ANSWERS TO REVIEWERS

April 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8493-edited.docx).

Title: GUT-LIVER AXIS AND PROBIOTICS: THEIR ROLE IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Author: Giulia Paolella, Claudia Mandato, Luca Pierri, Marco Poeta, Pietro Vajro

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8493

The manuscript has been modified according to the suggestions of reviewers:
1 Format has been updated
2 Revision has been made according to the suggestions of the reviewers

Reviewer 1

The authors failed to discuss thoroughly the issue of probiotic delivery and mucosal transport. This holds of great relevance when considering the newest technological proposals in the field of metabolic syndrome-related issues. Overall, this work adds little to other reviews on the subject.

Authors' answer: We thank the reviewer for the suggestion.

1. We have thoroughly updated the MS by adding the newest reports on most issues, in order to add more recent data *vs.* existing reviews on the subject (see yellow highlighted parts)

2. Regarding your specific request:
a. we have considered the probiotic delivery issue (page 8 lanes 249-260).
b. we have discussed newer microbiology techniques and given recent references for the traffic control of bacterial-derived molecules (ref. 79) in the host-bacterial cross-talk (page 11; lanes 347-352)

Reviewer 2

References and typesetting have been corrected and re-numbered (green highlighted)

Q1. The manuscript contains many inclusion boxes: "Commentaire" showing that the submitted document is not the final version but probably an "intermediate" version. This is not very professional.

A1. We apologize for the inconvenience due to a mismatch of software. The inclusion boxes have been eliminated

Q2. Title, modify as: "Gut-liver axis and probiotics: their role in non-alcoholic fatty liver disease (NAFLD)

A2. The title has been modified as requested by reviewer.

Q3. The part of the review Pages 1 to 6 is extremely difficult to read and not easily understandable since too fractionated in sub-sub-sub paragraphs. Please reorganize in a comprehensive fashion.

A3. We have reorganized the first part of the review (page 1-6) as requested.

Q4. The section "Intestinal barrier" is not very well written and contains too many approximations and omissions. Entirely revise.

A4. We have modified and revised the section "intestinal barrier" in the "gut-liver axis components section" (lanes 101-142, page 4-5)

Q5. Authors surprisingly give old or inappropriate references concerning the tight junctions. The reference 10 is particularly inappropriate: Farquhar MG, Palade GE. Junctional complexes in various epithelia. J Cell Biol. 1963;17:375-412; Balda MS, González-Mariscal L, Contreras RG, et al. Assembly and sealing of tight junctions: possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. J Membr Biol. 1991;122:193-202; Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev 2011;91:151-75 Replace by Turner JR. 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9:799-80 and Marchiando AM, Graham WV, Turner JR. 2010. Epithelial barriers in homeostasis and disease. Annu. Rev. Pathol. 5:119-144.

A5. We apologize for the old or inappropriate references. We have replaced the mentioned references as requested.

Q6. In line 124 insert the reference: Lievin-Le Moal V, Servin AL. 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin. Microbiol. Rev. 19:315-337.

A6. We thank the reviewer for his suggestion. We added the reference in the manuscript.

Q7. The description of tight junction in Lines 128-134 is not complete. Revise.

A7. We revised the description of tight junction in the manuscript (page 4, lanes 106-124)

Q8. Line 134, delete "PKC has also been implicated in a sensing pathway (TLR2)."

A8. We deleted the mentioned sentence.

Q9. Replace reference 12 by Kumar H, Kawai T, Akira S. 2011. Pathogen recognition by the innate immune system. Int Rev Immunol 30:16-34 and Kinnebrew MA, Pamer EG. 2012. Innate immune signaling in defense against intestinal microbes. Immunol Rev 245:113-131.

A9. We have replaced the reference as requested.

Q10. Replace reference 13 by Bevins CL, Salzman NH. 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 9:356-368.

A10. We have replaced the reference as requested.

Q11. Line 152, replace reference 4 by Littman DR, Pamer EG. 2011. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 10:311-323

A11. We have replaced the reference as requested.

Q12. For dysbiosis, include Littman DR, Pamer EG. 2011. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 10:311-323 and Stecher B, Maier L, Hardt WD. 2013. 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. Nat. Rev. Microbiol. 11:277-284.

A12. We have also included the two reference as requested.

Q13. In paragraphs line 155-160 and 161-166 include reference : Lievin-Le Moal V, Servin AL. 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin. Microbiol. Rev. 19:315-337

A13. We have also included the two reference as requested.

Q14. Since the Review focuses on the modulation of intestinal microbiota by probiotics it is surprising and very disappointing that authors shortened the description of intestinal microbiota as: "The commensal gut microbiota

A14. We thank the reviewer for the suggestion.
We have revised the description of gut microbiota on page 5 (lanes 147-154; 159-166)

We thank you for you kind and valuable assistance.

Sincerely yours,

Pietro VAJRO

Pietro Vajro, MD
Chair of Pediatrics
Dept of Medicine and Surgery
University of Salerno
Via Allende
84081 Baronissi (Salerno) - Italy
Tel +39.089.672409 cell. +39.339.236.1008
Fax:+39.089.672409
E-mail: pvajro@unisa.it