Effect of Oat and Tartary Buckwheat – Based Food on Cholesterol – Lowering and Gut Microbiota in Hypercholesterolemic Hamsters

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Abstract: The nutritional components in oat and tartary buckwheat had been assessed to have cholesterol-lowering effects. However, The effect of oat and tartary buckwheat based-food (OF) on cholesterol-lowering and gut microbiota in hypercholesterolemic hamsters was still limited studied because they are usually consumed in whole gran as well as after being processed. In this study, normal diets, high fat diet (HFD) with/without OF were fed to hamsters for 30 days respectively and growth parameters, metabolic parameters, and gut microbiota were investigated, respectively. It was found that OF significantly decreased plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-cholesterol), lowered liver TC, cholesterol ester (CE), and triglycerides (TG) concentrations, and increased fecal weight and bile acids (BA) concentrations, compared with HFD (p < 0.05). Moreover, the concentrations of acetate, propionate, butyrate and total short-chain fatty acids (SCFAs) were significantly increased in hamsters fed with OF, compared with HFD (p < 0.05). OF changed the overall structure of gut microbiota. The relative abundances of Erysipelotrichaceae, Ruminococcaceae, and Lachnospiraceae were decreased and the relative abundance of Eubacteriaceae was increased, compared with HFD. These results suggested that OF could reduce the concentrations of plasma lipid by inhibiting cholesterol absorption in liver and promoting excretions of fecal lipid and BA. And it also increased SCFAs and modulated the gut microbiota effectively to exert the hypocholesterolemic effects.

Key words: oat and tartary buckwheat-based food, cholesterol-lowering, gut microbiota, short-chain fatty acid

1 Introduction

It has been attracted great attention on oat and tartary buckwheat for their reduction in serum cholesterol. Currently, numerous studies have indicated that β-glucan, protein, and oil in oat can decrease the concentration of plasma cholesterol. β-Glucan, a dietary fiber from oat, can increase the viscosity of intestinal and decrease the concentrations of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-cholesterol)¹, ². Proteins in oats decrease plasma TC and LDL-cholesterol concentrations, and the main reason may be its reasonable amino acid³, and low Lysine/Arginin and Methionin/Glycine ratios⁴. Previous researches indicate high levels of polyunsaturated fatty acids, Vitamin E, and phytosterols in oat oils, isolate using supercritical carbon dioxide extraction, can lower the concentrations of serum cholesterol⁵. Meanwhile, tartary buckwheat flavonoid, resistant starch, and protein can also suppress plasma cholesterol. The lower digestibility of protein, balanced amino acid composition, the high content of resistant starch in tartary buckwheat groats after cooking, and the relatively high concentrations of flavonoids were all responsible for the cholesterol-lowering effect⁶-⁹. In addition, it has been demonstrated that oat meal and oat flour, could significantly decrease plasma lipid levels¹⁰. The current studies mostly focus on the hypolipidemic effects of the nutritional component in oat and tartary buckwheat. However, considering the high costs of purified β-glucan and extracted oil as well as flavonoid utilizing supercritical carbon dioxide and microwave extraction ap-
approach, it will seriously limit their use\textsuperscript{5,6,11}. In general, it is oat and tartary buckwheat products, not its purified nutritional components, make up our diets. It is, therefore, meaningful to investigate the hypocholesterolemic effects of oat and tartary buckwheat food.

The US Food and Drug Administration claims that the amounts of intake β-glucan to reduce chronic diseases is 3 g/d, which is equal to 100 g rolled oats. Moreover, 120 g tartary buckwheat can satisfy the content of rutin to reduce serum cholesterol levels\textsuperscript{30}. In animal study, More than 600 g oat need to use extract oil to meet 70 g/kg dose to study the hypocholesterolemic effect of oat oil\textsuperscript{20}. In those respects, it can be reasoned that more oat and tartary buckwheat are needed to meet the acceptable daily intake of nutritional components. Despite the contents of nutritional components of oat and tartary buckwheat are lower, oat and tartary buckwheat are composed of many functional components. Thus, it is not clear whether oat and tartary buckwheat food can lower the plasma lipid.

Short chain fatty acids (SCFAs), the principal fermentation products from substrates broken down by gut microbiota, are effective plasma cholesterol-lowering agents\textsuperscript{13}. It has been widely reported that dietary AXs reduced the plasma TC and LDL-cholesterol concentrations by increasing colonic SCFAs\textsuperscript{41}. Oat and tartary buckwheat contain abundant carbon and nitrogen substrates, and also have many other nutritional components to provide nutrition for gut microbiota. Whereas, the effects of oat and tartary buckwheat on the gut microbiota are still poorly understood. And further research is necessary to investigate the effect of gut microbiota changes on the cholesterol-lowering.

In the present work, oat and tartary buckwheat were manufactured, named oat-based food (OF), to feed the diet-induced hypercholesterolemic hamsters. The normal (Control) and hypercholesterolemic hamsters (HFD) were used as controlled designs to evaluate the effects of OF on cholesterol-lowering and the composition of the gut microbiota. And the roles of gut microbiota to lower cholesterol were also discussed.

2 Materials and methods

2.1 Materials

Oat and tartary buckwheat were provided by Hebei Academy of Agriculture and Forestry Sciences (Heibei, China). OF was composed of oat (65 g/100 g), tartary buckwheat (25 g/100 g) and others. Oat and tartary buckwheat were cleaned and tempered overnight to adjust the moisture. Then it was baked and crushed into flour. The contents of protein, lipid, starch, dietary fiber, resistant starch and β-glucan were measured according to the methods as described previously\textsuperscript{4}.  

2.2 Animals and diets

Four-week-old male gold hamsters were chosen in the present study because it was commonly used to form hyperlipidemia model and its hyperlipidemia was consistent with that in clinical\textsuperscript{15}. After acclimatized for 7 days, they were then divided into 3 groups (Control, HFD, and OF, n = 10) and fed with different diets and water ad libitum for 30 days in an air-conditioned room (temperature, 22 ± 2°C; humidity, 60 ± 5%; 12 h light-dark cycle). Animal diets compositions was shown in Table 1. The body weights and the food intakes were recorded every day, and the plasma lipids of hamsters were taken from the eye-veniplex every 10 day. The hamster feces were collected for 7 days before the hamsters were sacrificed. After 30 days feeding, they were sacrificed after 16 h fasting. Livers and the colonic contents were collected and then stored at −80°C for further study. All experiments were carried out in accordance with the P. R. China legislation regarding the use and care of laboratory animals and were approved by the Bioethics Committee of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

2.3 Analysis of metabolic parameters

The concentrations of plasma TC, plasma LDL-cholesterol, plasma high-density lipoprotein cholesterol (HDL-cholesterol), plasma triglycerides (TG), liver TC, liver TG, liver cholesterol ester (CE), liver free cholesterol (FC), fecal bile acids (BA) and fecal TC contents were measured using the method mentioned before\textsuperscript{16}. Fecal SCFAs were determined by ion chromatography (DIONEX ICS-3000, USA) equipped with that in clinical\textsuperscript{15}.

| Sample                  | Control | HFD  | OF   |
|-------------------------|---------|------|------|
| Casein                  | 200     | 200  | 189.5|
| Corn starch             | 397.5   | 397  | 319.6|
| Soybean oil             | 70      | 70   | 62.9 |
| Cellulose               | 50      | 50   | 45   |
| Sucrose                 | 100     | 38.48| 38.48|
| L-Cystine               | 3       | 3    | 3    |
| Maltodextrin 10         | 132     | 132  | 132  |
| t-Butylhydroquinone     | 0.014   | 0.014| 0.014|
| Mineral mix             | 35      | 35   | 35   |
| Vitamin mix             | 10      | 10   | 10   |
| Choline bitartrate      | 2.5     | 2.5  | 2.5  |
| Cholesterol             | 10      | 10   | 10   |
| Bile salt               | 50      | 50   | 50   |
| Lard                    | 2       | 2    |      |

HFD: high fat diet; OF: oat-based food.
with a high-performance capillary column (IonPac AS11-HC, 4 × 250 mm). Acetate, propionate, and butyrate provided by Sigma Co., Ltd (Sigma, USA) were used as standards. Each sample was analyzed in 3 replications.

2.4 Illumina MiSeq sequencing and bacterial data processing

DNA extractions from the colonic contents were performed using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) and evaluated by measuring the optical density (OD) value. The V3-V4 region of the bacteria 16S rRNA gene was amplified with the universal primers of the forward 338 F (5’-ACTTCTACGGGAGGCAGCAG-3’) and the reverse 806 R (5’-GACTACHVGGGTWTCTAAT-3’). These primers contained a set of 8-nucleotide barcodes sequence unique to each sample. Polymerase chain reaction (PCR) reactions were run and its products were purified and quantified. Purified PCR products were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Allwegene, Beijing, China) according to the standard protocols. The extraction of high-quality sequences was firstly performed with the QIIME package (Quantitative Insights Into Microbial Ecology) described by previous reports. OTUs with 97% similarity cutoff were clustered using UCLUST, and the shannon-wiener curves were analyzed by mothur v.1.21.1 to reveal the diversity indices (chao 1 and shannon). The hierarchical clustering was conducted by the Primer 6 software. The dynamics of gut microbial about the control group, HFD, and OF was shown by principal component analysis (PCA) analyzed with Caonoco 4.5. The microbial communities on 16S rRNA abundance were compared in the control group, HFD, and OF.

3 Statistical analysis

The data were represented by the means with standard errors. One-way analysis of variance (ANOVA) was performed using SPSS 19.0 software (Chicago, IL, USA). p < 0.05 was considered statistical significant.

3 Results and discussion

3.1 Nutritional components of OF

Nutritional components in the diet were related to its metabolism of hamsters. The contents of protein, lipid, and

| Table 2 | Effects of OF on the growth and metabolic parameters of hamsters fed a hypercholesterolemic diet for 30 days. |
|---------|--------------------------------------------------------------------------------------------------|
|         | Control     | HFD          | OF          |
| Growth parameters (g) |                  |              |             |
| Initial body weight | 96.19 ± 3.14  | 96.25 ± 2.85 | 96.21 ± 3.10 |
| Final body weight  | 122.11 ± 4.53  | 120.18 ± 1.82 | 143.35 ± 4.73 |
| Body weight gain   | 25.92 ± 1.15  | 24.93 ± 0.74 | 47.14 ± 2.06 |
| Liver weight      | 4.61 ± 0.19   | 4.76 ± 0.21  | 4.97 ± 0.24  |
| Food intake (g/day) | 64.58 ± 3.19  | 67.76 ± 3.50 | 66.54 ± 2.64 |
| Liver (µmol/g)    |                  |              |             |
| TC            | 41.17 ± 1.53   | 44.14 ± 1.74 | 38.22 ± 1.87 |
| CE            | 39.53 ± 1.96   | 42.69 ± 2.06 | 35.63 ± 2.82 |
| FC            | 1.95 ± 0.13    | 2.14 ± 0.19  | 1.83 ± 0.16  |
| TG            | 18.78 ± 1.56   | 22.87 ± 2.04 | 17.99 ± 0.67 |
| Feces          |                  |              |             |
| Feces weight (g/d) | 7.46 ± 0.34    | 7.76 ± 0.34 | 8.42 ± 0.27  |
| Total fat (g/d) | 59.25 ± 1.48   | 45.4 ± 0.57  | 37.65 ± 0.78 |
| TC (µmol/d)    | 26.45 ± 1.91   | 27.95 ± 0.07 | 30.55 ± 1.63 |
| Bile acid (µmol/d)| 3.43 ± 0.10    | 3.75 ± 0.07  | 3.81 ± 0.18  |

Means and standard errors were determined from ten hamsters per group. Different letters among different groups in the same row indicated significant differences at p < 0.05. HFD: high fat diet; OF: oat-based food. TC: total cholesterol; CE: cholesterol ester; FC: free cholesterol; TG: triacylglycerol.
starch in OF were 10.28 g/100 g, 5.56 g/100 g, and 75.08 g/100 g respectively. The content of total dietary fiber was reach up to 4.55 g/100 g. And the contents of β-glucan and resistant starch were 1.74 g/100 g and 1.77 g/100 g, respectively. OF was rich in total dietary fiber, especially β-glucan and resistant starch, which are beneficial for cholesterol-lowering and improving gut microbiota.

3.2 Growth and metabolic parameters of hamsters

It has been demonstrated that β-glucan, protein and oil in oat and flavone in tartary buckwheat could reduce the concentrations of cholesterol [10]. In the present study, oat and tartary buckwheat were mixed and processed into products to investigate its effect on cholesterol-lowering. The growth and metabolic parameters of hamsters were measured and shown in Table 2 and Fig. 1. The final body weight and body weight gain of OF were increased by 17% and 81% respectively, compared to the control group and HFD. In the previous study, it had indicated that there had no association between the body weight gain and fat deposition [10]. Compared with control and HFD, OF had composed with different proteins, amino acids and dietary fiber. The increased body weight might be due to the low feed conversion ratio in OF group. In addition, the diverse structure of gut bacteria might be a response to dietary as a result of different growth performance [10]. Apart from those, there were no difference in growth parameters among the control group, HFD, and OF.

Plasma lipids concentrations at 0, 10th, 20th, and 30th days were analyzed (Fig. 1). The concentrations of TC, LDL-cholesterol, and TG in control were decreased owning to the decreased food intake in order to keep the same food intake with other groups, which were not in this study. The concentrations of TC, LDL-cholesterol, HDL-cholesterol and TG at 0 days had no difference among the control group, HFD, and OF [p > 0.05]. The concentration of TC in hamster fed with HFD was increased significantly with time increased, compared to the control group [p < 0.05] (Fig. 1(A)). When OF were fed to hamsters, the concentrations of TC were decreased from 10th day compared with HFD, and had no significantly difference with the control group in 20th and 30th day. The concentration of plasma LDL-cholesterol in HFD was increased at 20th and 30th day compared to the control group [p < 0.05], and it was decreased in OF compared to HFD and had no significantly difference with the control group (Fig. 1(B)). The concentrations of TC and LDL-cholesterol in the control group and OF (Fig. 1(A) and (B)) at 30th day were significantly lower than HFD [p < 0.05], whereas all other differences in all groups were no-significant (Fig. 1(C) and (D)).

![Fig. 1](image)

**Fig. 1** Plasma TC(A), LDL-cholesterol(B), HDL-cholesterol(C), and TG(D) on the 0, 10th, 20th, and 30th days of hamsters fed with different diet. Mean and standard error were determined from 10 hamsters per group. ■: Control group; ●: HFD; ▲: OF. Different letters among different groups at the same day indicated significant differences at p < 0.05. HFD: high fat diet; OF: oat-based food. LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density lipoprotein cholesterol.
The concentrations of TC, TG, CE and FC in liver and fecal lipids concentrations were measured and the results were shown in Table 2. There were no significantly differences in the levels of FC in liver among the three groups \((p > 0.05)\). Compared with HFD, the concentrations of liver TC, TG, and CE in OF were reduced by 13\%, 21\% and 16\% respectively. The feces weight and the concentrations of TC and BA were 13\% lower than that in the control group (Table 2). Compared with HFD, the concentration of TC in feces was significantly increased in OF \((p < 0.05)\). This results indicated that OF significantly reduced plasma TC, LDL-cholesterol and liver TC, CE, TG concentrations by promoting the excretion of BA and TC. Thus, although the contents of total dietary fiber, protein and oil in OF were lower than the levels that could reduce the cholesterol according to the literatures, it had effect on cholesterol-lowering due to the cooperation of nutritional components. The intake of oat and tartary buckwheat – based food was more economically and practically, compared with the high costs to extract the nutritional components.

3.3 Fecal SCFAs concentrations

SCFAs were the principal fermentation products from undigested dietary substrates broken down by gut microbiota\(^{20}\). It had been demonstrated that SCFAs could lower plasma cholesterol and inhibit hepatic synthesis of cholesterol\(^{20, 22}\). As shown in Table 3, the concentrations of acetate, propionate, and total SCFAs in HFD were 18\%, 24\%, and 18\% lower compared with the control group. The concentrations of butyrate in HFD had no significantly difference \((p < 0.05)\). When fed hamsters with OF, the concentrations of acetate, propionate, butyrate, and total SCFAs were higher than those in control group and HFD \((p < 0.05)\). The levels in OF being 52\%, 62\%, 373\%, and 60\% higher than those in HFD and 24\%, 24\%, 300\%, and 31\% higher than those in the control group for the concentrations of acetate, propionate, butyrate, and total SCFAs respectively. This indicated that OF increased the SCFAs concentrations, which were concerned with cholesterol-lowering. It was well known that dietary fiber could be fermented by gut microbiota to produce SCFAs. And it had also shown that moderate dietary protein could improve gut microbiota and the concentrations of SCFAs\(^{15}\). Increased SCFAs contents, which was increased by high dietary fiber and moderate dietary protein contents in OF, may be one explanation for the hypcholesterolemic effects.

3.4 Overall structure of gut bacteria

The modulating of gut microbiota by ingestion of OF was revealed in this study by characterized gut bacteria structure and composition. The multy samples shannon-wiener curves were measured and shown in Fig. 2(A). It was suggested that all samples were sufficient for the majority of the bacterial communities because they all reached a stable plateau. The OUT numbers of bacterial community, Chao estimate and Shannon index in OF were lower than control and HFD (Fig. 2). In Fig. 2(D), the OUT community comparisons by hierarchical clustering showed that samples in three groups clustered, and OF was separated from HFD. Meanwhile, the control samples could not be well separated with OF and HFD. PCA revealed a substantial inter- and intra-group variation of gut bacteria as a response to samples (Fig. 2(E)). The first two components accounted for 68.85\% of the total variation. Principal component 1 (PC1) interpreted both inter-group and intra-group variations, while principal component 2 (PC2) mainly explained the intra-group variation from hamsters fed with samples\(^{30}\). The compositions of gut bacteria in hamsters fed with OF had relatively small intra-group variation, but hamsters fed with three samples showed great variations.

3.5 Composition of gut bacteria

The compositions of colonic bacteria at the phyla, family, and genus levels were measured and shown in Fig. 3. When HFD was fed to the normal hamsters, there was no significantly difference in the abundance of Bacteroidetes and Firmicutes, compared with control. However, hamsters fed with OF had lower abundance in Bacteroidetes and the Bacteroidetes/Firmicutes ratio \((B/F)\), compared with HFD. The ratio of \(B/F\) in gut bacteria has relationships with

### Table 3

| Sample | Acetate \((\mu g/g)\) | Propionate \((\mu g/g)\) | Butyrate \((\mu g/g)\) | Total SCFA \((\mu g/g)\) |
|--------|-----------------|-----------------|-----------------|-----------------|
| Control | 5.45 ± 0.11b | 0.21 ± 0.02b | 0.13 ± 0.01b | 5.79 ± 0.05b |
| HFD | 4.46 ± 0.02c | 0.16 ± 0.02c | 0.11 ± 0.01c | 4.73 ± 0.12c |
| OF | 6.79 ± 0.27a | 0.26 ± 0.02a | 0.52 ± 0.02a | 7.57 ± 0.29a |

Data are mean ± SD and were determined from ten hamsters per group. Different superscript letters among different groups in the same row indicated significant differences at \(p < 0.05\). HFD: high fat diet; OF: oat-based food. SCFAs: short-chain fatty acids.
lipid metabolism and other metabolic disorders\(^{23}\). The result of OF in B/F ratio seem to contradict with the previous studies. However, it was not if carefully studied. At family level, *Firmicutes* of bacteria community were mainly composed of *Erysipelotrichaceae*, *Eubacteriaceae*, *Ruminococcaceae*, *Lachnospiraceae*, and *Lactobacillaceae*. Compared with HFD, the concentrations of *Erysipelotrichaceae*, *Ruminococcaceae*, *Lachnospiraceae*, and *Lactobacillaceae* were decreased in OF \((p<0.05)\). Similarly, several studies had indicated that the level of *Erysipelotrichaceae* was positively correlated with increased in liver cholesterol, and negatively correlated with fecal cholesterol excretion\(^{24-26}\). *Lachnospiraceae* and *Ruminococcaceae* could degrade cellulose and hemicellulose components and produce SCFAs\(^ {27}\). However, it was also revealed that the abundance of four *Lachnospiraceae* species and three *Ruminococcaceae* species was positively correlated with plasma HDL-cholesterol\(^ {28}\). As can be seen from the present research, in the genus levels, the concentrations of *Ruminococcus-1*, *Ruminococcus-2*, and *Ruminococcaceae-UGG-014* were decreased in hamsters fed with OF, compared with HFD (Fig. 3(C)). Moreover, the increased abundance of *Eubacteriaceae* was also investigated and this may be the main reason for the increased abundance of *Firmicutes*. It was shown that the cholesterol could be metabolized by *Eubacterium* bacteria. And its
productions, coprostanone and coprostanol, could be not absorbed in the intestine\(^{29,30}\).

From the above, it could be deduced that OF could change the compositions of gut microbiota to metabolize the cholesterol. And it also could be utilize by gut microbiota to produce SCFAs and other metabolites to reduce the levels of cholesterol. β-Glucan, resistant starch, and dietary protein that could no digest in the small intestine, entered into the large intestine to ferment that could affect gut microbiota composition and SCFA concentrations\(^{31}\). The results from nutritional compositions showed that the contents of total fiber and protein were 4.55 g/100 g and 10.28 g/100 g respectively, which might be one explanation for the promising effects of OF on the gut microbiota and SCFAs production.

4 Conclusion

The present study clearly showed that OF exerted cholesterol-lowering by inhibiting cholesterol absorption, and promoting excretions of fecal lipid and bile acids. And it also increased SCFAs through modulating the gut microbiota effectively to exert the hypocholesterolemic effects.

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