

List of responses to reviewer's comments

Dear Ma:

We have studied the reviewers' comments carefully and have made correction to our manuscript entitled "Alleviate *Acanthopanax senticosus* polysaccharides-induced intestinal tight junction injury via inhibiting NF- κ B/MLCK pathway in a mouse model of endotoxemia (manuscript NO 31819)". The revised portion have been formed "list of corrections" and marked in the "revised manuscript version" in red. These corrections will not influence the content and framework of the paper. We hope that the revision is acceptable for publication.

Reviewer # 1.

1. Question: There are several awkward sentences such as: Page 4, first sentence in Introduction, Page 13, in Discussion TJ losses are involved.. The text should be corrected for the English.

Answer: Thanks for reviewer's good proposal. We have re-written the awkward sentence as "and developed rapidly into fatal systemic infections" in introduction, and the sentence "TJ losses are involved" in page 13 in discussion has been proved to be redundant and deleted.

2. Question: "In introduction, page 5, Why mention efficacy of ASPS when you have not studied it?. In the last chapter, the question addressed is not clearly presented and should be reorganized.

Answer: Thanks very much for the advice of reviewers. We have been aware of the inappropriate use of the word "efficacy", which has been replaced by the word "effect" to more exactly express our meaning. And we have reorganized the last paragraph in introduction as "In the present study, we determined the effects of ASPS on MLCK activation and TJ barrier breakdown in LPS-induced endotoxemia to evaluate whether the administration of ASPS alleviates endotoxemia-induced epithelial TJ breakdown by suppressing the NF- κ B/MLCK signaling pathway"

3. Question: Figures In general the lettering in most of the figures cannot be read, please improve them. Figure 1. There is no interest in showing figure B if you do not discuss the data. Discuss or Remove the data. Figure 2. It is very difficult to see anything in figure C. Show bigger figures and comment them.

Answer: Thanks very much for the advice of reviewers. We have resized the figures 1, 2 and 3 to make them clearly. And figure 1B as the histopathologic representative photographs of the distal ileums and colons after LPS injection can be used to address the condition of intestinal inflammation in each group, and we have added some description of figure 1B in discuss.

4. Question: In discussion. You can try to explain why ASPS is only a preventive agent as opposed to a cure. Please also mention your recent work whereby ASPS has several ways of protecting the intestinal mucosal barrier such as raising EGFR expression (Asian

Australas J. Anim Sci Jan 2016) .In this context, how is ASPS acting on the epithelial cells to mediate the different effects, please comment.

Answer: Thanks very much for the advice of reviewers. We have added some discuss aiming to reviewers good proposal as “This situation may be attributed to pharmacokinetic features of ASPS although little understanding about it. Interestingly, our recent work might provide some clues where regulatory expressions of TLR4 and EGF/EGFR occurred with pre-treatment of ASPS^[18, 33]. We infer ASPS administration prior to endotoxemia can function via EGF/EGFR dependent regulation of TLR4[34], whereby EGFR mediates intestinal epithelium growth and differentiation. More attention to the relationship between EGFR and TJ proteins should be focused. However in case of endotoxemia, ASPS is unavailable due to LPS combining more TLR4 to activate NF-κB than EGF/EGFR”.

Reviewer # 2.

Question: Several factors influence intestinal tight junction damage. Some limitations might be occurred. Please add more details of the pathophysiology of these effects? What are the new knowledges from this study? Please add the limitations of the study?

Answer: Thanks for reviewer’s good proposal. Just like what the reviewer 2 said, there exist several influencing factors on TJ damage, and we have supplement relevant content such as in discuss, as “It is worth noting that our present study obtaining some new understanding about the influencing mechanism of ASPS on TJ damage solely from MLCK/NF-κB pathway. Further attention to other modulation between TJ damage and Protein kinase pathway, calcium ion pathway, G protein and so forth will bring more comprehensive discoveries to ASPS action”.

Question: This is an animal study. Further studies need to be done. Finally, please recommend the readers “How to apply this knowledge for routine clinical practice?

Answer: Thanks for reviewer’s good proposal. Indeed, more clinical research should be done. We added clinical suggestion in discuss, as “In addition, ASPS intake preceding any upcoming stressful and infectious condition are likely suggested to be done in routine clinical practice. Further clinical research should be operated to bring available evidence to support commenced treatment of ASPS”.