Effect of Oat Soluble and Insoluble β-glucan on Lipid Metabolism and Intestinal *Lactobacillus* in High-fat Diet-induced Obese Mice

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Abstract In this study, high-fat diet-induced obese mice were administered oat soluble (SOG) and insoluble β-glucan (IOG) at different doses. Mice were sacrificed after 6 weeks, body weight, serum lipid level, fecal pH value, fecal bile acid excretion and total colonic short-chain fatty acid (SCFA) concentration was measured. Mesenteric adipocyte count and size were also evaluated histologically. The population of *Lactobacillus* in colon was determined. Compared with obese mice administered normal saline, body weight, serum cholesterol, triglycerides and the lipoprotein profile were significantly decreased \((p<0.05)\) in mice administered SOG and IOG. The fecal pH value was significantly decreased \((p<0.05)\), while both fecal bile acid excretion and total colonic SCFA concentration were significantly increased \((p<0.05)\). In addition, it exhibited an increase of fat cell count and a decrease of cell size. SOG and IOG restored the number of *Lactobacillus* in colon \((p<0.05)\). IOG was more effective on weight-loss while SOG might play a more important role in improving serum lipids and the efficacy of promoting growth of *Lactobacillus* is similar. A dosage of 2 g kg\(^{-1}\)BW (Body Weight) produced the most significant effect. These data were anticipated to support the prebiotic property and anti-obesity effect of oat β-glucan.

Keywords: oat β-glucan, obesity, serum lipids, Lactobacillus, short-chain fatty acid


1. Introduction

In recent years, the prevalence of obesity has reached alarming levels, which is not only observed in developed western countries, but also in developing ones [1]. There is a metabolic link between obesity and a series of chronic diseases including of hypertension [2], type 2 diabetes mellitus [3], coronary heart disease [4] as well as metabolic syndrome [5]. In addition, the body fat deposition plays an important role in the dyslipidemia, such as high serum cholesterol (TC), high triglycerides (TG), and lipoprotein disorders. Elevated levels of TC are associated with atherosclerosis [6]. Bile acid could act as physiological detergents that facilitate intestinal fat and sterols, thereby a stimulation of bile acid synthesis has cholesterol-lowering effect [7]. Furthermore, previous study suggested that the increased production of short-chain fatty acid (SCFA) in intestinal may affect lipogenesis. Propionate, in particular, has been demonstrated to inhibit hepatic cholesterol synthesis [8]. On the other hand, acetate is generally considered as signaling molecules in metabolic pathways of lipogenesis [9].

Dietary interventions and development of nutrient digestion inhibitors would be the important strategies in treatment of obesity [10,11]. There is mounting evidences that consumption of oat products would cause well-known positive effects, such as reduction in the serum low-density lipoprotein cholesterol (LDL-C) [12], postprandial glycemia and insulin response [13,14]. An inverse association between oat product intake and body weight gain has also been observed. β-glucan, a natural polymer comprised of individual glucose molecules that are linked together by a series of β-(1-3) and β-(1-4) linkages [15], is thought to be the active component for these physiological functions of oats. On the other hand, a prebiotic effect of oat β-glucan has been proposed and proven by both *in vivo* and *in vitro* experiments [16,17]. It suggested that β-glucan could promote growth of probiotic bacteria in the gastrointestinal tract. Xie et al. discovered the hypcholesterolemic effects of *Lactobacillus* supplementation in rats [18]. Therefore, the relationship between the prebiotic effect and the lipid-lowering effect of oat β-glucan has become a focus of research.

Nowadays, most of the proposed protective mechanisms of oat products consumption are likely to be relevant with oat soluble β-glucan (SOG), and few research on oat insoluble β-glucan (IOG) have been conducted. In this study, the anti-obesity and hypolipidemic effects of different doses of SOG and IOG on the high-fat diet-induced obese mice were evaluated from body weight, serum lipid level, fecal pH value, fecal
bile acid excretion, fecal total colonic SCFA concentration and histomorphological changes of adipose tissue in the process of fatty metabolism. The population of Lactobacillus in colon was also determined.

2. Methods and materials

2.1. Oat Soluble and Insoluble β-glucan

Oat bran was a commercial product purchase from Jinlvhe Bio-technology Co., Ltd (Shanxi, China). SOG and IOG used in this experiment were prepared by the method of Johansson et al. [19] with minor modifications. Commercial oat bran was comminuted with a HL-100 mill (Shanghai Sainai Instruments Co., Ltd., Shanghai, China), the powder that passed through a 0.3 mm screen was defatted with ether. Polysaccharides were suspended in water to a final concentration of 0.1 g/ml (w/v) at 80°C and starch was hydrolyzed by α-amylase (≥10 units/mg). The sample was then centrifuged (3000 r/min, 25 min) (TP5A-WS Table Centrifuge, Hunan Xiangyi Lab Instrument Development Co., Ltd., Hunan, China). The insoluble fraction was collected. Pancreatin (≥1.0 units/mg) was added into the supernatant to degrade proteins. The water-soluble β-glucan was precipitated by 70% ethanol, removed by centrifugation and dissolved in water at 80°C. The solution was concentrated (RE-52AA, Shanhai Yarong Lab Instrument Development Co., Ltd., Shanghai, China) and freeze-dried. Water-insoluble β-glucan was obtained from the precipitation collected before. It was extracted by alkaline solution (adjust the initial pH value to 9 with 0.1 mol/L Ba(OH)_2).

The total β-glucan contents of SOG and IOG samples were determined using reagent kits obtained from Megazyme International Ireland Ltd (Bray, Ireland) according to the manufacturer’s instructions, which gave values of 80.10% and 81.72%, respectively.

Pancreatin and α-amylase were purchased from Sigma Chemical Company (St. Louis, USA). All other reagents and chemicals used were from Kermel (Tianjin, China) and were analytical reagent grade.

2.2. Animals and Experimental Diets

The experiment was conducted in compliance with the guidelines for animal experimentation of the biological/pharmacological research laboratories. And this study has obtained the approval of the Animal Experiment Committee of China. A total of 130 male KM mice (Weighing 15.00±3.00 g) obtained from the Laboratory Animal Centre of Henan Province (Zhengzhou, China) were used. The mice were allowed to acclimatize for 3 days in an environmentally controlled room at 22°C with a 12:12-h light-dark cycle. They were provided with a commercially available basic diet (Laboratory Animal Centre of Henan Province, Zhengzhou, China) prior to the dietary manipulation. The compositions of the basic diet are shown in Table 1.

After the acclimation, the mice were randomly divided into two groups: mice (n=15) which fed the basic diet was considered as normal control group (NC); the other mice (n=115) were fed first with a high-fat diet (Table 1) for 3 weeks to establish obesity model. After the dietary manipulation, 105 obese mice (Mice that were at least 110% of the weight of mice of NC group) were subdivided into seven different groups including the model control group (MC), three SOG groups (High-dose, SH; Middle-dose, SM; Low-dose, SL) as well as three IOG groups (High-dose, IH; Middle-dose, IM; Low-dose, IL) of fifteen each. The NC group was continually fed with the basic diet and given an oral administration of normal saline every day. The other five groups were given the high-fat diet besides the oral administration of normal saline (MC group). Meanwhile, the SH group, SM group and SL group were administered SOG by intragastric gavage at dose of 2 g·kg⁻¹·BW, 1 g·kg⁻¹·BW and 0.5 g·kg⁻¹·BW daily for 6 weeks as well as IH group, IM group and IL group were administered IOG. All of the treatments lasted for 6 weeks, and the mice had free access to water and diet during this period.

2.5. Measurements of Fecal pH Value and Fecal bile Acid Excretion

Faeces samples from each group were collected quantitatively at week 6, stored at 4 °C, and weighed on an electronic balance (BSA224S-CW, Sartorius Lab.
Instrument Development Co., Ltd., Germany). The weighed aliquot of fecal samples were freeze-dried and used for further analysis. Bile acids in faeces were measured by accurate enzymatic method with a spectrophotometer (UV-1600, Shangai MAPADA Lab Instrument Development Co., Ltd., Shanghai, China) according to the method by Porter et al. [20]. Another quantified fecal sample of each group was diluted 10-fold (w/v) in distilled water. They were homogenized thoroughly with a vortex mixer (Vortex-Genie 2, Service Science & Technology, Beijing, China). The pH was measured with a pH meter (PHS-3C, Sheng Ci, Shanghai, China).

2.6. Analysis of SCFA in Colon

At the end of the treatment, animals were sacrificed under ether anaesthesia after the serum sample collection. The colonic contents and the mesenteric fat pads was collected and stored at -70 °C prior to analysis. The frozen colonic contents (0.1-0.2 g) were diluted with 2 ml physiological saline and 1 ml of 50% H2SO4. Then 2 ml of ether was added into the solution to extract SCFA [21]. The analysis was performed by gas chromatography according to Shen et al. [16]. Acetic acid, propionic acid, iso-butyric acid and butyric was used as a standards, crotonic acid was used as internal standard. The analysis was performed using A GC-14A gas chromatograph equipped with a flame ionization detector (Shimadzu Corp., Kyoto, Japan). Besides, a 30 m × 0.53 mm ID fused silica capillary column (PEG-20M stationary phase) was used and nitrogen was used as the carrier gas with a flow rate of 3 mL/min. Column temperature was 80°C and the detector temperature was set at 240°C.

2.7. Histomorphometric Analysis of Mesenteric Fat Tissue

The mesenteric fat pads were thawed on ice and washed within ice-cold saline solution. Then the fat pads were immediately fixed in 10% buffer formalin for 48 h. Tissues were then processed with tissue processors (BT-300A Dehydrator, CS-V Spreading machine and ZHB1-ZT-HB Baking machine from JinHua YiDi Medical Appliance Co., Ltd., HESTION Embedded machine from TexLab Precision Instruments Co., Ltd., YD-1508R Slicer from Shanghai Leica instruments Co., Ltd.) and tissue sections were prepared and stained with hematoxylin-eosin, as previously described [22]. Histopathologic examination was performed by a light microscopy (OlympusBX51 Microscopy from Japan) and the fat cells were observed at a 10×40 magnification.

2.8. Determination of the Number of Lactobacillus in Colon

The frozen colonic contents (approximately 1 g) were diluted in 9 mL of phosphate buffer (pH 7.4, 1.36 g KH2PO4, 79 mL of 0.1 mol/L NaOH and 121 mL of distilled water) and homogenized. Then, the homogenates were serial 10-fold diluted in phosphate buffer under anaerobic environment (Model 1029 Forma Anaerobic System, Thermo Fisher Scientific, USA). Fifty microliters of dilution sample was spread on selective media (LBS agar) in triplicate. And the plates were incubated anaerobically (Model 1029 Forma Anaerobic System, Thermo Fisher Scientific, USA) at 37°C for 48 hours. Growth of Lactobacillus evaluated by colony counts and results were expressed as log10 CFU (Colony Forming Unit) per milliliter medium.

2.9. Statistical Analysis

Data from the control and other experimental groups were analyzed with the SPSS 11.5 program following the one-way ANOVA. Duncan’s new multiple range test was performed to determine the significant difference between samples. p<0.05 was considered as a significant difference. The results were presented as the mean ± standard deviation (X±SD).

3. Results

3.1. Effects of SOG and IOG on Body Weight

![Figure 1](https://via.placeholder.com/150)

Figure 1  Body weight of (mean ± SD) of NC, MC, SH, SM, SL, IH, IM and IL mice during 6 weeks of the protocol. Statistical results were omitted for clarity. Each group contained 15 mice. SOG groups and IOG groups exhibited lower BWs than MC and IOG was superior to SOG. High-dose was more efficient. NC group always exhibited the lowest body weight until the 6th week.

Figure 1 shows changes in body weight during the experiment. As shown, high-fat diet significantly increased the body weight of mice (p<0.05) in the initial stage. Both SOG and IOG inhibited the body weight gain after treatment for 2 weeks. At the end of the experiment, body weight of SM group, SH group, IL group, IM group and IH group was not statistically significantly different with that of NC group, especially for SH group (p≥0.05). Results suggested that both SOG and IOG might have potential effect on weight-loss of obese mice. It seemed that IOG was more effective.

3.2. Effects of SOG and IOG on Serum Lipids

![Figure 2](https://via.placeholder.com/150)

Figure 2  Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels of (mean ± SD) of NC, MC, SH, SM, SL, IH, IM and IL mice during 6 weeks of the protocol. Statistical results were omitted for clarity. Each group contained 15 mice. SOG groups and IOG groups exhibited lower TC levels and HDL-C levels than MC and IOG was superior to SOG. High-dose was more efficient. NC group always exhibited the lowest TC level until the 6th week.

There were significant differences between the SOG groups and the IOG groups (Table 2) concerning TC, TG, LDL-C, HDL-C and the lipoprotein profile (LDL-C/HDL-C). The serum TG, TC levels were elevated by high-fat diet (p<0.05). After treatment for 6 weeks, both SOG and IOG exerted remarkable effect on decreasing serum TG and TC levels. On the other hand, the high-fat diet...
increased the serum LDL-C level and decreased the serum HDL-C level, thus significantly enhanced the LDL-C/HDL-C ratio (p<0.05). However, administration of SOG and IOG improved the lipoprotein profile. Furthermore, SOG was superior to IOG on improving serum lipids and there was a dose-dependent effect.

<table>
<thead>
<tr>
<th>Table 2. Effect of oat β-glucan on serum lipid and fecal bile acids excretion in experiment period</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>NC</td>
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<tr>
<td>MC</td>
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<tr>
<td>SL</td>
</tr>
<tr>
<td>SM</td>
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<tr>
<td>SH</td>
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<td>IL</td>
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<tr>
<td>IM</td>
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<td>IH</td>
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Data are mean ± SD, n=15 in each group. Values in the same row that do not share the same capital letter are significantly different (p<0.05).

3.3. Effects of SOG and IOG on Fecal Bile Acids

Fecal bile acid excretion in MC group was higher than that in NC group (Table 2), which might be caused by dietary cholesterol in the high-fat diet. At the end of the experiment, SM, SH, IM and IH groups showed significantly increased bile acid excretion compared with both the NC group and the MC group (p<0.05), especially for SOG groups. The bile acid excretion of high-dose groups was significantly higher than that of other groups.

3.4. Effects of SOG and IOG on Fecal pH Value

Fecal pH values in MC group were higher than that in NC group (p<0.05). As shown in Figure 2, fecal pH value was lowered throughout administration of oat β-glucan and it presented no significant difference between middle-dose groups and NC group at week 6 (p>0.05). Fecal pH values in the high-dose group were even lower than that in NC group. In addition, results showed that SOG was more efficient in terms of lowering the fecal pH value in obese mice than the same dose of IOG. The change of pH values might be caused by increase of intestinal SCFA production, so the SCFA formation in colon was next analyzed.

<table>
<thead>
<tr>
<th>Table 3. Effect of oat β-glucan on the total SCFA concentrations and relative proportions of acetate, propionate iso-butyrate and butyrate in colon</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>NC</td>
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<tr>
<td>MC</td>
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<tr>
<td>SL</td>
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Data are mean ± SD, n=15 in each group. Values in the same row that do not share the same capital letter are significantly different (p<0.05).

Abbreviation: SOG, oat soluble β-glucan; IOG, oat insoluble β-glucan; NC, normal control; MC, model control; SH, high-dose SOG group (2 g · kg⁻¹ · BW); SM, middle-dose SOG group (1 g · kg⁻¹ · BW); SL, low-dose SOG group (0.5 g · kg⁻¹ · BW); IL, high-dose IOG group (2 g · kg⁻¹ · BW); IM, middle-dose IOG group (1 g · kg⁻¹ · BW); IH, low-dose IOG group (0.5 g · kg⁻¹ · BW).

As shown in Table 3, the high fat diet induced a significant decrease (p<0.05) in the total amount of colonic SCFA compared to the NC group. However, a statistically significant increase (p<0.05) was observed in SM group, SH group, IM group and IH group compared to the NC group. Furthermore, mice of high-dose group had higher colonic SCFA concentrations than those of middle-dose groups (p<0.05), and the total SCFA concentrations in mice administered SOG tended to be higher than that administered IOG when at a high-dose (p<0.05). Besides, mean SCFA ratios (acetate: propionate: butyrate: iso-butyrate) were also be changed throughout the administration of oat β-glucan. Acetate constituted the major part of the colonic SCFA of each group, ranging from 62.77 to 67.33%. Consumption of oat β-glucan resulted in higher proportions of propionate and butyrate compared to the MC group, especially for propionate, but it had no obvious effect on the proportion of iso-butyrate.
3.6. Effects of SOG and IOG on the Population of *Lactobacillus* in Colon

Table 4 presented the change of the population of colonic *Lactobacillus*. The mean *Lactobacillus* counts in the MC group was significantly lower than that in the NC group (p<0.05). After administrated with SOG and IOG for 6 weeks, the mean *Lactobacillus* counts in oat β-glucan groups were significantly increased compared with the MC group (p<0.05), especially for that in SM, SH, IM and IH groups, which were even significantly higher than that in NC group (p<0.05). It seemed that the effect of SOG and IOG on increasing the number of *Lactobacillus* in colon was almost similar.

Table 4. Effects of oat β-glucan on the population of *Lactobacillus* in the colonic sample of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Lactobacillus (×10^7)</th>
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</thead>
<tbody>
<tr>
<td>NC</td>
<td>9.07±0.10</td>
</tr>
<tr>
<td>MC</td>
<td>8.39±0.15</td>
</tr>
<tr>
<td>SL</td>
<td>8.88±0.10</td>
</tr>
<tr>
<td>SM</td>
<td>9.68±0.13</td>
</tr>
<tr>
<td>SH</td>
<td>9.98±0.11</td>
</tr>
<tr>
<td>IL</td>
<td>8.86±0.17</td>
</tr>
<tr>
<td>IM</td>
<td>9.79±0.12</td>
</tr>
<tr>
<td>IH</td>
<td>9.94±0.18</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n=15 in each group. Values in the same row that do not share the same capital letter are significantly different (p<0.05).

Abbreviation: NC, normal control; MC, model control; SH, high-dose SOG group (2 g · kg^-1 · BW); SM, middle-dose SOG group (1 g · kg^-1 · BW); SL, low-dose SOG group (0.5 g · kg^-1 · BW); IH, high-dose IOG group (2 g · kg^-1 · BW); IM, middle-dose IOG group (1 g · kg^-1 · BW); IL, low-dose IOG group (0.5 g · kg^-1 · BW).

3.7. Observation of Histomorphology of the Mesenteric Fat Tissue

After the experiment, sections of mesenteric fat tissue of mice in all groups were prepared and observed (Figure 3). From the figure, we could see that the number of fat cells in the MC group was decreased significantly compared with the NC group, while cell size was increased. After administration of oat β-glucan, the number of fat cells was increased and the cell size was reduced at different degree. Furthermore, the number of fat cells and the cell size of IH group were similar to that of NC group. Results showed that both SOG and IOG could help increase the number of fat cells and decrease the cell size, which might contribute to the metabolism of fat. The effect of IOG was more obviously, especially for the high-dose groups.

4. Discussion and Conclusion

In this present study, we verified that oat β-glucan inhibited obesity, which was consistent with previous studies [15]. One explanation is that, oat β-glucans could absorb large quantities of water and increase stomach distension. In the intestine, the incorporation of fiber may complicate the union between digestive enzymes and their substrate, thus slowing down the absorption of nutrients [23]. Other studies have shown that the effects of oat β-glucan on body weight might also be related to different gut hormones which regulate satiety, energy intake and pancreatic functions [14]. On the other hand, the sections of mesenteric fat tissue of mice in all groups were observed. We found that the number of fat cells of experimental groups increased and the fat size decreased, especially for IL group, which might contribute to the metabolism of fat cell and eventually achieved the purpose of losing weight.

Results of this experiment also showed that oat β-glucan could decrease the serum cholesterol including TG, TC enhanced by high-fat diet. The LDL-C level and HDL-C level were not improved by oat β-glucan, however, both SOG and IOG showed a tendency to decrease the LDL-C/HDL-C ratio. Prospective studies have suggested that a high LDL-C/HDL-C ratio combined with hypertriglyceridermia is associated with coronary heart disease risk [24]. Our data also showed that SOG might play a more important role improving serum lipids. There are several mechanisms proposed to explain the beneficial effect of oat β-glucan on lowering serum cholesterol. Firstly, Katie et al [15] believed that oat β-glucan may decrease absorption of dietary cholesterol by altering the composition of the bile acid pool. Furthermore, hypcholesterolemic action of dietary fiber is partly mediated by a lower absorption of intestinal bile acid because the interruption of the enterohepatic bile acid circulation [25]. Hence we measured bile acid excretion in faeces. We found that it was much higher in oat β-glucan groups than that in MC group indicating that there was binding of bile acids by the oat β-glucan. Besides, higher fecal bile acid content in SOG groups was observed than that in IOG groups. The elevated faecal bile acid excretion followed by a stimulation of hepatic bile acid synthesis from circulating cholesterol thereby lowering blood cholesterol levels [26].

In addition, oat β-glucan is fermented in the colon to SCFA, which may contribute to the slightly acidic gut
environment and inhibiting activity of intestinal harmful enzymes [16]. On the other hand, SCFA would enter the portal vain and attenuate hepatic cholesterol [15]. In this study, significant difference was seen in the total SCFA concentration and the SCFA profile of oat β-glucan groups compared to the MC group. We found that the colonic total SCFA concentration was positively correlated with the TC, TG levels and LDL-C/HDL-C ratio. Among the studied SCFA, the proportion of acetate was decreased while proportion of propionate and butyrate was increased, especially in SOG groups. Acetate is a kind of energy source for the liver and it seems to contribute to lipid and cholesterol synthesis in the liver. Propionate can inhibit the effects of acetate thereby lowering the serum cholesterol. Butyrate is metabolized by the colonic epithelium and utilized by enterocytes. It could help regulate apoptosis and lower the risk of colon cancer [27]. We also found that the total SCFA concentration was negatively correlated with the body weight of the high-fat diet-induced obese mice. It has been reported SCFA stimulated the secretion of peptide hormone related to obesity [28].

Oat β-glucan was fermented by gut bacteria with the production of SCFA and colonic fermentation varies between individuals as a result of species of gut bacteria. Lactobacillus is generally recognized as a probiotic that has beneficial effect on maintaining human health. It suggested that the Lactobacillus strains could produce a kind of cholesterol reductase which could convert the cholesterol into coprostanol [18]. Results of this study revealed that oat β-glucan could increase populations of Lactobacillus. It seemed that SOG and IOG exhibited similar efficacy in terms of stimulation of growth of Lactobacillus. It can be assumed that both SOG and IOG could pass undigested into the large intestine and act as a prebiotic promoting the growth of Lactobacillus and the formation of SCFA, thus caused a hypocholesterolemic effect. Besides, Lactobacillus plays an important role in the prevention of colitis and antibiotic-associated diarrhea [29,30], hence the interest in the relationship between the prebiotic effect and the health benefits of oat β-glucan would increase in the near future.

In conclusion, both SOG and IOG dose-dependently improved obesity, serum lipids via increasing fecal bile acid excretion, colonic SCFA production, population of Lactobacillus in colon as well as metabolism of fat cell. Our data showed that IOG was more effective on weight-loss while SOG might play a more important role in improving serum lipids, and the efficacy of promoting growth of Lactobacillus is similar. A dosage of 2 g·kg⁻¹·BW for 6 weeks produced the most significant effect in high-fat diet-induced obese mice.

Acknowledgment

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List of Abbreviations

TC, serum cholesterol; TG, serum triglycerides; SCFA, short-chain fatty acid; LDL-C, serum low-density lipoprotein cholesterol; SOG, oat soluble β-glucan; IOG, oat insoluble β-glucan; BW, body weight; NC, normal control group; MC, model control group; SH, high-dose SOG group; SM, middle-dose SOG group; SL, low-dose SOG group; IH, high-dose IOG group; IM, middle-dose IOG group; IL, low-dose IOG group; HDL-C, high-density lipoprotein cholesterol.

References


