Wheat Gluten Regulates Cholesterol Metabolism by Modulating Gut Microbiota in Hamsters with Hyperlipidemia

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Abstract: The objective of this research was to evaluate the effect of wheat gluten on gut microbiota from hamsters and also analyse whether alterations in microbiota could result in wheat gluten’s lipid-lowering properties. Four weeks male hamsters were divided into 3 groups (n=10). Two hypercholesterolemic groups were fed for 35 days with hypercholesterolemic diet, containing 20% (w/w) wheat gluten or casein. Wheat gluten significantly reduced serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) concentrations, and also decreased the liver total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE), triglycerides (TG) concentrations. Wheat gluten group had a higher fecal lipids, total cholesterol (TC) and bile acids (BA) than that of casein group (p < 0.05). Moreover, wheat gluten significantly increased total short-chain fatty acids (SCFA) concentrations in feces. Sequencing of 16S rRNA gene revealed that intake of wheat gluten decreased the relative abundances of Firmicutes and Erysipelotrichaceae, but to increased the relative abundances of Bacteroidetes, Bacteroidales_S24-7_group and Ruminococcaceae. The lipid lowering properties of wheat gluten was associated with the lower ratio of Firmicutes/Bacteroidetes, the lower of the bacterial taxa Erysipelotrichaceae and the higher of the bacterial taxa Bacteroidales_S24-7_group and Ruminococcaceae. These results suggest that wheat gluten modulate cholesterol metabolism by altering intestinal microflora.

Key words: wheat gluten, hypocholesterolemia, cholesterol metabolism, gut microbiota

1 Introduction

Obesity is still a major cause of death in developed countries¹, and it can induce several metabolic diseases, such as colorectal cancers, type 2 diabetes and cardiovascular disease (CVD). Human health is largely dependent on diet. Proteins are the main nutrients in the dietary ingredients, and play a very important role in the human health. Compared to plant protein, higher animal protein intake was positively associated with mortality, particularly the red meat and processed red meat. It indicated that the importance of protein source on the human health has been aroused people’s concern². Using soy proteins instead of red meat proteins would reduce the risk of mortality resulting from CVD³. These studies suggest that when compared with animal proteins, plant proteins are more beneficial to human health.

Studies have shown that oat⁴, soy⁵,⁶, rice⁷,⁸, buckwheat⁹ and other plant dietary proteins can effectively reduce serum cholesterol levels and prevent CVD. There is little information available about lipid-lowering effect of wheat gluten on human. However, studies have shown that wheat protein peptide have effects of antioxidant, antihypertensive and immunomodulatory¹⁰,¹¹. Nevertheless, what the exact mechanism of wheat protein reduces plasma lipids has not been fully clarified.

Undigested protein undergoes microbial fermentation,
resulted in produce a series of metabolites, such as short chain fatty acids (SCFAs), Ammoniaceous nitrogen (NH3-N), microbial crude protein (MCP). Among them, SCFAs were including acetate, propionate and butyrate. Acetate can promote the secretion of gastric juice to help digestion, lower cholesterol, dilate the blood vessels, delay atherosclerosis and other functions; propionate can inhibit cholesterol synthesis; butyrate was considered to be the most important source of energy, it contributed to the colon cell of differentiation and proliferation. Moreover, the production of SCFAs can induce the alterations of gut microbiota. Thus, it is hypothesized that the alteration of microbiota could result in wheat gluten’s lipid-lowering characteristic.

In recent years, significant interest has focused on gut microbiota-host metabolism. Because some evidence has revealed that gut microbiota act as an indispensable role in human health and diseases. Diet is a major aspect that can change intestinal microbiota, particularly when transitioning between plant- and animal-based diets. The changes of microbial communities have a wide-ranging of effects on host metabolism and have an important implications for human health. Some evidence suggests that food can play a physiological role by regulating the composition of intestinal microbiota. A number of studies have indicated that the different sources of proteins have a significant effect on the composition of intestinal microflora. The differences among three proteins of casein, soybean protein, and fish protein on intestinal microflora were evaluated. Moreover, effects of the pork protein, beef protein, chicken protein, fish protein, and soybean protein on gut microbiota were compared. These researches have suggested that there were marked differences on the composition of intestinal microbiota from different source of proteins.

The aim of this study was to examine the impact of casein and wheat gluten on the composition of intestinal microflora in hamsters. And determine whether these alterations could lead to the wheat gluten of hypolipemic characteristic. Wheat gluten and casein were fed to the hamsters. After 35 days feeding, the lipids and enzymes concentrations in serum, liver, faeces, the SCFAs of faeces were determined, and the compositions of gut microbiota were examined.

### Table 1 Amino acid composition of casein and wheat gluten (mg/g proteins).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Casein</th>
<th>Wheat gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>52.81</td>
<td>41.00</td>
</tr>
<tr>
<td>Threonine</td>
<td>20.53</td>
<td>17.56</td>
</tr>
<tr>
<td>Serine</td>
<td>33.57</td>
<td>22.63</td>
</tr>
<tr>
<td>Glutamine</td>
<td>142.30</td>
<td>138.48</td>
</tr>
<tr>
<td>Glycine</td>
<td>15.42</td>
<td>22.16</td>
</tr>
<tr>
<td>Alanine</td>
<td>34.96</td>
<td>23.77</td>
</tr>
<tr>
<td>Cystine</td>
<td>10.18</td>
<td>7.95</td>
</tr>
<tr>
<td>Valine</td>
<td>36.22</td>
<td>28.89</td>
</tr>
<tr>
<td>Methionine</td>
<td>13.45</td>
<td>9.29</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>31.69</td>
<td>22.46</td>
</tr>
<tr>
<td>Leucine</td>
<td>48.37</td>
<td>44.00</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>32.28</td>
<td>21.17</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>47.09</td>
<td>32.46</td>
</tr>
<tr>
<td>Lysine</td>
<td>27.51</td>
<td>20.22</td>
</tr>
<tr>
<td>Histidine</td>
<td>19.13</td>
<td>13.69</td>
</tr>
<tr>
<td>Arginine</td>
<td>31.26</td>
<td>39.96</td>
</tr>
<tr>
<td>Proline</td>
<td>104.1</td>
<td>/</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6.58</td>
<td>4.49</td>
</tr>
</tbody>
</table>

### 2 Materials and Methods

#### 2.1 Materials

Wheat gluten was provided by Anhui Ruifuxiang Co., Ltd. (Anhui, China), and it contents (wt%) was 85.46%; Casein was provided by Beijing Nuokangyuan Biotechnology Co., Ltd. (Beijing, China), and it contents (wt%) was 86.88%. Amino acid compositions of casein and wheat gluten was shown in Table 1.

#### 2.2 Animals, diets and sample collection

Every experiments were conducted to the Chinese legislation, what’s more, the selection and rearing of hamsters were approved by the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, and the authorization number of animal experiment was SYXX (JING) 2017-0020. Four weeks old male golden hamsters were acclimatized to the laboratory conditions (12 hours light/dark cycle, and 22 ± 2°C, 55 ± 5% humidity) for 1 week. The hamsters were segmented into 3 groups (Wheat, Control, Casein, n = 10), so that the initial body weight and cholesterol concentration...
Wheat Gluten Regulates Cholesterol Metabolism

Table 2 Composition of the experimental diets (2.5 g/2500 g diet).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control</th>
<th>Casein</th>
<th>Wheat gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Corn starch</td>
<td>993.7</td>
<td>992.5</td>
<td>992.5</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>175</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>Cellulose</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Sucrose</td>
<td>250</td>
<td>96.2</td>
<td>96.2</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>330</td>
<td>330</td>
<td>330</td>
</tr>
<tr>
<td>t-Butylhydroquinone</td>
<td>0.035</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>87.5</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lard</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Bile salt</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
</tr>
</tbody>
</table>

of each group were no significant difference. The hamsters were provided purified water, and fed three kinds of diets for 5 weeks. According to the American Institute of Nutrition (AIN)-93G, three group’s diets were made, and it was shown in Table 2. The experimental diets were prepared by Beijing Nuokangyuan Biotechnology Co., Ltd. (Beijing, China).

The blood were collected on the 0th, 10th, 20th, 30th days. Hamster feces were taken for 3 days before they were slaughtered, the collection of hamster feces for testing of fecal’s weight, total lipids, total cholesterol and bile acids. The animals were fasted for 16 hours before slaughtered. Livers frozen were placed at −20°C. The colonic feces were taken and then kept at −80°C for testing of SCFAs and genomic DNA isolation.

2.3 Determination the metabolic parameters in the serum, liver, feces

Serum lipids concentrations were analyzed by Automatic Chemistry Analyzer (Hitachi, Tokyo, Japan). The liver total cholesterol, free cholesterol, triglyceride levels were measured with Elisa kits provided by Applygen Technologies Co., Ltd. (Beijing, China). The concentrations of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase and cholesterol 7α-hydroxylase (CYP7A1) in liver were measured with Elisa Kits (Applygen Technologies Co., Ltd, Beijing, China). The fecal lipids were determined by the Soxhlet method, fecal total cholesterol was measured using total cholesterol assay kit E1015, fecal bile acids content was measured according to bile acids Elisa kit from Applygen Technologies Co., Ltd. (Beijing, China).

2.4 Determination the concentrations of SCFAs

The concentrations of SCFAs were determined by a gas chromatographic method according to the procedures of Reeves and others with modifications.

2.5 Characterization of the colonic fecal microbiota

2.5.1 DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from samples using the E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s instruction. The quality of extracted DNA was checked by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm ratio). All extracted DNA samples were stored at −20°C for further analysis. The V3–V4 hypervariable regions of the 16S rRNA gene were subjected to high-throughput sequencing by Beijing Allwegene Tech, Ltd (Beijing, China) using the Illumina Miseq PE300 sequencing platform (Illumina, Inc., CA, USA). The V3-V4 region of the bacteria 16S rRNA gene was amplified with the universal primers of the forward 5′-ACTCTACGGGAGGCAGCAC-3′ and the reverse 806 R (5′-GACTACHVGGGTWTCTAAT-3′). These primers contained a set of 8-nucleotide barcodes sequence unique to each sample. The PCR program was as follows 95°C for 5 min, 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension of 72°C for 10 min. PCR reactions were performed in triplicate 25 μL mixture containing 2.5 μL of 10
Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer’s instructions and quantified using QuantiFluor™-STM (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Allwegene, Beijing, China) according to the standard protocols.

2.5.3 Processing of sequencing data

The extraction of high-quality sequences was firstly performed with the QIIME package (Quantitative Insights into Microbial Ecology) (v1.2.1). Raw sequences were selected based on sequence length, quality, primer and tag. The raw sequences were selected and the low-quality sequences were removed: (i) Raw reads were shorter than 110 nucleotides; (ii) The 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, discarding the truncated reads that were shorter than 50 bp exact barcode matching, two nucleotides mismatched in primer matching, reads containing ambiguous characters were removed; (iii) Only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded. The unique sequence set was classified into operational taxonomic units (OTUs) under the threshold of 97% identity using UCLUST. Chimeric sequences were identified and removed using Usearch (version 8.0.1623). The taxonomy of each 16S rRNA gene sequence was analyzed by UCLUST against the Silva 119 16S rRNA database using confidence threshold of 90%.

2.6 Statistical analysis

The differences in the concentrations of lipids in serum, liver, feces and the contents of HMG-CoA reductase, 7α-hydroxylase (CYP7A1) in liver were analyzed using one-way analysis of variance (ANOVA) by SPSS 22.0 software (Chicago, IL, USA). Tukey-Kramer’s multiple comparison post hoc test was also used to express significant differences among three groups. A Mann-Whitney U test was used to assess the differences taxonomy of fecal microbiota. P value of less than 0.05 was considered significant. Rarefaction analysis and alpha diversities were performed using Mothur (version v1.30.1, http://www.mothur.org). Community richness was evaluated by Chao and Shannon. Bray Curtis similarity clustering analysis was performed by R package (R 3.0.2, http://cran.r-project.org/).

3 Results and Discussion

3.1 Effects of proteins on growth parameters and cardio metabolic markers in hamsters

3.1.1 Growth parameters in hamsters

As shown in Table 3, there was no difference in initial body weight and food intake among the three groups (control, casein, wheat gluten), this ensure that the accuracy of the experiment. The hamsters, fed with wheat gluten, showed significantly lower body weight, and liver weight, compared with casein group. We could speculate that the weight loss of hamsters fed the wheat gluten diet due to the wheat gluten is not easily to digest, this was in consistent with the reports of Qiu and others[25], they suggested that wheat gluten utilisation is limited by its low digestibility for its large content of nonpolar amino acid residues and glutamine residues.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Effects of wheat gluten and casein on the growth parameters of hamsters fed a hypercholesterolemic diet.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth parameters (g)</td>
<td>Control</td>
</tr>
<tr>
<td>Initial body weight</td>
<td>94.79 ± 2.85</td>
</tr>
<tr>
<td>Final body weight</td>
<td>117.49 ± 4.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight gain</td>
<td>22.70 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver weight</td>
<td>3.99 ± 0.11</td>
</tr>
<tr>
<td>Food intake (g/group/day)</td>
<td>65.12 ± 3.19</td>
</tr>
</tbody>
</table>

Means and standard errors were determined from ten hamsters per group. Different superscript letters indicate significant differences at p < 0.05.

3.1.2 Cardio metabolic markers of serum and liver in hamsters

Serum lipid concentrations were measured on the 0th, 10th, 20th, and 30th days. Four indexes (TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglycerides) appeared in the trend of first increased then decreased. On the 30th day, casein group had a higher serum total cholesterol, but other indexes were not significant differences.
terol concentration than control group \( p < 0.05 \), this showed that the establishment of the high-fat model group was successful. The group of wheat gluten lowered serum TC concentration significantly \( p < 0.05 \) (Fig. 1A). In addition, as to the concentration of serum of LDL-C in the hamster, wheat gluten was significantly lower than that of casein group on 30th day \( p < 0.05 \) (Fig. 1B). There were not significantly different about the serum of concentrations of HDL-C and TG among three groups \( p > 0.05 \) (Fig. 1C, D).

Liver lipid concentrations of total cholesterol (TC), free cholesterol (FC), cholesterol ester (CE), triglycerides (TG) are shown in Fig. 2. The level of liver TC in casein group was higher than that of control group. The level of liver TC was decreased in wheat gluten group as compared with casein group \( p < 0.05 \) (Fig. 2A). Meanwhile, liver FC concentration in control group were decreased compared with the casein group, but there was no significant difference between wheat gluten group and casein group (Fig. 2B). Liver CE and TG concentrations in wheat gluten were markedly lower than that in casein group \( p < 0.05 \) (Fig. 2C, D).

As shown in Fig. 3, the enzyme of level of CYP7A1 and HMG-CoA reductase in liver were changed after fed with different sources of protein diet. The level of HMG-CoA reductase in wheat gluten was lower than that of casein group \( p < 0.05 \). Moreover, wheat gluten was significantly increased the level of CYP7A1 \( p < 0.05 \).

As we all know, CYP7A1 and HMG-CoA reductase in the liver as rate-limiting enzymes that can regulate the cholesterol synthesis and excretion. Studies shown that the 7a-hydroxylase can transform the cholesterol into bile acid, resulting in the reduction of cholesterol in the liver. And that when inhibits the production of the rate-limiting enzyme HMG-CoA reductase can reduce cholesterol synthesis by hepatocytes catalyzes. We found that the wheat gluten group was significantly increased the level of CYP7A1 and decreased the level of HMG-CoA reductase (Fig. 3). These datas suggest that wheat gluten could play an important roles in the cholesterol metabolism of hamsters with hyperlipidemia.

3.1.3 Fecal lipids and bile acids

Fecal weight did not show significant difference between wheat gluten and casein groups (Fig. 4A). Wheat gluten

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Fig. 1  (A) Serum total cholesterol, (B) LDL-cholesterol, (C) HDL-cholesterol, (D) triglycerides on 0th, 10th, 20th, and 30th day of hamsters fed different sources of dietary protein. Mean and standard error were determined from 10 hamsters per group. Different superscript letters indicate significant differences at \( p < 0.05 \). (Tukey-Kramer’s multiple comparison post hoc test).
and casein group were significantly increased the excretion of fecal lipids compared with the control group \( (p < 0.05) \). There had no significant difference between wheat gluten group and casein group \( (\text{Fig. 4B}) \). Nevertheless, the fecal excretion of cholesterol was increased in wheat gluten group as compared with casein group, moreover casein group was lowered as compared with control group \( (p < 0.05) \) \( (\text{Fig. 4C}) \). What’s more, the concentration of bile acids in the feces from wheat gluten group was higher than in the casein group and control group \( (p < 0.05) \), and casein group was the lowest \( (\text{Fig. 4D}) \).

Nowadays, the mechanisms of wheat gluten decreased
plasma lipids have not been fully clarified. In the small intestine, inhibition of cholesterol absorption could increase the excretion of fecal bile acid, thereby inhibiting the synthesis of cholesterol in the liver, it was considered to be a hypocholesterolaemic mechanism of cholesterol-lowering functional foods. Therefore, we determined the effects of wheat protein and casein on the excretion of fecal cholesterol. As a result, wheat gluten group was significantly increased the concentrations of bile acids and TC (Fig. 4C, D), and wheat gluten intake also had a higher the fecal total lipids (Fig. 4B). These results suggest that wheat gluten could decrease the level of the serum of TC, LDL-C and the liver of TC, as a result of the wheat gluten could promote the excretion of fecal total lipids, TC and bile acids. Moreover, bile acids act as signaling molecules and intermediates between the host and the gut microbiota.

3.1.4 Fecal short chain fatty acids (SCFA)

There were no significant difference in the concentration of acetate among three groups (p > 0.05). But the concentration of propionate in the colonic fecal samples were significantly different three groups (p < 0.05). It was higher in wheat gluten group than those in casein group and control group, and was more in casein group than in control group (p < 0.05). As for the concentrations of butyrate, valerate and total acid were higher in wheat gluten group than those in the casein group and the control group (p < 0.05) (Fig. 5). From Fig. 5D, it can be found that there was more proportion of propionic acid, butyric acid and valeric acid in wheat gluten group than casein group.

SCFAs are the principal fermentation products from substrates broken down by gut microbiota. There was a close association between gut microbiota and host. They maintain energy homeostasis through metabolic products such as short chain fatty acids in the gut. It has been found that wheat gluten group also had a higher the concentrations of propionate, butyric acid, valeric acid and total SCFAs than that of casein group (Fig. 5). Acetate could result in the synthesis of cholesterol and lipid in the liver, but propionate which could inhibit the action of acetate and lowers serum cholesterol levels. Butyrate was re-
ported to decrease the intestinal mucosal permeability; it also can increase transepithelial electrical resistance. Our study indicated that there was a possible association between the intestinal microflora and cholesterol metabolism in hamsters after fed with different source of proteins.

3.2 Effects of proteins on intestinal microbiota

3.2.1 Overall sequence statistics

After size filtering, quality control and chimera removal, 105196 clean tags and 916062 sequences were obtained. On the operation taxonomy unit (OTU) level, 404 OTUs were detected for all 30 hamsters. According to the rarefaction curve analysis (Fig. 6A), all samples reached a steady level, indicating that sampling was sufficient for

Fig. 5 (A) Acetic acids, (B) Propionic acids, (C) Butyric acids, (D) Valeric acids, (E) Total acids, (F) Proportions (%) of the fatty acids to the total acids of feces fed different sources of dietary protein. Mean and standard error were determined from 10 hamsters per group. Different superscript letters indicate significant differences at $p < 0.05$. (Tukey-Kramer’s multiple comparison post hoc test).
most microbiota communities.

The wheat gluten group microbiota community had less OTU numbers, lower Chao estimate and Shannon index than other two groups. While there were no significant differences between control and casein group. Nevertheless, within-group difference of Shannon index in wheat gluten group was smaller than the other two groups (Fig. 6B, C).

The OTU community comparisons by hierarchical clustering showed that samples in three groups clustered, and most of wheat gluten group samples were separated from control and casein group. Control and casein group samples could not be well separated (Fig. 6D). While principal component analysis (PCA) plot observed that all three group samples were apparently clustered separately (Fig. 6E). This indicated that there were significant differences in intestinal flora after intake of different source of proteins.

3.2.2 Colonic bacterial community structure

On the phylum level, the colonic samples from wheat gluten group could be separated from those of control and casein groups. *Firmicutes* and *Bacteroidetes* constituted the vast majority of colonic microbiota in all three diet groups. *Firmicutes* were ranged from 70% to 77%, and *Bacteroidetes* were ranged from 21% to 26%. Furthermore, wheat gluten group had a higher of *Bacteroidetes* and a lower of *Firmicutes* (Fig. 7A). Thus, it resulted in the lowest ratio of *Firmicutes* to *Bacteroidetes* in wheat gluten group. However, wheat gluten group had a significantly higher abundance of *Proteobacteria* (1.63%) than control and casein group (0.5% and 0.6%, respectively) ($p < 0.05$).

The composition and structure of intestinal microbiota plays an important role in nutrition, immunity and disease, such as obesity, type 2 diabetes, and cardiovascular disease (CVD). Numerous studies indicated that the ratio of *Firmicutes* and *Bacteroidetes* to *Proteobacteria* plays a significant role in the development of obesity and type 2 diabetes. The results of this study suggested that wheat gluten could regulate cholesterol metabolism by modulating the intestinal microbiota.

**Fig. 6** Alpha-diversity and similarity of colonic bacterial community of hamsters fed different sources of dietary protein. (A) Rarefaction curve for each sample. (B) The bacterial richness in colon estimated by the Chao 1 value. (C) The bacterial diversity estimated by Shannon index. (D) Hierarchical clustering analysis of colonic bacterial community. (E) Principal component analysis (PCA) of colonic bacterial community on the OTU level.
micutes to Bacteroidetes (the F/B ratio) in intestinal microbiota was associated with the obesity, lipid metabolism and other metabolic diseases. It was reported that the intake of a high fat diet decreased the relative abundance of Bacteroidetes, but increased the relative abundance of Firmicutes in rat feces. However, after fed with wheat gluten, hamsters with hyperlipidemia was found to have an increased relative abundance of Bacteroidetes in feces, but a decreased relative abundance of Firmicutes. And this led to a lower ratio of Firmicutes to Bacteroidetes (F/B ratio) in wheat gluten group when compared with casein group. These results demonstrated that there was a correlation between the ratio of Firmicutes to Bacteroidetes and wheat gluten’s lower cholesterol in hamsters. Researchers indicated that gut microbiota can alter the risk of food allergies. A recent study found that many patients have an immune reaction to wheat, it also suggested that wheat gluten or another substance may alter intestinal permeability, and led to the production of foodborne pathogenic and inflammatory bacteria. This can speculate that the wheat gluten sensitivity may cause the increase of pathogen bacteria, such as Proteobacteria.

At family level, Firmicutes in colon were mainly included the Erysipelotrichaceae, Eubacteriaceae, Lachnospiraceae, Lactobacillaceae and Desulfovibrionaceae. Bacteroidetes mainly consisted of Bacteroidales_S24-7_group and Prevotellaceae. Wheat gluten group had a higher proportion of Bacteroidales_S24-7_group and Ruminococcaceae than the other two groups (p < 0.05) (Fig. 7B). Proportions of Erysipelotrichaceae in wheat gluten group were significantly lower than that of control and casein group (p < 0.05) (Fig. 7B). The proportions of other bacterial families were not significantly influenced by different proteins.

Recent studies showed that bacterial taxa within Erysipelotrichaceae family were highly related to dyslipidemia phenotypes, metabolic syndrome, obesity, and hypercholesterolemia in mice and human. There were strong
correlations between \textit{Erysipelotrichaceae} family, and host cholesterol concentrations in serum, liver, and fecal samples\textsuperscript{50}. Moreover, there was a negative correlation between \textit{Erysipelotrichaceae} and the excretion of fecal cholesterol after fed with sorghum grains in hamsters\textsuperscript{53}. In this research, intake of wheat gluten reduced the relative abundance of \textit{Erysipelotrichaceae} as compared with casein group (\textsuperscript{7B}). Combined with the increases of fecal cholesterol excretion in wheat gluten, these data suggested that the bacterial taxa \textit{Erysipelotrichaceae} and host cholesterol metabolism had a highly correlation.

What’s more, the relative abundance of butyrate-producing bacteria, within \textit{Bacteroidales S24-7 group} and \textit{Ruminococcaceae} families were increased after wheat gluten interventions. Combined with the results of lowering blood lipids, these data suggested that \textit{Bacteroidales S24-7 group} and \textit{Ruminococcaceae} might be a biomarker for improving hyperlipemia.

Down to genus level, \textit{Allobaculum} was the dominant genera and its proportion was more than 20% in all three groups. The colonic samples of wheat gluten group had lower abundance of \textit{Allobaculum} (25.4%) than control and casein group (35.1% and 29.9%, respectively) \textsuperscript{(p<0.05)}. However, wheat gluten group had higher abundance of \textit{Ruminococcus _2} (2.5%) than control and casein group (0.4% and 1.3%, respectively) \textsuperscript{(p<0.05)}, and casein group was observed higher than control group \textsuperscript{(p<0.05)}. While the \textit{Lactobacillus} was more abundant in casein group as compared to control and wheat gluten groups \textsuperscript{(p<0.05)}. In addition, \textit{Desulfovibrio} in colonic content samples were observed more abundant in wheat gluten group (\textsuperscript{7C}). However, approximate half of colonic bacterial community was unidentified at the genus level.

Studies have reported that choline in food can induce gut microbiota to produce Trimethylamine oxide (TMAO), including \textit{Coriobacteriaceae}, \textit{Erysipelotrichaceae} and \textit{Allobaculum}\textsuperscript{54}. As we all know, TMAO can increase the risk of cardiovascular disease. It is hypothesized that wheat gluten may lower the levels of atherosclerotic by lowering \textit{Allobaculum}, \textit{Lactobacillus} and \textit{Bifidobacterium} has been considered as a key player in host metabolic balance\textsuperscript{55,56}. However, it has been found lower level of \textit{Lactobacillus} in wheat gluten group \textsuperscript{(p<0.05)}. A recent study suggested that there may be a link between \textit{Desulfovibrio} (DSV) and intestinal diseases\textsuperscript{57}. But, \textit{Desulfovibrio} in colonic content samples was observed higher abundance in wheat gluten group \textsuperscript{(p<0.05)} (\textsuperscript{7B, C}). Studies have shown that \textit{Ruminococcus} can help cells absorb too much sugars, leading to obesity or overweight\textsuperscript{58}. While wheat gluten had higher abundance of \textit{Ruminococcus}. Therefore, the effects of gut microbiota on cholesterol metabolism may be more complex, not just studying the variation of these flora.

4 Conclution

In summary, wheat gluten could play an important role in cholesterol metabolism in hyperlipidemic hamsters (Fig. 8).
8), and it could decrease the level of serum TC, LDL-C and liver TC. It might partly due to wheat gluten had more excretion of fecal total lipids, TC and bile acids. The data of SCFAs demonstrated that there were closely association between intestinal microbiota and cholesterol metabolism in hamsters treated with different source of proteins. There was a correlation between ratio of Firmicutes to Bacteroidetes and cholesterol metabolism in hamsters. Moreover, the data suggested the strong association between butyrate-producing bacteria (Bacteroidales_S24-7_group, Ruminococcaceae), Erysipelotrichaceae and host cholesterol metabolism. Therefore, it can be concluded that the ability of wheat gluten reduce lipids of hamsters by modulating gut microbiota.

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