Baohe Pill Decoction Regulated Intestinal Mucosal Bacteria of Mice to Cure Diarrhea Caused by High-fat and High-protein Diet

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Received December 01, 2021; Revised January 07, 2022; Accepted January 16, 2022

Abstract The study aimed to investigate the effect of Baohe pill decoction on intestinal mucosal microbiota of mice with diarrhea caused by feeding high fat (HFD) and high protein diet (HPD). Fifteen SPF KM male mice were randomized into three equal groups of five mice, namely Control group (ftcm), Natural recovery group (ftmm), and Baohe pill Treatment group (fttm). The ftcm were general feed diet, ftmm and fttm were treated with HFD and HPD. After successful diarrhea, mice in fttm were gavaged with 0.35mL Baohe pill decoction at a dose of 6.67 g · kg⁻¹ day⁻¹ twice a day, ftmm were given with an equal amount of sterile water. 4 days later, the mice's intestinal mucosa was collected for microbial analysis. Compared to ftcm and ftmm, the OTU number, Chao1, ACE, Simpson and Shannon index increased in fttm (p>0.05). The relative abundance of Proteobacteria, Actinobacteria, and Streptophyta were increased in ftmm, and the Firmicutes, Bacteroidetes, and Verrucomicrobia in fttm were higher than that in the other two groups. The genus of Ralstonia had the highest abundance in ftmm and the lowest abundance in fttm. Additionally, bacterial species such as Lactobacillus (Lactobacillus johnsonii, Lactobacillus murinus, Lactobacillus acidophilus, Lactobacillus intestinalis, Lactobacillus sp. C30An22, Lactobacillus sp. C30An7) in fttm were significantly higher than those of the ftmm, and close to those in the ftcm (p>0.05). Our results indicated that Baohe pill decoction could promote the growth of probiotics Lactobacillus, inhibit the growth of opportunistic pathogens Ralstonia, regulate the balance of gut microbiota, to treat diarrhea caused by HFD and HPD.

Keywords: baohe pill decoction, high-fat and high-protein diet, gut microbiota, 16S rRNA sequencing, diarrhea


1. Introduction

Gut microbiota colonizes and attach to the surface of human intestinal mucosa in a mutualistic symbiosis, constituting the physical barrier to the intestinal mucosa [1]. Gut microbiota is structurally complex and numerous, and more than 50 phyla have been reported, with Firmicutes and Bacteroidetes being the dominant phyla accounting for 90% [2]. While Actinobacteria, Proteobacteria, Verrucomicrobia, and Methanogenic Archaea are less abundant. The gut microbiota of healthy humans maintains a complex dynamic balance with its host, which provides an environment for material metabolism and also serves as a physiological barrier to protect the host intestine from pathogenic bacteria. When the composition and abundance of intestinal flora change, the balance between flora and host is disrupted, leading to the development of disease [3,4].

Many factors can affect the gut microbiota, such as host genetics, diet, environment, and drugs [5]. Dietary factors, especially the dietary proteins and fats, not only the major dietary nutrients but also a source of substances for gut microbial metabolism, are becoming important factors affecting gut microbiota composition, abundance, function, and people's health [6,7]. A new study published in “M Systems”, a journal of the American Society of Microbiology, showed that HFD and HPD could accelerate the reproduction of Clostridium difficile, and inhibit the growth of probiotics such as Lactobacillus and bifidobacteria of mice. Clostridium difficile is closely correlated with diarrhea, which can produce toxin A, toxin B, and binary toxins causing diarrhea or pseudomembranous colitis, about 20% of antibiotic-associated diarrhea is mainly caused by Clostridium difficile [8]. HFD could cause intestinal microbiota dysbiosis, lead to an increase in the number of Bacteroidetes, Actinobacteria, and Proteobacteria, and a decrease in the number of Firmicutes [9,10], a significant increase in the levels of inflammatory factors IL-6, TNF-α, and LPS, which induce intestinal inflammation, disrupting intestinal mucosal integrity, thereby triggering diarrhea.
Additionally, existing evidence also shows that the HPD could lead to the overgrowth of *Clostridium, Enterococcus, Shigella, Escherichia coli*, and *Bacteroides*, at the same time decreased populations of *Bifidobacterium and Roseburia* [12], which would favor the development of a more pathogenic and pro-inflammatory microbial profile.

Dysbiosis of the bacteria induces a range of intestinal diseases. As inflammatory bowel disease, irritable bowel syndrome, colorectal cancer, and antibiotic-associated diarrhea [3, 13], they all have diarrhea as the main clinical manifestation, which is commonly reflected by a reduction of the probiotics and an increment in pathogenic microbiota [14, 15]. Therefore, the key aspect of diarrhea treatment is re-establishing the balance of gut microbiota. Many TCM scholars actively explore the association between intestinal bacteria and diarrhea, a variety of TCM classical famous prescriptions represented by Baohe pill decoction have achieved good results in the treatment of diarrhea [16].

Baohe pill decoction derived from the "Dan xi Xin Fa", and is a classical Chinese medicine prescription for the treatment of food accumulation syndrome caused by improper diet and excessive dietary intake. Modern pharmacological studies have shown that Baohe pill decoction has the effects of promoting digestion, regulating gastrointestinal function, anti-ulcer, and antibacterial [17]. Recent studies have also found that Baohe Pill decoction has a regulatory effect on intestinal bacteria, such as regulating the composition of intestinal flora in dextran sulfate sodium-induced Ulcerative Colitis model mice, increasing the abundance of *A. muciniphila* flora, relieving intestinal inflammation [18]; increasing the number of intestinal *Bifidobacteria* and reduce the Red bug fungus, *Bacteroidetes*, *Bacillus liver fungus*, and *Verrucomicrobia* bacteria in hyperlipidemic rats, thereby reducing TC, TG, and LDL-C [19]. Our research team previously investigated the effect of Baohe pill decoction on the intestinal mucosa of mice fed HFD and HPD from the perspective of *lactobacillus and Escherichia coli* [20], found that it could reduce the number of intestinal bacteria, *Lactobacilli* and *Escherichia coli* in mice. The above results provided strong evidence for the regulatory effect of Baohe pill decoction on gut microbiota, less is known about the effect of Baohe pill decoction on global intestinal mucosal bacteria in mice with diarrhea induced by HFD and HPD.

This study aimed to explore the effect of Baohe pill decoction on intestinal mucosal bacteria in mice with diarrhea caused by HFD and HPD by high-throughput sequencing. Interpret the therapeutic mechanism of Baohe pill decoction in treating diarrhea from the perspective of intestinal microecology, Promote the application of traditional Chinese medicine formula in the treatment of diarrhea.

## 2. Materials and Methods

### 2.1. Animals

Fifteen 4-week-old specific pathogen-free (SPF) Kunming (KM) mice (all mice are male), weighing 18-22 g, were purchased from Hunan Slaccas Jingda Laboratory Animal Co., Ltd (SCXK (Xiang) 2016-0002). Feeding under stable conditions (temperature 23–25°C, relative humidity 50–70%, 12 h light / dark cycles, free access to diet and water) in the laboratory animal center of Hunan University of Chinese Medicine, the study was approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine.

### 2.2. Fodder

The normal fodder is 100% basal feed (protein: 20%, fat: 4%), which was provided by the Laboratory animal center of Hunan University of Chinese Medicine. The high-fat, high-protein diet consisted of Nestlé milk powder (300 g/bag, product execution standard number: GB 19644, 30% protein and 20% fat), Huiyi soybean powder (350 g/bag, product execution standard number: GB/T17878, 33% protein, and 18% fat), Huiyi low gluten flour (1000 g/bag, Product Execution Standard No.: GB/T 8608, 13% protein and 2% fat), and Anhui Lizheng meat pine (500 g/bag, 30% protein, and 25% fat) in a ratio of 1:2:1.

### 2.3. Preparation of Baohe Pill Decoction

Baohe pill decoction: the hawthorn 18g (Hebei, number: HY 20081206), medicated leaven 6g (Sichuan, number: 190503), pinellia ternata 9g (Sichuan, number: 2004260132), poria cocos 9g (Hunan, number: CK20072102), forsythia suspensa 6g (Shanxi, number: HH20070604), pinellia ternata 6g (Sichuan, number: 2004260132), and radish seed 6g (Anhui, number: 1909180412) were weighed. They were purchased from the First Affiliated Hospital of the Hunan University of Chinese Medicine. Put the above medicinal materials into a porcelain pot with 500 mL water, and then filter after boiling for 30 minutes. Then add 400 mL water to the residue, continue to boil for a further 15-20 minutes, the 2 decoctions were mixed, evaporated, and concentrated into 180 mL (0.28 g/mL crude drug) and preserved at 4°C.

### 2.4. Experimental Design

After adaptive feeding for 2 days, fifteen Kunming mice were randomly divided into three groups with five males in each group: Control group (fcmc), Natural recovery group (ftmm), and Baohe pill decoction treatment group (ftm). Diarrhea model was established according to the method explored by previous studies [21, 22]. The mice in the ftmm and ftm were fed with HFD and HPD, then gavaged with vegetable oil (0.4 mL) from day 4, twice a day for 3 days, and the mice in ftcm were gavaged with the same amount of sterile water. When mice in ftmm and ftm were successfully developed diarrhea, the ftmm was gavaged with 0.35mL Baohe pill decoction at a dose of 6.67 g·kg⁻¹·day⁻¹ twice a day, mice in the ftcm and ftm were gavaged with an equal amount of sterile water. After 4 days of treatment, all the mice were sacrificed by cervical dislocation. The small intestine mucosa in each group was collected under sterile condition [23]. After extruding the intestinal contents, the wall of the small intestine was washed with sterile saline.
to remove the contents and fat adhered, and then the intestinal mucosa was scraped by using coverslips and cooled to – 80°C refrigerator.

2.5. DNA Extraction and PCR Amplification

Intestinal mucosal bacterial metagenomic DNA was extracted with CTAB or SDS, the full-length bacterial 16S rRNA gene sequence was amplified, using the extracted DNA as a template. Primer pair 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-ACCTTGTTACGACTT-3') were provided by Shanghai Pesseno Biotechnology Co., Ltd. (Fig. http://www.personalbio.cn/). The amplification program was: pre-denaturation at 98°C for 2 min, 25–30 cycles (denaturation at 98°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s) and a final extension at 72°C for 5 min. Amplification system: The PCR amplification system included 2.0 µL of dNTPs (2.5 mmol L−1), 5.0 µL of 5×Q5 reaction buffer, 5.0 µL of 5× Q5 high enhancer, 1.0 µL of forwarding primer (10 µmol L−1), 1.0 µL of reverse primer (10 µmol L−1), 0.25 µL of Q5 polymerase (5 U µL−1), 2.0 µL of template DNA (0.2 ng µL−1), and 8.75 µL of sterilized ddH2O. Measured the PCR products by 2% agarose gel electrophoresis, The target fragment was recovered with AxyPrepDNA Gel Extraction Kit (AXYGEN Company) and quantified using QuantiFluorTM-ST Handheld Fluorometer with Blue Channel (Promega Compan).

2.6. Bioinformatic Analyses

Selected sequences were clustered into operational taxonomic units (OTUs), with a threshold similarity of 97%, were rowed into one OTU, the OTU table finally used for analysis is obtained [24]. The abundance of bacteria was reflected with Chao1 (http://scikit-bio.org/docs/latest/generated/skbio.diversity.alpha.chao1.html) and ACE (http://scikit-bio.org/docs/latest/generated/skbio.diversity.alpha.ACE.html), Simpson (http://scikit-bio.org/docs/latest/generated/skbio.diversity.alpha.simpson.html) and Shannon (http://scikit-bio.org/docs/latest/generated/skbio.diversity.alpha.Shannon.html) are the indexes that reflect community diversity. Principal component analysis (PCA), multidimensional scaling (NMDS), and clustering analysis were carried out by R package (https://www.R-project.org/) to analyze the main distribution characteristics and the similarity of community samples.

2.7. Statistical Analysis

SPSS 21.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Data were expressed as mean±SE. Significant differences in the variance of parameters were evaluated either with ANOVA or Kruskal-Wallis test, depending on the data normality distribution. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Behavioral Changes

Mice in the ftcmm had normal food intake, mental state, and weight intake, and had brown stools and smooth coat color; Compared with the ftcmm, the mice in the fttm showed darken fur, poor mental condition, abdominal distension, wet and soft feces, and dirty perianal area, half of the mice successfully developed diarrhea after three days of HFD and HPD feeding. Compared with the fttm, the mental status and feces of the mice in the fttm gradually returned to normal after the intervention of Baohe Pill decoction for 4 days.

![Figure 1](image.png) Effects of Baohe pill decoction on the number of intestinal mucosal bacteria OTUs in mice fed with HFD and HPD. (n=5). The larger the number was, the more the number of intestinal microbes. a: OTU numbers. b: Mean OTUs at each classification level.
3.2. Effects of Baohe Pill Decoction on the Number of Intestinal Mucosal Bacteria OTUs in Mice Fed with HFD and HPD

As shown in Figure 1, there were 192 OTUs in the ftcm, 121 in the ftmm, and 212 in the fttm. Compared to the ftcm and ftmm, the number of OTUs observed at the bacterial phylum, class, order, family, genus, and species levels were higher in the fttm. Among them, the number of OTUs at each taxonomic level of the ftmm was the lowest, which was close to that of the ftcm. These results suggested that Baohe pill decoction had a restorative effect on the intestinal mucosal bacteria diversity in mice with diarrhea caused by HFD and HPD, after removing the HFD and HPD diet intervention, the diversity of intestinal mucosal bacteria in mice has decreased but gradually returned to normal levels with time.

Figure 2. Sparse curves (The abscissa represents the total number of sequences randomly selected per sample, and the ordinate represents the number of OTUs found at the corresponding depth. OTU numbers. Ftcm 1-5: Control group; ftmm 1-5: Natural recovery group; fttm 1-5: Baohe pill decoction treatment group.)

Figure 3. Effects of Baohe pill decoction on intestinal mucosal bacteria in diarrhea mice induced by feeding HFD and HPD. (n=5) Alpha diversity was evaluated based on the Shannon (a) and Chao1 (b) indices of the OTU levels. a Chao1 index. b ACE index. C Shannon index. d Simpson index. Data were no statistical differences between the above (p>0.05)
3.3. Alpha Diversity Analysis

Sparse curves are used for the detection of sequencing depth, to measure the diversity of each sample to a certain extent. The smoothness of the curve in the sparse curve reflects the effect of sequencing depth on the diversity of each sample. As shown in Figure 2, the sparse curve fattens after 100 bps each curve gradually tends to be flat, indicating that the amount of sequencing data of our study is large enough to estimate the vast majority of microbial species information in the sample. The Alpha diversity index includes Chao1, ACE, Simpson, and Shannon index, which can estimate the richness and diversity of intestinal mucosal for a [25]. Chao1 and ACE indexes show the abundance of intestinal mucosal fora (Figure 3a, b), Shannon and Simpson's indexes reflecting the community diversity (Figure 3c, d). As shown in Fig 3, compared with the ftcm, the Alpha diversity was increased in the ftmm and fttm, but there were no significant changes among the three groups ($p > 0.05$). These results indicate that both HFD, HPD, and Baohe pill decoction treatment can increase the diversity of intestinal mucosal bacteria in mice.

3.4. Beta Diversity Analysis

Beta diversity can comparative analysis of the microbial community composition of different samples. In Beta diversity, principal component analysis (PCA) was used to reflect the difference and distance of sample composition in each group [26], Unweighted UniFrac measure was used to presence/absence of bacteria or OTUs to compare community composition [27]. We can know from Figure 4, most of the samples in the ftmm were close to each other and could be significantly distinguished. Compared with the ftmm, each sample in the ftcm was more scattered, suggesting that the difference within the samples may be relatively large. The reasons may be affected by individual differences in animals, anatomical sampling, DNA extraction, sequencing on the machine, and other factors. The results showed that the difference was the largest between the ftcm and fttm. However, there was little difference between the ftcm and the fttm, which was consistent with the OTU classification results and Alpha diversity results.

3.5. Effect of Baohe Pill Decoction on the Taxonomic Composition of Intestinal Mucosal Bacteria OTUs in Mice Fed with HFD and HPD

From the gene sequences, OTUs were identified into 9 prokaryotic phyla, (the sequences that could not be classified into any known groups were specified as unidentified). From Figure 5 a, the relative abundance of 9 phyla varied between three groups. The dominant (relative abundance>5%) phyla in ftcm, ftmm and fttm were Proteobacteria (55.6% vs. 59.94%vs. 45.73%), Firmicutes (32.95% vs. 25.80%vs. 41.71%).

![Figure 4. Principal component analysis (PCA) of intestinal mucosal microbiota. (n=5) (Points of different colors or shapes represent sample groups under different conditions. The scales on the horizontal and vertical axes are relative distances. The more similar the sample composition, the closer the distance reflected in the PCA graph. Data were no statistical difference ($p>$0.05))](image-url)
At the genus level, a total of 75 genera of bacteria were detected from samples of three groups. Figure 5b is the relative abundance of the top 20. The dominant (relative abundance $>1\%$) genera in ftcm, ftmm, and fttm were *Ralstonia* (53.32\% vs. 56.05\% vs. 43.32\%), *Lactobacillus* (12.27\% vs. 8.67\% vs. 11.03\%), and *Afipia* (1.76\% vs. 1.71\% vs. 1.68\%).

Additionally, at the species level, a total of 134 species were detected from samples of three groups. In Figure 5c is the relative abundance of the top 20. Compared with the fttm, the relative abundance species of reduction in the ftmm were a filamentous bacterium, *Lactobacillus johnsonii*, *Limosilactobacillus reuteri*, *Lactobacillus murinus*, *Staphylococcus lentus*, *Limosilactobacillus sp. BG-AF3-A*, *Staphylococcus lentus*, *Limosilactobacillus sp. c9Ua_26_M*, *Pelomonas saccharophila*, and *Bacterium cL10-2b-4*. The species increased in the ftmm were *Ralstonia insidiosa*, *Bacterium MNFS-9*, *Afipia genosp. 1*, *Ralstonia sp. 1F2*, *Bacillus cereus group*, *Bradyrhizobium sp.*, *Ralstonia pickettii*, *Solanum nigrum* and *Aerococcus urinaeequi*.

<table>
<thead>
<tr>
<th>OTU No.</th>
<th>genus</th>
<th>species</th>
<th>Contribution value</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>otu3786</td>
<td><em>Ralstonia</em></td>
<td><em>Ralstonia pickettii</em></td>
<td>0.005132</td>
<td>0.005500</td>
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<tr>
<td>otu352</td>
<td><em>Bradyrhizobium</em></td>
<td><em>Bradyrhizobium sp.</em></td>
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<td>0.006021</td>
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<td>otu1459</td>
<td><em>Lactobacillus</em></td>
<td><em>Lactobacillus murinus</em></td>
<td>0.002473</td>
<td>0.003672</td>
</tr>
<tr>
<td>otu2792</td>
<td><em>Ralstonia</em></td>
<td><em>Ralstonia sp. 1F2</em></td>
<td>0.000645</td>
<td>0.000951</td>
</tr>
<tr>
<td>otu1299</td>
<td><em>Ralstonia</em></td>
<td><em>Ralstonia insidiosa</em></td>
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<td>0.000968</td>
</tr>
<tr>
<td>otu1450</td>
<td><em>Pseudomonas</em></td>
<td><em>Pseudomonas sp.</em></td>
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<td>0.000871</td>
</tr>
<tr>
<td>otu631</td>
<td><em>Lactobacillus</em></td>
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</tr>
<tr>
<td>otu1302</td>
<td><em>Ralstonia</em></td>
<td><em>Ralstonia insidiosa</em></td>
<td>0.000164</td>
<td>0.000709</td>
</tr>
<tr>
<td>otu1349</td>
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<td><em>Staphylococcus lentus</em></td>
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<td>otu2628</td>
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<td><em>Lactobacillus intestinalis</em></td>
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<td>otu959</td>
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<tr>
<td>otu2245</td>
<td><em>Phocaeicola</em></td>
<td><em>Bacteroides coprocola DSM 17136</em></td>
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<td>0.000133</td>
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<tr>
<td>otu2473</td>
<td><em>Paenirhodobacter</em></td>
<td><em>Paenirhodobacter enshiensis</em></td>
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<td>0.000221</td>
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<tr>
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<td><em>Stenotrophomonas</em></td>
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<td>otu826</td>
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<td><em>Lactobacillus johnsonii</em></td>
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<td>0.001207</td>
</tr>
</tbody>
</table>
3.6. Key Biomarker for Baohe Pill Decoction on the Mice Fed with HFD and HPD

As shown in Table 1, the main genera contributing greatly in the experiment of Baohe pill decoction on intestinal mucosal microbiota in mice with diarrhea induced by feeding HFD and HPD were Ralstonia (0.006607), Lactobacillus (0.003315) and Bradyrhizobium (0.002598). The corresponding species are mainly Ralstonia pickettii, Ralstonia insidiosa, Ralstonia sp. 1F2, Lactobacillus johnsonii, Lactobacillus johnsonii, Lactobacillus intestinalis, and Bradyrhizobium sp.

4. Discussion

Dietary habits have a huge impact on the formation of the gut microbiota pattern, changes in the composition and function of intestinal bacteria have gradually become potential new targets for the prevention and treatment of diarrhea. Therefore, clarifying the association between diet, intestinal bacteria, and diarrhea is a prerequisite for treating it. Traditional Chinese medicine (TCM) attaches importance to the overall regulation of the body to achieve the effect of treating both the symptoms and the root causes and has a long history of application. Chinese herbal medicines are rich in substances such as fibers, polyphenols, and polysaccharides, and most herbs or formulas are orally administered and interact with bulky intestinal bacteria in the gastrointestinal tract, to achieve the effect of regulating intestinal bacteria and relieving inflammatory inflammation [28].

The main mechanisms of action of intestinal bacteria on the body are endotoxin mechanism, short-chain fatty acid mechanism, bile acid mechanism, and gene expression mechanism [13]. A high-fat diet stimulates inflammatory signaling pathways, High levels of fatty acids and inflammatory mediators are the key factors inducing colitis [29,30]. Long-term HPD can also disrupt the intestinal micro ecological balance, mainly as shown by increased abundance of Bacteroidetes, Proteobacteria, and Actinobacteria [31]. In our experiment, compared with ftcm, the diversity of intestinal mucosal bacteria in mice was more abundant after being fed with HFD and HPD, which also means that the intestinal bacteria are overgrown. Specifically, the relative abundance of Proteobacteria, Actinobacteria, Bacteroidetes, and Streptophyta were increased and the Firmicutes was decreased, which was consistent with the previous studies [10]. Further analysis at the genus level revealed that HFD and HPD mainly increased the relative abundance of genera belonging to the phylum of Proteobacteria (Afipia, Ralstonia, and Bradyrhizobium), and both Firmicutes and Bacteroidetes increased after Baohe pill decoction intervention, and further analysis at the genus level revealed that it mainly increased the relative abundance of genera belonging to the phylum of Firmicutes (Lactobacillus), among them, the species and abundance of Lactobacillus johnsonii, Lactobacillus murinus, Lactobacillus acidophilus, Lactobacillus intestinalis, Lactobacillus sp. C30An22 and Lactobacillus sp. C30An7 was significantly increased and similar to the ftcm. Lactobacillus species are common probiotics, animal studies and clinical findings suggest that they help prevent and treat a variety of gastrointestinal diseases [32]. Among them, Lactobacillus johnsonii is resistant to gastric and bile acids and can inhibit the adhesive ability of pathogens on the host intestinal mucosa [33], Lactillobacus murinus reduces the number of cells associated with an inflammatory response — TH17 [34]. Remarkably, Helicobacter, the dominant bacteria caused by the modeling, was even not detected in the ftcm. This can be speculated that although both HFD and HPD modeling, as well as Baohe pill decoction treatment, can increase the diversity of intestinal mucosal bacteria in mice, the emphasis is different between the two. HFD and HPD mainly increase the species and number of a variety of opportunistic pathogens, while Baohe pill treatment can increase the abundance of beneficial flora and genera. And Baohe pill treatment may inhibit the development of inflammatory flora during diarrhea and thus relieve diarrhea.

Baohe pill decoction consists of seven classical herbs of hawthorn, medicated leaven, pinellia ternata, poria cocos, clay bark, forsythia suspensa, and radish seed. Hawthorn powder, the main component of Baohe pill decoction, can regulate blood lipids in HFD model mice by changing the gut microbiota in mice [35]; and another classical herb in Baohe pill decoction, massa medicata fermentata, was found to increase the amount of intestinal Lactobacillus, Bifidobacteria, and Bacteroides and achieve the purpose of promoting gastrointestinal propulsion in food accumulation mice [36]. From this point of view, Baohe pill decoction can regulate intestinal bacteria, the regulatory effect of Baohe pill decoction on intestinal bacteria is mainly reflected in the inhibition of a variety of opportunistic pathogens, to increasing the abundance of beneficial bacteria and subordinate species.

Random forest analysis showed that Ralstonia, Akkermansia, Helicobacter, Duncaniella, Parasutterella, and Muribaculum contributed greatly to the diarrhea model mice induced by HFD and HPD. Ralstonia, Lactobacillus, and Bradyrhizobium contributed greatly to the Baohe Pill decoction treatment. The genus in which the two intersected was Ralstonia. Studies have found that the Ralstonia genus is opportunistic pathogens capable of causing bacteremia, meningitis, and septic arthritis [37]. More importantly, whether it is modeling experiment or treatment experiment, some species under Ralstonia have higher abundance after feeding HFD and HPD, such as Ralstonia insidiosa, and Ralstonia picketti, which are waterborne bacteria that can survive and grow in various water sources, have recently been considered as emerging pathogens of infectious diseases, in particular as the pathogens responsible for nosocomial infection in immunocompromised patients [38]. Therefore, it is speculated that Ralstonia may be a key genus to diarrhea caused by HFD and HPD, more in-depth validation experiments are needed to establish characteristic bacteria associated with diarrhea caused by HFD and HPD, at the same time, Helicobacter, Akkermansia, and Lactobacillus should be included in the study.
5. Conclusions

To sum up, in our study, Baohe pill decoction can regulate intestinal mucosal bacteria of mice to cure diarrhea caused by a high-fat and high-protein diet. Its regulatory effect is mainly reflected in the inhibition of opportunistic pathogens Ralstonia, to increase the abundance of probiotics Lactobacillus. So, the Baohe pills decoction has a good application prospect and in-depth research value in the treatment of diarrhea caused by a high-fat and high-protein diet.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81874460).

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Author Contributions

Author contributions were as follows: study design (Zhoujin Tan), data collection (Tao Zheng), statistical analysis (Lili Huang, Yawei Liu), data interpretation (Lili Huang, Tan Guo), manuscript preparation (Lili Huang), and funds collection (Zhoujin Tan, Tan Guo). All authors have read and approved the final manuscript.

Human and Animal Rights Statement

The study was approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine (LL2020062302).

References


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