October 21, 2017

Re: “Fructo-oligosaccharide intensifies stress-induced visceral hypersensitivity and intestinal inflammation in IBS mouse model” (Manuscript ID-36331)

Dear Editor of World Journal of Gastroenterology:

Thank you very much for your email of October 19, 2017 regarding this manuscript. We have revised the manuscript to address the comments of the reviewers. Our point-by-point responses are listed below. In the revised manuscript we have highlighted the changes that were made from the original manuscript by using the track changes mode.

We eagerly look forward to your response.

Sincerely,

Ning Dai MD, PhD

**Reviewer 1**

1. **In material and methods I suggest that the number of rats studied in each group be emphasized.**

   We randomly assigned 32 mice into four groups of eight mice (sham-WAS+saline administration, sham-WAS+FOS administration,

WAS+saline administration, WAS+FOS administration). Prior to the outcome analysis at 14 days, five mice (sham-WAS+saline administration group in one, sham-WAS+FOS administration in one, WAS+saline administration group in two, WAS+FOS administration group in one) died due to gavage trauma and were excluded from the outcome analysis

We have now provided the number of mice randomized to the four groups in the Methods section. We also provided the number of mice included in the outcome analysis for each group in the Results section and Figure legends.

“To evaluate the effects of FOS on WAS induced-visceral hypersensitivity and intestinal inflammation, thirty-two mice were randomly assigned into four groups of eight mice (sham-WAS+saline administration, sham-WAS+FOS administration, WAS+saline administration, and WAS+FOS administration).”
(Page 6, line 28)

“Of the 32 randomized mice, five (sham-WAS+saline group in one, sham-WAS+FOS group in one, WAS+saline group in two, WAS+FOS group in one) died due to gavage trauma and were excluded from the outcome analysis.”
(Page 10, line 3)

“Figure 1. Effects of water avoidance stress (WAS) on rate of weight gain and visceral sensitivity. (A) Rate of weight gain (g) was lower in WAS (n=13) compared to the sham-WAS (n=14) group.”
(Page 23, line 2)

“*P<0.05; sham+saline (n=7), sham+FOS (n=7), WAS+saline (n=6),

WAS+FOS (n=7); Kruskal–Wallis one-way ANOVA."
(Figure 2, Page 24, line 6)

"Values represent median with 5th and 95th percentiles; sham+saline (n=7), sham+FOS (n=7), WAS+saline (n=6), WAS+FOS (n=7); Kruskal–Wallis one-way ANOVA."
(Figure 3, Page 25, line 7)

"Values represent mean±SD, *P<0.05, NS P>0.05; sham+saline (n=7), sham+FOS (n=7), WAS+saline (n=6), WAS+FOS (n=7); one-way ANOVA."
(Figure 4, Page 26, line 7)

"Values represent mean±SD; *P<0.05, NS P>0.05; sham+saline (n=7), sham+FOS (n=7), WAS+saline (n=6), WAS+FOS (n=7); one-way ANOVA."
(Figure 5, Page 27, line 2)

"Values represent mean±SD; *P <0.05, NS P>0.05; sham+saline (n=7), sham+FOS (n=7), WAS+saline (n=6), WAS+FOS (n=7); one-way ANOVA."
(Figure 6, Page 27, line 14)

2. **In the discussion I suggest the inclusion of the results obtained by our research group in Brazil, where we highlight the importance of nonspecific food intolerance triggering symptoms in patients with IBS and the difficulty of correlation between symptoms and a single specific food group such as fructo-oligosaccharide. Soares, R. L., Figueiredo, H. N., Santos, J. M., Oliveira, R. F., Godoy, R. L., & Mendonça, F. A. (2008). Discrepancies between the responses to skin prick test to food and respiratory antigens in two subtypes of patients with irritable bowel syndrome. World Journal of Gastroenterology : WJG, 14(19), 3044–3048. http://doi.org/10.3748/wjg.14.3044 and**

**Soares, R.L.S., Figueiredo, H.N., Maneschy, C.P., Rocha, V.R.S., & Santos, J.M. (2004). Correlation between symptoms of the irritable bowel syndrome and the response to the food extract skin prick test. Brazilian Journal of Medical and Biological Research, 37(5), 659-662. https://dx.doi.org/10.1590/S0100-879X2004000500005**

We have now referenced previous studies that highlight the role of food intolerance triggering IBS symptoms despite difficulty demonstrating specific correlation with a single food group in the discussion section.

“Although the role of food intolerance-induced IBS symptoms have been long recognized, correlations with a specific food group have been difficult to demonstrate [21, 22]. A key observation in our study is that FOS consumption further intensified visceral hypersensitivity already present in mice subjected to WAS.”
(Page 13, line 11)

3. **In conclusion, it is necessary to emphasize the difficulties of constructing an experimental model of IBS. IBS has biopsychosocial components and experimental models do not contemplate these aspects, which makes it difficult to interpret the results.**

We have now acknowledged the limitations of constructing an experimental model that is able to encompass the full complex biopsychosocial components of IBS in the Discussion section.

“Finally, although the WAS-induced mouse model exhibited visceral hypersensitivity and low-grade inflammation, experimental models are not able to fully encompass the complex biopsychosocial components of IBS, and our findings should be interpreted with caution.”

(Page 16, line 15)

**4. I suggest a general review of the language.**

We have made minor grammatical revisions, especially the Comments section, to improve the clarity of the manuscript.

**Reviewer 2**

**1. The article is informative and well-presented. The reviewer has no comments.**

**References**


1. Soares RL, Figueiredo HN, Santos JM, Oliveira RF, Godoy RL, Mendonca FA. Discrepancies between the responses to skin prick test to food and respiratory antigens in two subtypes of patients with irritable bowel syndrome. World Journal of Gastroenterology 2008; 14(19): 3044-3048 [PMID: 18494056]

2. Soares RL, Figueiredo HN, Maneschy CP, Rocha VR, Santos JM. Correlation between symptoms of the irritable bowel syndrome and the response to the food extract skin prick test. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 2004; 37 (5): 659-662 [PMID: 15107926]