Author's response to reviews

Title: Effects of two Lactobacillus strains on lipid metabolism and intestinal microflora in rats fed a high-cholesterol diet

Authors:

Ning Xie (laziowin@126.com)
Yi Cui (cuiyi1000@126.com)
Ya-Ni Yin (niniyaya123@sina.com)
Xin Zhao (109860810@qq.com)
Jun-Wen Yang (695399512@qq.com)
Zheng-Gen Wang (wzg987@yeah.net)
Nian Fu (fulian365@sina.com)
Yong Tang (1037411437@qq.com)
Xue-Hong Wang (wxh05271@sina.com)
Xiao-Wei Liu (liuxiaoweiabc@126.com)
Chun-Lian Wang (wcl0747@163.com)
Fang-Gen Lu (lufanggenyao@163.com)

Version: 2 Date: 3 June 2011

Author's response to reviews: see over
Cover letter for responding

List of Actions:

1. We addressed the points of revision according to the reviewers’ comments.

2. The manuscript has been copyedited by a professional language editing service as recommended by the editorial office.

3. All changes are underlined to make it easier for the Editors and Reviewers to check them. Thank you very much for your insightful comments and valuable suggestions. We hope that we have successfully addressed them according to your standards.
Responses to Editors,

We asked Edanz group China to help us with the English editing work on our manuscript, the work includes correcting spelling, grammatical, punctuation and syntax errors. The certification is provided. Concerns of the reviewers are also responded point-by-point.
Response to Professor Min-Tze Liong:

Thank you very much for your valuable comments and suggestions to our manuscript. Here we want to express our extreme gratitude for everything you have done (the valuable comments and suggestions) for our manuscript.

In the revised edition, we carefully checked and corrected the spelling and grammatical errors, and organized the information and evidence to make the presentation more fluent. We also modified the discussion section. Minor points have been amended point by point according to your comments.
Response to Professor Namjoo Ha,

We wish to express our deep indebtedness to you for your kindness. In the revised edition, we further improved our statistical comparisons and significance, and strengthened the analysis of the results. There are some studies which also assigned 10 rats to each group, we took three samples from each rat and three replicates for each sample, but we agree that this sample size may be relatively small and we are currently preparing to use these strains on another animal model, to review their hypocholesterolemic effect in vivo with more samples. Again please allow us to extend our thanks to you. We are grateful for your conscientious academic attitude and professional encouragement toward our article.
Response to Professor Katsumi Imaizumi,

We are grateful for your conscientious academic attitude, professional encouragement and precise corrections toward our article. We have made revisions according to your comments as follows.

**Major revisions:**

1. Serum total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured enzymatically with commercially available kits produced by Daiichi Chemicals (Tokyo, Japan) and a chemical analyzer 7020 produced by Hitachi (Tokyo, Japan) in the clinical laboratory of our hospital. After putting the samples into the tubes, the machine can automatically classify and analyze the lipids profiles and LDL-C results can be achieved directly.

2. We have modified some related sentences and emphasized the effect of diet on the serum LDL-C levels.

**Minor revisions:**

1. We deleted the last unnecessary significant figure but we kept one significant figure after the decimal point because we believe that it is necessary for body weight gain, though not compulsory.

2. Spaces between sentences were added throughout the manuscript.
3. Expression of the statistical probability was changed for consistency. 
   \( p<0.05 \)
4. Spelling errors and inappropriate sentences were corrected and rephrased.
5. Conventional clean rats were used.
6. All references were checked again carefully.
7. Expression of the statistical difference is now consistent among the tables.
8. Legends to all figures were edited where necessary.

Again please allow us to extend our thanks to you!
Response to Professor Alejandra de Moreno de LeBlanc,

Thank you very much for giving us so much advice. The revision made according to your comments are as follows:

1. Typographical and grammatical errors in the text were carefully corrected by a professional language editing service recommended by the editorial office.

2. “Normal saline” means 0.9% NaCl solution, we clarified the concentration in the revised text.

3. Some studies failed to find an obvious influence on the HDL-C level with LAB supplementation [1-3], while others declared a reduction in HDL-C of hamsters fed fermented milk [4] and in humans [5-6], and there are also other studies reported LAB supplemented diets raise serum HDL-C [7-10]. After reviewing these articles in addition to our own study results, we propose that different factors influence the probiotic strains effect on serum HDL-C levels, including properties of probiotic strains, administration dosage, clinical characteristic of subjects, and duration of treatment period.

4. The finding that the TCH/HDL-C and TG/HDL-C ratios were reduced in LAB supplemented rats suggests that using the two strains may help to reduce atherosclerosis and may have a cardiac preventive effect. We are now preparing to use these strains on a hamster model to confirm this hypocholesterolemic effect in vivo. In the subsequent
study, the treatment period will be much longer to determine whether these strains could influence HDL-C. In the present study we provided pathological differences which are seldom seen in previous reports with the similar aim, we also plan to observe the pathological differences (for example, atherosclerotic plaque and foam cells) of the aorta of hamsters in different groups to confirm our presumption.

5. It is a very good idea that using these same LAB strains in animals fed a conventional diet, in order to determine if body weight and other parameters can be modified in normal animals. As we mentioned in the background section, alternative management strategies for the treatment of hypercholesterolemia is required for people with borderline cholesterol levels. Park et al. [11]. found that a supplementation of Lactobacillus acidophilus ATCC 43121 in conventional diet could slightly reduced body weight and serum TCH. We are also eager to see if our LAB supplementation could have a preventative effect on normal models. These groups will be included in upcoming experiments.

6. We changed the sentence in page 17, line 7 accordingly, we consider that you got the very point.

7. In our present study, L. plantarum 9-41-A had a more robust effect in lowering serum lipid profiles and body weight. Since rat feces treated with L. 9-41-A included more fecal cholesterol and bile acids, we
presume that cholesterol was incorporated, tightly bound to bacteria or coprecipitated with bile acids and excreted from feces. *In vitro* studies have proven this ability in LAB cells [12,13]. Because biliary secretion of cholesterol, either in the form of free cholesterol or bile acids is the only significant efflux pathway for eliminating cholesterol in mammals [14], so more excretion of cholesterol and bile acids mean better ability to lower cholesterol.

8. The intestinal microbial balance might influence cholesterol metabolism. Intestinal microbiota may not only improve intestinal transit time but also consume energy and lipids in their metabolism. Stepankova et al. [15] recently reported that absence of microbiota accelerates atherosclerosis in ApoE-deficient mice fed a standard low cholesterol diet. We have improved the discussion and final conclusion of our manuscript according to your suggestions.

Our group is grateful for your valuable comments. Our colleagues are engaged in a study to attempt to clarify the relationship between probiotics and experimental colitis have referred to your work: “The application of probiotic fermented milks in cancer and intestinal inflammation.”, “Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice.” and “Yoghurt consumption regulates the immune cells implicated in acute
intestinal inflammation and prevents the recurrence of the inflammatory process in a mouse model.”. We hope that you continue in your progresses and success and that you would be willing to collaborate with us.

References


11 Park YH, Kim JG, Shin YW, Kim SH, Whang KY: Effect of dietary inclusion of Lactobacillus acidophilus ATCC 43121 on


