Dear Professor Asim Duttaroy,

Editor-in-Chief, Food & Nutrition Research

Thank you for considering the manuscript entitles "The effects of orally administered *Bacillus coagulans* and inulin on prevention and progression of rheumatoid arthritis in rats ". Following, please find our response to the comments of reviewers. The amendments have made and highlighted in the "revised manuscript". We will appreciate if you reconsider it for publication.

Sincerely yours

S.S. Shekarforoush, Ph.D.,

Department of Food Hygiene and Public Health,

School of Veterinary Medicine,

Shiraz University

Reviewer’s comment:

1) It is noted that other commercial strain of *B. coagulans* has been reported to for the similar study in Human. David R Mandel et al., BMC Complementary and Alternative Medicine DOI: 10.1186/1472-6882-10-1 describes that adjunctive treatment with *Bacillus coagulans* GBI-30, 6086 LAB probiotic appeared to be a safe and effective for patients suffering from RA. One can ask if *B. coagulans* is reported for similar application in Human then why do we need to study in animal (rat). This should be addressed properly in the manuscript.

Our response:

1.The objective of Mandel et al. study was to evaluate the effects of *Bacillus coagulans* on RA symptoms and the functional ability of patients with RA using clinical examination, Assessment Questionnaire Disability Index, erythrocyte sedimentation rate and C-reactive protein, but in our study we evaluate the serological and inflammatory markers including Fibrinogen, Serum Amyloid A, TNF-α and Alpha-1-acid glycoprotein.

2. The mentioned paper focused on treatment of RA but in this study Bacillus coagulans was used as a preventive agent.

3. The mentioned paper suggested larger trials to verify their results.

4. In spite of Mandel et al., in the current study, *Bacillus coagulans* spores have administered alone and in combination with inulin.

The following text have inserted to Introduction of the manuscript: A pilot study by Mandel et al. demonstrated the effectiveness of *Bacillus coagulans* on RA symptoms using clinical examination, and laboratory tests for erythrocyte sedimentation rate and C-reactive protein. They also suggested larger trials, because of their low study population size to verify their results (2).

To the best of our knowledge, there is lack of information about the effects of *B. coagulans* and inulin, on progression of RA using serological markers and considering our previous findings indicated significant improvement in gastrointestinal microbiota following administration of probiotic *B. coagulans* and inulin (9), we conducted this study to evaluate the *in vivo* effects of probiotic *B. coagulans* and inulin, separately and in combination on progression of RA using complete Freund’s adjuvant (CFA) induced arthritis rat model.

2) What was the rational to select single dose of 1×109. Manuscript discusses more about the other probiotic strains (Lactobacillus) but not about *B. coagulans*. There are number of commercial probiotic strains of B*. coagulans* which have been studied for their safety and efficacy in animal and rats. Literature on other *B. coagulans* strains will enhance the paper.

Our response:

1. According to the FDA maximum level of 2 x 109 cfu/serving of *B. cogulans* specified as a GRAS ([http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/Notice Inventory/UCM413003](http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/Notice%20Inventory/UCM413003)).

2. According to Kliger (2008) a wide range of dosages for probiotics have been studied in clinical trials, ranging from 1 ×108 to 1.8 ×1012 CFUs per day. Most studies examined dosages in the range of 1 ×109 to 2 ×1010 CFUs per day, although exact dosages for specific indications varied within this range (Kliger B. Am Fam Physician. 2008 Nov 1;78 (9):1073-1078).

3) Page 5, line 5; mycobacterium tuberculosis; this should be italicized.

Our response: Done.

4) Similar abstract is available on web-link: https://www.waset.org/abstracts/39259 which was published in January 1999. This should verify if it is same group, if it is so then this should be included in the manuscript.

Our response: Part of this work was previously presented as abstract in The World Academy of Science, Engineering and Technology Conferences, San Francisco, 2015, which is mentioned in the declaration of the manuscript.

Seyed Shahram Shekarforoush, DVM, Ph.D

Department of Food Hygiene and Public Health,

School of Veterinary Medicine,

Shiraz University, Shiraz, Iran

*E-mail: shekar@shirazu.ac.ir*