Dear editor and reviewer,

Thanks for considering our manuscript and all of the useful comments. The reviewer’s comments are very important for our future research, so we revised the manuscript, and gave responses to the comments as follows.

Reviewer: This manuscript described a role of polysaccharide-enriched fraction from *Amillariella mellea* fruiting body on insulin resistance. An overall effect of AAMP on glucose metabolism is quite mild although AAMP may affect lipid metabolism more significantly. This work would eventually have a scientific merit; however, there are issues which should be addressed properly by authors in the current form.

Major Issues:

1. No information on diet composition of HFD as well as control diet was provided.

Response to comments: We have provided the informations of HFD as well as control diet in lines 196-197.

2. Rats fed HFD/DEX gain much less weight than those on control diet and showed severe hyperglycemia compared to controls in Figure 3. Please show how the reduced body weight gain in rats on HFD/DEX compared with those on control diet began and how the onset associated with their diabetic states, food intake, and/or energy expenditure during the course of the experiment. Rat on HFD alone for 25 days gain more weight than those on control diet? To evaluate the effect of DEX alone in control diet and how AAMP modifies the outcome, did the authors do the experiments with rats fed control diet/DEX? How did they choose the dose of DEX at the concentration of 0.8mg/kg i.p. for the last 10 days of the experiment? How did AAMP treatment affect food intake and energy expenditure in both rats on control diet and HFD/DEX?

Response to comments:

The purpose of this project is to screen an active fraction from *Amillariella mellea* fruiting body that lowers blood glucose and develop it into an oral hypoglycemic health food. Therefore, we established a diabetic rat model according to “National Handbook for Implementing Technical Specification for Evaluation of Health Food”, and choose the 0.8 mg/kg i.p. of DEX for the last 10 days. Based on the references [1-3] and handbook, the rats showed hyperinsulinemia after HFD/DEX treatment, and our result of serum
insulin content was consistent with the references (Figure 5). During the course of the experiment, the food intake was no significant changes between control rats and diabetic rats, suggesting AAMP did not affect food intake in rats. But energy expenditure was still unknown at present due to lack of the examination tools. The increase of body weight gain in rats fed with HFD alone was similar to that in rats fed with control diet.

In fact, a diabetic rat model can also be established if HFD is fed alone for a long period of time (at least 12 weeks). However, short-term treatment of HFD and DEX can accelerate insulin resistance in rats and establish diabetic rat model faster. The reviewer’s suggestions are very meaningful, and we perhaps examine the effect of AAMP on DEX alone treated rats in subsequent trials.

References:


3. Serum TG levels are mainly regulated by lipoprotein lipase (LPL). Did the authors measure LPL activity? How did the serum lipoprotein component change in the rats on HFD/DEX vs. control diet? Did the authors test serum TG clearance in those rats?

Response to comments:

Based on the reviewer’s suggestions, we have measured LPL activity (Figure 6D). The serum LPL of diabetic rats was lower than that of control rats, but AAMP treatment could increase the LPL level.

We examined the contents of LDL and HDL in rats of control and HFD/DEX. As shown in the table below, HFD/DEX treatment significantly increased the levels of LDL and HDL. HFD/DEX increased the contents of TG, TC and FFA, resulting in increased
lipoprotein production. Our results showed that AAMP could decrease serum TG and FFA (Figure 6A and C), but the clearance of TG was still not detected so far.

<table>
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<tr>
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<th>Control (µmol/L)</th>
<th>HFD/DEX (µmol/L)</th>
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<tbody>
<tr>
<td>LDL</td>
<td>8.40±1.22</td>
<td>38.2±0.83 ***</td>
</tr>
<tr>
<td>HDL</td>
<td>6.06±2.12</td>
<td>45.98±1.57 ***</td>
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4. Did the authors measure FFA levels?

Response to comments: We have measured FFA level (Figure 6C). HFD/DEX treatment increased FFA level compared to the control diet, but AAMP treatment could significantly decrease the FFA level.

5. Quantitative analysis is required for Figure 7A to make the authors’ point.

Response to comments: We have added the quantitative analysis in Figure 7A.

6. In line 147 it is not clear in what triglyceride decreased.

Response to comments: We have revised the description “reducing serum insulin, TG and FFA levels” in line 150.

Minor Issues:

7. FT-IR should be spelled out in line 58.

Response to comments: We have revised the description in line 59.