

## **Author's response to reviews**

**Title:** Role of serotonin on the intestinal mucosal immune response to stress-induced diarrhea in weaning mice

### **Authors:**

Yulan Dong (ylbcdong@cau.edu.cn)

Yanan Han (34178062@qq.com)

Zixu Wang (zxwang2007@163.com)

Zhuoming Qin (qinzm1997@163.com)

Chenyu Yang (945373007@qq.com)

Jing Cao (caojing315@126.com)

Yaoxing Chen (yxchen@cau.edu.cn)

**Version: 1 Date:** 04 May 2016

### **Author's response to reviews:**

#### COVER LETTER

Dear Editor and Reviewers:

I'm very glad to hear from you, and thank you for your works in dealing with my paper (BMGE-D-16-00135), and give us a potentially acceptable for publication in BMC Gastroenterology. All of those comments are valuable and very helpful for improving the quality of our paper, as well as the important guiding to our research. We have carefully checked the manuscript and revised it according to the suggestions [red font color represented the revised text in revised manuscript (R2)]. Now, I'm submitting here with a reply of queries.

Sincerely yours,

Prof. Yaoxing CHEN, Ph.D.

Laboratory of Anatomy of Domestic Animal,

College of Animal Medicine, China Agricultural University

Haidian, Beijing, 100193, China

E-mail: yxchen@cau.edu.cn

Reviewer #1: The authors have revised the manuscript according to the reviewer's comments on the first submission and improved the manuscript greatly. However, the following points should be concerned by the authors before publication.

QUESTION 1. The tense used in "statistical analysis" on Page 8 is not consistent.

ANSWER: Thank you very much.  $P \approx 0.000$  was represented as  $P < 0.001$ .

QUESTION 2. What's the unit of vertical axis in figure 3B and 3C?

ANSWER: Thank you very much. The B lymphocytes proliferation data of vertical axis in figure 3B is a ratio of Stimulation index (SI) = OD570 (stimulated cells)/OD570 (unstimulated cells), so there is no unit. The unit of vertical axis in figure 3C is  $\mu\text{g}/\text{mL}$ , and it had been revised in Fig. 3C.

QUESTION 3. The font and size of subtitles in the manuscript should be consistent.

ANSWER: Thank you very much. They had been consistent.

QUESTION 4. The expression of CD3+CD4+ and CD3+CD8+ should be consistent in the manuscript. Sometimes they are expressed as CD3+CD4+ and CD3+CD8+.

ANSWER: Thank you very much. They had been consistent as CD3+CD4+ and CD3+CD8+.

QUESTION 5. In figures, the font and size of labels for "A", "B" and "C" are not consistent.

ANSWER: Thank you very much. The figures were consistent.

QUESTION 6. The authors have described 204 mice used in this study. But they didn't describe how many mice were used in each group.

ANSWER: Thank you very much. The total of 204 was separated into 3 groups (n=68).

QUESTION 7. In figure 4A, did the authors make sure the difference among groups were significant? It looks like no significant differences among groups according to the figure.

ANSWER: Thank you very much. One-Way ANOVA with a LSD post-hoc test was performed to statistical analyses. It was true that there were significant difference among groups. Detailed as: In Figure 4A, T lymphocyte proliferation is very significant difference in duodenum between control and stress diarrhea ( $P=0.000$ ), but  $p=0.266$  between control and PCPA+stress diarrhea, and  $p=0.000$  between stress and PCPA+stress diarrhea. In jejunum, it is very significant difference between control and stress diarrhea ( $P=0.006$ ), and it is not different between control and PCPA+stress diarrhea ( $P=0.091$ ), and  $p=0.535$  between stress and PCPA+stress diarrhea. In ileum, there is no difference. In colon, it is very significant difference between control and stress diarrhea ( $P=0.009$ ), and it is not different between control and PCPA+stress diarrhea ( $P=0.533$ ), and  $p=0.015$  between stress and PCPA+stress diarrhea.

QUESTION 8. In discussion, there is no need to use subtitles.

ANSWER: Thank you very much. Subtitles had been cancelled.

Reviewer #3: The manuscript entitled "Role of serotonin on the intestinal mucosal immune response to stress-induced diarrhea in weaning mice" by Dong et al, the authors aimed to demonstrate the effect of serotonin (which is also known as 5-hydroxytryptamine (5-HT)) on the development of diarrhea. To explore this study, the authors performed stress induced diarrhea in weaning mice model. Three groups of weaning mice were used to perform the experiments in this study. Those mice were (a) control group, (b), stress induced diarrhea group (by performing intragastric administration of folium sennae) and (c) the group pretreated with para-

chlorophenylalanine (PCPA) following stress-induced diarrhea group. To determine the role of 5-HT in stress induced diarrhea, the authors performed measurement of different biochemical markers in plasma such as blood glucose level, cortisol level and 5-HT level as well in three groups of weaning mice. The authors also measured the levels of IEL, B lymphocytes and sIgA in different part of small intestine and ileum of those three groups of mice. The authors also measured T cell markers (T lymphocytes proliferation, CD3, CD4 etc) as well as cytokines (such as TNF- $\alpha$ , IL-2, IL-4, and IL-10) in the small intestine and ileum of those three different groups of mice. This work is very interesting and well done. This resubmitted manuscript is well described and well written. The authors nicely addressed each concerns made by the reviewer. Just the following minor concerns are needed to be addressed.

Minor Correction:

QUESTION 1. In the methods section, there is a sentence, "The first group of mice was subjected to stress-induced diarrhea by treating the mice with folium sennae (0.4g/mL, 15 mL/Kg body weight) via intragastric administration at 2:00 PM after intraperitoneal injection of sterile saline at 10:00AM. The second group was the PCPA+stress-induced diarrhea group, which was treated by intraperitoneal injection of sterile saline containing PCPA (30mg/mL, 300mg/kg body weight) at 10:00AM followed by the intragastric administration of folium sennae (0.4g/mL, 15 mL/Kg body weight) at 2:00 PM" in here no need to mention the time.

It is better to write the sentence like as "The first group of mice was subjected to stress-induced diarrhea by treating the mice with folium sennae (0.4g/mL, 15 mL/Kg body weight) via intragastric administration 4 hours after intraperitoneal injection of sterile saline. Similarly, the second group was the PCPA+stress-induced diarrhea group, which was treated by intraperitoneal injection of sterile saline containing PCPA (30mg/mL, 300mg/kg body weight) followed by the intragastric administration of folium sennae (0.4g/mL, 15 mL/Kg body weight)."

ANSWER: Thank you very much. It had been revised that: The first group of mice was subjected to stress-induced diarrhea by treating the mice with folium sennae (0.4 g/mL, 15 mL/kg body weight) via intragastric administration 4 hours after intraperitoneal injection of sterile saline. The second group was the PCPA+stress-induced diarrhea group, which was treated by intraperitoneal injection of sterile saline containing PCPA (30 mg/mL, 300 mg/kg body weight) followed by the intragastric administration of folium sennae (0.4 g/mL, 15 mL/kg body weight).

## Editorial Requests

QUESTION 1.Ethics: If your study involves humans, human data or animals, then your article should contain an ethics statement which includes the name of the committee that approved your study.

If ethics was not required for your study, then this should be clearly stated and a rationale provided.

ANSWER: All animal procedures were approved by the China Agricultural University Institutional Animal Care and Use Committee (XK20110915).

QUESTION 2. Authors Contributions: Your 'Authors Contributions' section must detail the individual contribution for each individual author listed on your manuscript.

ANSWER: Authors' contributions YL DONG designed the study project, analyzed data, and created the manuscript; YN HAN acquired data, analyzed data; ZX WANG made manuscript drafting and gave final approval for the draft; ZM QIN revised it critically for important intellectual content and made important modifications to the manuscript; CY YANG made manuscript drafting; J CAO revised the study design; YX CHEN made important contributions to the manuscript and given final approval of the version to be published. All authors read and approved the final version of the manuscript.