ANSWERING REVIEWERS

January 19, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7529-review.doc).

Title: Protective effect of glutamine on intestinal injury and bacterial community in rats exposed to hypobaric hypoxia environment

Author: Chunlan Xu, Rui Sun, Xiangjin Qiao, Cuicui Xu, Xiaoya Shang, Weining Niu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7529

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) List of correction made with the comments of reviewer No.00068418

Page 5 Line 143: changed "group C" to "Group control"

Page 8 Line 214-245: changed original content to "Genomic DNA in cecal contents were extracted using E.N.Z.A. ® DNA Kit (Omega Bio-Tek) according to the manufacture's protocol with slight modification, then identified by 1% agarose gel electrophoresis. DNA purity and concentration were analyzed by ultraviolet spectrophotometer (HITACHI, Japan). According to the specific sequence region (533R-27F) in the 16S rRNA gene that covering the V1–V3 region, the bar-coded primers 27F and 533R containing the A and B sequencing adaptors were synthesized and used to amplify this region. The forward primer (B-27F) was 5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG-3', where the sequence of the B adaptor is shown in italics and underlined. The reverse primer (A-533R) was 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG NNNNNNNNNNTTACCGCGGCTGCTGGCAC-3', where the sequence of the A adaptor is shown in italics and underlined and the Ns represent an eight-base sample specific barcode sequence. The identified DNA was subjected to polymerase chain reaction (PCR) using TranStartFastpfu DNA Polymerase (MBI. Fermentas, USA) in a 20 μL volume containing 5 mM each of the primer, 10 ng of

template DNA, and 5×FastPfu Buffer, 1 U of FastPfu DNA Polymerase. PCR was performed in a thermocycler (Gene Amp® PCR System 9700, ABI, USA). The PCR profile included denaturation at 95 ℃ for 2 min, followed by 25 cycles of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 72 ℃ for 30 s, and a final extension at 72 ℃ for 5 min. Triplicate PCR products of the same sample were mixed, and then detected by 2% agarose gels electrophoresis containing ethidium bromide. PCR products were recycled and purified with a AxyPreDNA gel extraction kit (Axygen, China) according to the manufacture's instruction. The recycled PCR products were visualized on agarose gels. Furthermore, the PCR products were quantitatively determined using QuantiFluorTM-ST Fluoremeter (Promega, USA) and PicoGreen® dsDNA Quantitation Reagent (Invitrogen, Germany) Following quantitation, the amplification from each reaction mixture were pooled in equimolar ratios based on concentration and subjected to emulsion PCR (emPCR) using RocheGS FLX Titanium emPCR kits to generate amplification libraries. Amplification pyrosequencing was performed from the A-end using a 454/Roche A sequencing primer kit on a Roche Genome Sequencer GS FLX Titanium platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China."

Page 9 Line 250-261: changed original content to "The pyrosequencing data were subjected to bioinformatics analysis. Prior to analyze, the original pyrosequencing data must be filtered and optimized to obtain the valid and trimed sequences through Seqcln and Mothur(http://sourceforge.net/projects/seqclean/&http://www.mothur.org/wiki/Main_Page). Then, these trimed sequences were analyzed from two aspects : operational taxonomic units (OTUs) cluster (97% similarity) and Taxonomy which mainly performed on Mothur (http://www.mothur.org) and compared with the Bacterial SILVA database (http://www.arb-silva.de/), and by methods of kmer searching (http://www.mothur.org/wiki/Align.seqs) and UCHIME (http://drive5.com/uchime). Rarefaction analysis and Good's coverage for the nine libraries were determined. Community figure was generated using R tools according to the data from document "tax.phylum.xls". Heatmap figure were generated using Vegan-package (distance measure: Bray-Curtis; cluster analysis : complete). "

Page 10 Line 281 : changed "Figure 2A" to "Figure 2"

Line 290: deleted "(Figure 2B)"

Page 2 line 32: changed "Gln" to "glutamine"

Line 35: changed "Gln" to "glutamine (Gln)"

Line 43: changed "TLR4" to "Toll-like receptor 4 (TLR4)"; changed "NF-κB p65" to nuclear factor- κB p65 (NF-κB p65); changed "MyD88" to "myeloid differentiation factor 88 (MyD88)"

Page 6 Line 165: changed "% bw" to "percentage of body weight"

Line 169: changed "PBS" to phosphate buffered solution (PBS)

Page 7 line 185: changed "ELISA" to enzyme-linked immunosorbent assay (ELISA) and deleted "(DuoSet ELISA development system, R&D systems, USA)"

Page 8 line 228: changed "PCRs" to "polymerase chain reactions (PCRs)"

Page 9 Line 257: changed "OTUs" to "operational taxonomic units (OTUs)"

Page 16 Line 460: changed "MAPK kinase" to "mitogen-activated protein kinase (MAPK)"

Line 461: changed "TIR" to "Toll/IL-1 receptor (TIR)"

(2) List of correction made with the comments of reviewer No.00033010

- All abbreviations that appear in the manuscript have been give the full spelling at the first mention. (See the revised manuscript or the list of List of correction made with the comments of reviewer No.00068418)
- Glutamine is synthesized by the enzyme glutamine synthetase from glutamate and ammonia. In catabolic states of injury and illness, glutamine becomes conditionally essential amino acid that need to be supplemented. Because in these severe physiologic stress conditions such as trauma, infection, fatigue and so on, endogenous synthesis glutamine was soon exhausted, which caused glutamine deficiency.
- The suggestion is very valuable. It may be a lack of professional pathology knowledge and understanding of characteristics of the intestinal pathological changes. In the current study, we only conducted the intestinal histology examine through reference to relevant literature, which is also our preliminary study. The important minor inflammatory change need further investigation.

(3) List of correction made with the comments of reviewer No.00008736

- The study was designed to investigate the protective effect of glutamine on intestinal barrier function in rats exposed to hypobaric hypoxia environment. The starting point of this research is based on the role of glutamine in maintaining the structure and function of intestine. We didn't take the role of the antioxidant activity of glutamine in the protective effect of glutamine on the intestinal barrier function under hypobaric hypoxia environment

as our research focus. Therefore, in the experimental design we did not set another antioxidant treatment group. Certainly, the suggestion is very reasonable and worthy of our consideration. Hypobaric hypoxia as a kind of special environmental factors can cause oxidative stress. Effect of glutamine on ROS and ROS-mediated signaling pathway may be one of the molecular mechanism of the protection of glutamine on intestinal barrier function exposed to hypobaric hypoxia. Those need to be further investigated.

Page 4-5 line 113-120: deleted this paragraph

Page 5 Line 143: changed "group C" to "Group control"

Page 10 Line 281 : changed "Figure 2A" to "Figure 2"

Line 290: deleted "(Figure 2B)"

- In experiment, each group was with ten rats. However, after experiment, seven rats from each group were sacrificed and then used to collect samples. Therefore, in table 1 and 2, n=7 is given per group.
- We have supplemented error bars in Figure 1:

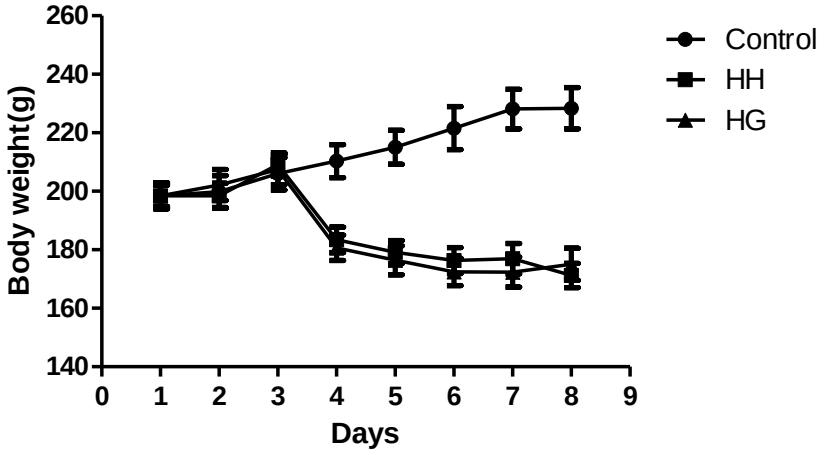


Figure 1. Effects of Gln treatment on body weight in rats from different groups.

- If the intestinal morphology was observed by fluorescence microscope at high power magnification, we can only observe local scope which will not benefit to the measurement of villous height and area. Therefore, in the manuscript , we presented the pictures with magnification 200 X.
- We have carefully checked the statistical indicators.

Moreover, we made other essential revision(the revised manuscript have shown them).

Note: 1. We state our reasons according to the manuscript evaluation form for referees

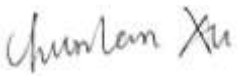
2. The line numbers and page numbers in the correction list are all in accordance with the

original manuscript.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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