would be richer if the results from the different experiments were connected with each other. It does not mean to make the discussion longer, since I think that there are paragraphs that could be deleted or resumed, such as lines 392-403.
Discussion was improved according to the reviewer suggestion

Finally I have found some typographycal errors:
- Line 35: 'nutrient' should be changed by 'nutrients'
- Line 46: 'know' should be changed by 'known'
- Line 56: 'evaluate' should be changed by 'to evaluate'
- Line 108: 'isolates' should be changed by 'isolated'
- Line 205: 'pot' should be changed by 'post'
- Line 245: 'in same' should be changed bu 'in the same'
- Line 323: an space between 5 and days is lacking.
- Line 338: 'of' should be changed by 'from'
- Line 427: 'high even' should be changed by 'even high'

All the typographical errors were corrected in the new version of the manuscript

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I work for a company that could be competitor of the company supporting the present work, but I declare that this has not influenced my review

Editorial Requests:
a) We recommend that you ask a native English speaking colleague to help you copyedit the paper.

The text was corrected for typographical and grammatical errors and an English speaker helped to correct the manuscript.

b) For research carried out on animals we encourage all authors to comply with the "Animal Research: Reporting In Vivo Experiments" (ARRIVE) guidelines. Relevant information should be included in the appropriate section of the article as outlined in the guidelines, paying particular attention to the Methods section.

This information was added (lines 95-97)

c) Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.
Competing interests section was added (lines 495-500)

d) Please review our criteria for authorship, as written in your contributions section, author RW does not fulfil the criteria.
It was a mistake and it was corrected. RW also conceived the study.