

Response to Reviewers

1. **Reviewer's comment:** ABSTRACT: I would suggest more details in the abstract, regarding Methods (number of participants, explanation about pre-prandial and post-prandial groups, doses of the two probiotics).

Authors' response: We followed Reviewer's recommendations and we are very grateful for these suggestions. Now more information about pre- and -post prandial groups, as well as probiotic doses have been added in the Abstract ("METHODS" section).

2. **Reviewer's comment:** INTRODUCTION 1. Definition of probiotics – not correct. The recent consensus statement reference should be used: "Hill C, Guarner F, Reid G, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506-514". According to it, probiotics are "NON-PATHOGENIC live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host" The used reference is from 2001 and it was NOT included in the reference list.

Authors' response: Many thanks to Reviewer for his observation. We have carefully read the article cited by Reviewer and we have now changed "definition of probiotics" in the manuscript by uploading the reviewer's suggestion. Accordingly, references were updated as suggested by Reviewer.

3. **Reviewer's comment:** INTRODUCTION 2. Instead of mentioning a very old reference (Kaur - nr. 3 – from 2002): many recent papers are available, written by experts in the field, regarding the use of probiotics in preventing and/or treating some diseases – like "Floch MH. Recommendations for probiotic use in humans—a 2014 update. Pharmaceuticals. 2014;7:999-1007". Or "Floch MH, Walker WA, Sanders ME, Nieuwdorp M, Kim AS, Brenner DA, Qamar AA, Miloh TA, Guarino A, Guslandi M, Dieleman LA, Ringel Y,

Quigley EM, Brandt LJ. Recommendations for Probiotic Use - 2015 Update: Proceedings and Consensus Opinion. J Clin Gastroenterol. 2015 Nov-Dec;49 Suppl 1:S69-73."

Authors' response: We have appreciated the comment by the reviewer. Following Reviewer's suggestion, reference 3 has now been replaced by a recent paper of Floch MH (Floch MH. Recommendations for probiotic use in humans—a 2014 update. Pharmaceuticals. 2014;7:999-1007).

4. **Reviewer's comment:** INTRODUCTION 3. Reference nr. 6 is old. Many recent reviews are available about safety. Like "Doron S, Snyderman DR. Risk and safety of probiotics. Clin Infect Dis. 2015 May 15;60 Suppl 2:S129-34".

Authors' response: We definitively agree with this suggestion. Now, reference 6 has been replaced in the manuscript by a recent paper about safety of probiotics.

5. **Reviewer's comment:** INTRODUCTION 4. I think the sentence "Nowadays, hundreds of different bacterial strains are available in the global probiotic market and consequently, the choice of the most suitable probiotic product becomes very complex and dispersive." should be rephrased. A choice cannot be very complex, but maybe difficult.

Authors' response: The aforementioned sentence has now been changed in the manuscript as suggested by Reviewer.

6. **Reviewer's comment:** INTRODUCTION 5. The sentence "Alone or in combination, these microorganisms exhibit several healthy beneficial properties, such as antagonistic activities against microbial pathogens and enhancement of the anti-inflammatory response [8,9]." should be inserted after the one mentioning the beneficial effects of probiotics.
- INTRODUCTION 6. The sentence "Moreover, they are often associated with alleviation of lactose intolerance [10], improvement of bacterial, antibiotic or radiotherapy induced diarrhea [11-13], anti-carcinogenic effects [14] and even blood cholesterol reduction [15]."

should be inserted after mentioning about probiotic use in preventing/treating some diseases.

Authors' response: Many thanks to the Reviewer for his suggestion. These parts have now been modified and rephrased in the introduction session accordingly.

Reviewer's comment: INTRODUCTION 7. The sentence "Even if in literature there is few and contentious information about the ability of probiotics to modulate the host's gut microbiota composition and maintaining the intestinal homeostasis, these latter have been proposed as one of the main mechanisms by which probiotics exert their beneficial effects [16]." is too long and confusing.

Authors' response: We have followed Reviewer's suggestion; the aforementioned sentence has now been rephrased and clarified in the manuscript.

7. **Reviewer's comment:** In short, "INTRODUCTION" paragraph is too long, some data are redundant, some sentences are too long and confusing. I suggest shortening and reviewing the full paragraph. Also, not all the studies described in detail the "INTRODUCTION" are really needed there. On the contrary, the authors could (maybe) mention more published studies that used *L. rhamnosus* HN001 and *B. longum* BB536.

Authors' response: Many thanks to Reviewer for his suggestions that we followed very carefully. For this reason, we shortened Introduction, adding more specific references about *L. rhamnosus* HN001 and *B. longum* BB536 strains characteristics.

8. **Reviewer's comment:** MATERIALS AND METHODS: "During the month of probiotic consumption, some problems of product resuspension have been highlighted." – please explain.

Authors' comment: Thanks again for this additional request. During the probiotic resuspension and before oral intake, some volunteers described the formation of lumps inside the solution. This part has now been clarified in the manuscript.

9. Reviewer's comment: DISCUSSION 1. The auth

Authors' response: Unfortunately, it seems that Reviewer's comment was incomplete, consequently we could not revise this part of manuscript.