Author’s response to reviews

Title: Synbiotic therapy decreases microbial translocation and inflammation and improves immunological status in HIV-infected patients: a double-blind randomized controlled pilot trial

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Author’s response to reviews: see over
Title: Synbiotic therapy decreases microbial translocation and inflammation and improves immunological status in HIV-infected patients: a double-blind randomized controlled pilot trial

Date: September 19, 2012.

Reviewer: Shunji Fujimori

Points:

1) It is no wonder that total bacterial load in faces are increased in prebiotic group. If total bacterial load in faces are increased in also probiotic and synbiotic group, there are no wonder. The results of total bacterial load in faces mean a little. So, Figure 3 is unnecessary. And, P=0.05 should be described such as P=0.052 or P=0.048
   In page: 8, line: 4-5. We decided to delete the phrase “Unexpectedly” and the figure 3, and add the p value:
   “Additionally, the probiotic and synbiotic groups demonstrated a decrease in total bacterial load in feces (p = 0.045 and 0.048, respectively). The prebiotic group showed a tendency toward higher bacterial load”.

2) The results of decreasing total bacterial load in faces in probiotic and symbiotic groups, should be discussed.
   Theses results are now discussed briefly (page: 9, line 22-25, and page: 10, line: 1-2)

3) Meanwhile, the results of increasing Bifidobacterium and decreasing Clostridium are very important. The data of changing these bacteria should be described in exact detail using a Figure.
   We add the figure 3 with these data.

4) Figure 5 should be included all four groups of IL-6 cytokine level.
   We change the figure 5, and add the IL-6 cytokine levels in all groups.

5) Detailed contents of placebo should be stated
   The placebo was described, was a product of Biogel without the two types of probiotics and without the prebiotic (fiber), but with the same flavor and characteristics, and was made by biotechnology company called Biotek in Guadalajara city (page: 4, lines: 2-10).

6) Introduction line 2, Ca. should be spelled out
   We change the phrase “Ca.”, by “Approximately” (Page: 1, line 3).

7) P 15 line 14, ART should be spelled out
   We made a correction; “ART” is “ARV” (antiretroviral). Page 10, line: 11.
Title: Synbiotic therapy decreases microbial translocation and inflammation and improves immunological status in HIV-infected patients: a double-blind randomized controlled pilot trial

Date: September 19, 2012.

Reviewer: Kingsley Chidozie Anukam

Points:

1) **The authors should explain the rationale for using synbiotic 2000.**

   In this study we did not use the synbiotic 2000. Our Synbiotic, Probiotics and Prebiotic were made by biotechnology company called Biotek in Guadalajara city in Mexico (page: 4, line 2-10).

   We decide to use this synbiotic because we wanted to take advantage of the inulin from the agave pines of Agave tequilana Weber var. azul that are widely employed to produce the national drink, tequila, and could also be a potential source of prebiotics and part of synbiotic with accessible prices (page 10, lines 15-17).

2) **The authors should provide evidence showing the 4 bacterial strains contained in the synbiotic are proven probiotics?**

   As probiotics, we employed just 2 bacterial strains: *Lactobacillus rhamnosus* HN001 plus *Bifidobacterium lactis* Bi-07 at $10^9$ cfu/mL (page: 4, lines 7-8). The concentration was confirmed by culture, in the company that manufactured them (Biotek).

3) **The authors claimed to have used standard curve for each of the four lactobacilli strains? But only 2 lactobacillus species are present in the synbiotic 2000. (Lactobacillus paracasei subsp paracasei 19 and Lactobacillus plantarum).**

   A standard curve was created from serial dilutions of plasmid DNA containing known copy numbers of the template. The count was absolute using this standard curve with dilutions of concentrations known: 30 copies/µL, 300 copies/µL, 3,000 copies/µL, 30,000 copies/µL, 300,000 copies/µL, 3,000,000 copies/µL, 30,000,000 copies/µL of plasmid pGEM®-T vector from Promega Systems. So, we did not use curves for each strains.

4) **The authors should also explain why they used four lactobacilli 16S rRNA gene sequences, They should separate the lactobacilli from Leuconostoc mesenteroides and Pediococcus pentosaceus.**

   Universal primers were used to amplify DNA templates encoding 16S rRNA. We test them with several bacterial DNA (see the next figure):
To detect changes in stool bacterial composition, we measured the bacterial loads, dividing as beneficial bacteria the *Bifidobacterium spp.* Load, and as harmful bacteria, the *Clostridium spp.* Load. We did not use primers to quantify lactobacilli (as beneficial bacteria), we just used primer to detect *Bifidobacterium spp.* and primers were the following: forward 5’-CGC GTC YGG TGT GAA AG-3’ and reverse 5’-CCC CAC ATC CAG CAT CCA-3’. Page 6, lines 18-20. See the next figure:

1. MPM
2. *Bifidobacterium adolescentis*
3. *Clostridium difficile*
4. *Bacteroides fragilis*
5. *Lactobacillus acidophilus*
6. *E. coli* TOP10
7. Negative control