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Research Article

Effects of Black Quinoa Polysaccharides on Obesity and Intestinal Flora Dysbiosis in T2DM Mice

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To explore the mechanism of the hypoglycemic effect of BQP in type 2 diabetic mice induced by the combination of high-fat diet (HFD) and streptozotocin (STZ). Treatment with BQP significantly ameliorated blood glucose and lipid levels and improved oxidative stress levels and liver injury levels. The results of the intestinal flora study showed that the concentration of short-chain fatty acids (SCFAs) in cecum contents of BQP group mice was significantly higher than those of the diabetic mice. BQP treatment improved microbial disorders in feces, altered the diversity of intestinal flora, reduced the *Bacteroidota* to *Firmicutes* (B/F) ratio in diabetic mice, and increased the abundance of *Dubosiella*, *Akkermansia*, *Faecalibaculum*, and *Allobaculum*. Spearman correlation analysis showed that changes in intestinal microorganisms were closely related to biochemical parameters. The function's prediction of gut microbiota indicated that the microbial compositions involving chemoheterotrophy, aerobic chemoheterotrophy, Gram-negative, potential pathogenicity, and sulfur cycle were changed. *Conclusion*. Intake of BQP can effectively regulate the blood glucose level of diabetic mice through improving glucose and lipid metabolism, and its mechanism may be related to the improvement of obesity and intestinal microecological balance.

1. Introduction

The incidence of type 2 diabetes mellitus (T2DM) is increasing every year as people's lifestyles change (high-fat food intake and sedentary lifestyle) [1]. In addition, the increase in the number of patients with T2DM has put tremendous pressure on healthcare services [2]. The economic burden of diabetes is mainly due to the development of diabetes-specific complications, including retinopathy, nephropathy, neuropathy, and cardiovascular disease [3]. The glucose-lowering drugs currently on the market have certain therapeutic effects, but they have been also proved to have some inevitable side effects [4]. Therefore, natural, efficient, and less toxic compounds have become the focus of researchers' attention.

Polysaccharides are a class of structurally diverse macromolecules. They have attracted much attention from scholars because of their antidiabetic, antioxidant, and immunomodulatory functions, as well as their ability to reduce the toxicity, teratogenicity, and potential carcinogenicity of synthetic chemical drugs [5, 6]. An increasing number of studies have shown that polysaccharides can be involved in changes in the composition and function of the intestinal flora, which is a large microbial community in the intestine, and that polysaccharides can influence the glucose homeostasis of the host by improving the intestinal flora [7–10]. Meanwhile, polysaccharides can ferment in the intestinal flora and alleviate T2DM by producing metabolites such as SCFAs [11, 12].

Quinoa is an annual herb native to the Andean region of South America and has been introduced in recent years as a cold- and drought-tolerant plant for cultivation in many countries [13]. In addition to the main nutritional ingredient carbohydrates, quinoa also contains many phytochemicals, including phenolic compounds, bioactive peptides, and polysaccharides [14]. Quinoa polysaccharide has been

reported to have antioxidant and antidiabetic activity and immunomodulatory activity against RAW264.7 cells [15]. However, there are fewer studies on the ameliorative effects of BQP on type 2 diabetic mice.

The present study was undertaken to investigate the antidiabetic properties of the polysaccharide from black quinoa in HFD and STZ-induced T2DM models. Furthermore, the effect of polysaccharides on the imbalance of intestinal flora in T2DM was investigated by 16S rRNA.

2. Materials and Methods

2.1. Materials. STZ was obtained from Beijing Coolaber Technology Co., Ltd. Basal feed and HFD were obtained from Shenyang Maohua Biotechnology Technology Co., Ltd. Black quinoa was obtained from Golmud Namu Blue Trading Co., Ltd. (Qinghai, China).

2.2. Preparation of BQP. Some modifications were made to BQP extraction as described by Ren and Liu [16, 17]. After crushing black quinoa, soaked in petroleum ether to degrease and filtered. The filtrate was soaked in 95% ethanol for 24 h and then filtered. After drying, the filter residue was soaked in distilled water (1 : 21, w/v) for 2 h at room temperature and then placed in an ultrasonic device. The temperature was 61°C for 51 min. After removing the protein with trypsin. Collect the supernatant, and then adjust the pH to 7.0 and concentrate. Soak in 95% ethanol at 4°C for 1 day. The precipitate was dissolved in an appropriate amount of distilled water, left for dialysis for 3 days, and then freeze-dried to obtain BQP.

2.3. Chemical Composition Analysis. The polysaccharide content was measured by the phenol-sulfuric acid method [18]. The monosaccharide composition was determined by high-performance liquid chromatography [19]. Molecular weight (Mw) of BQP was determined by gel permeation chromatography (GPC) [20]. The molecular morphology of the polysaccharides was characterized by SEM [19].

2.4. Animal and Experimental Design. Eight-week-old male C57BL/6 mice (21 ± 1 g) were acquired from Liaoning Changsheng Biotechnology Co., Ltd. (Liaoning, China, SCXK2020-0001). All operations were approved by the Animal Care Committee of Heilongjiang Bayi Agricultural University and carried out as per ethical standards (Reference number: SPXY2023008).

All mice were caged at 24 ± 2°C with lights on from 8:00 a.m. to 8:00 p.m. After 1 week of acclimation, 28 mice were randomly selected to be fed HFD for 6 weeks and then injected with STZ (50 mg/kg). Injection was given once every day for 3 consecutive days. Mice were free to eat and drink during the modeling period. After 3 days of modeling, the mice were made to fast on water for 6 h, and fasting blood glucose (FBG) measured by tail-tip blood ≧ 11.1 mmol/L was considered successful modeling. After successful modeling, the mice were divided into 4 groups ($n = 7$): NC group (treated with basal diet), diabetic control (DC) group (treated with HFD), BQP-L group (treated with HFD + BQP 400 mg/kg), and BQP-H group (treated with HFD + BQP 800 mg/kg).

During the experiment, mice were free to eat and drink. After 28 days of BQP intervention, the mice were anesthetized and dissected after fasting for 12 h. Blood was taken from the abdominal aorta and centrifuged. The liver was cleaned with saline. Serum, liver, and fecal contents were stored at −80°C (Figure 1).

2.5. Determination of Body Weight (BW) and Oral Glucose Tolerance Test (OGTT). The BW, water intake, and food intake of mice were measured every 7 days. After 21 days of oral administration, the mice were made to fast for 12 h, and the glucose solution was administered at a dose of 2 g/kg. Tissue weight was divided by mouse BW to calculate liver and heart indices [4]. The blood glucose concentration was measured at 0, 30, 60, 90, and 120 min, and the area under the curve (AUC) was calculated.

2.6. Analysis of Fasting Blood Glucose (FBG), Glycated Hemoglobin (GHb), Insulin (INS), and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). FBG was measured from the tail vain after fasting for 10 hours a week. The levels of GHb and INS were measured according to ELISA instructions (Shanghai Enzyme-Link Biotechnology Co. Ltd.). HOMA-IR was calculated using the following formula [21]:

$$\text{HOMA-IR} = \frac{\text{FBG (mmol/L)} \times \text{INS (mIU/L)}}{22.5}. \quad (1)$$

2.7. Determination of Lipid Parameters. The serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were measured with commercial kits (Nanjing Jiancheng Bioengineering Institute).

2.8. Determination of Oxidative Stress. Malondialdehyde (MDA), nitric oxide (NO), and glutathione peroxidase (GSH) levels were measured according to the kit instructions (Nanjing Jiancheng Bioengineering Institute). The catalase (CAT) assay was performed using kit instructions (Beijing Solarbio Science & Technology Co., Ltd.).

2.9. Determination of Inflammation and Liver Injury. Interleukin-1β (IL-1β) was measured according to the ELISA instructions with the standard (Shanghai Enzyme-linked Biotechnology Co. Ltd., Shanghai China). The assessment of liver function was performed by detecting alanine transaminase (ALT) activity using commercial kits (Nanjing Jiancheng Bioengineering Institute).

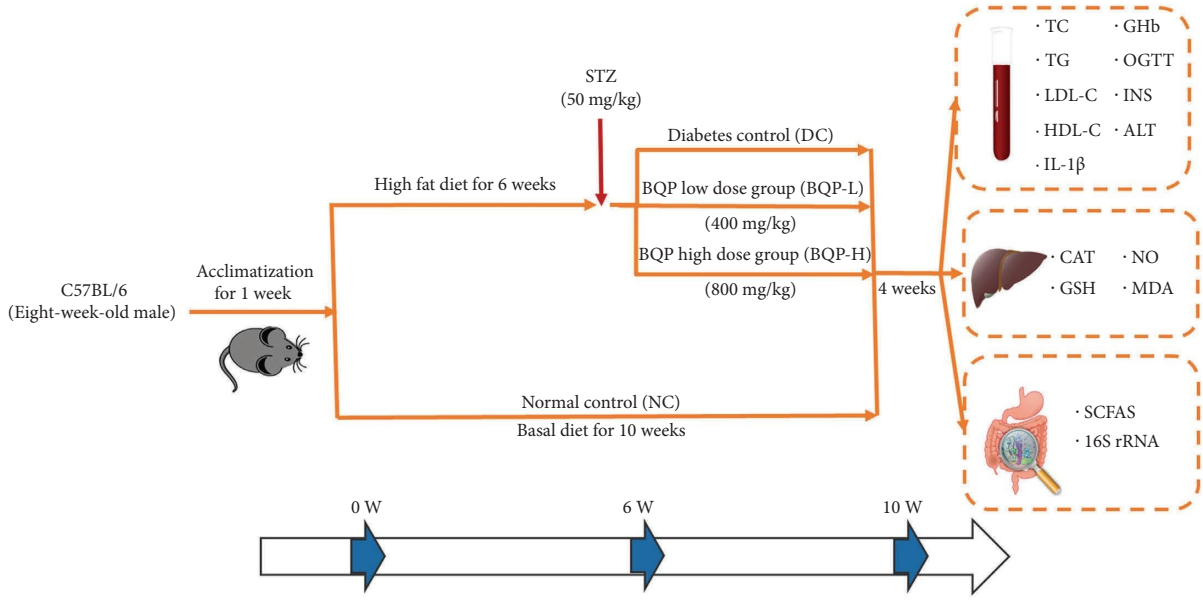


Figure 1: Animal experimentation.

2.10. Quantification of SCFAs in Cecal Samples. The mouse feces weighing 0.2 g were added to 0.24 mL 2.5 mM sulfuric acid solution, and the 0.2 mL supernatant was obtained after centrifugation. Adding 25% (V/V) metaphosphoric acid solution 0.04 mL, fully mixed and placed in refrigerator overnight. Then it was completely thawed, and the sample was centrifuged at. The supernatant was filtered by a 0.22 μm aqueous membrane for high-performance liquid chromatography (HPLC) analysis. The contents of SCFAs were determined using an Agilent 1200 high-performance liquid chromatographer equipped (Agilent Technologies Co. Ltd.).

2.11. Gut Microbiota Analysis. After genomic DNA was extracted from feces, 1% agarose gel electrophoresis was used to check the DNA extract, and DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′) by using an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by Fastp, and merged by FLASH. Operational taxonomic units (OTUs) with 97% similarity cutoff were clustered using UPARSE. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (https://rdp.cme.msu.edu/) against the 16S rRNA database using a confidence threshold of 0.7.

2.12. Statistical Analysis. All values were presented as the mean ± SD for each group. Statistical analysis was performed using SPSS 26.0.

3. Results

3.1. Physicochemical Characteristics of BQP. The total sugar content of BQP was 78.23 ± 0.38%. The relative percentages of monosaccharide composition of BQP were 0.560% (mannose), 0.418% (ribose), 0.467% (rhamnose), 1.889% (glucuronide), 0.388% (galacturonic acid), 91.169% (glucose), 2.512% (galactose), 0.305% (xylose), 2.031% (arabinose), and 0.262% (fucose) (Figure 2(a)). The GPC spectrum showed a peak in BQP (15.823 min). Mw was 8.087×10^3 Da (Figure 2(b)). At low magnification, the BQP consists of irregular and fragmented structures. As the magnification increases, it can be observed that the BQP surface is rough and forms irregular aggregates with abundant porosity (Figure 3).

3.2. Effects of BQP on BW, Food Intake, Water Intake, and Organ Weight. The BW, water intake, food intake, heart index, and liver index of mice in the DC group were increased. After 4 weeks of administration, the BQP of both groups inhibited the upward trend, and the BQP-H effect was better. The results showed that BQP could improve obesity, food intake, water intake, and organ index in diabetic mice (Table 1).

3.3. Effects of BQP on FBG, GHb, INS, and HOMA-IR. FBG, GHb, INS, and HOMA-IR were significantly higher in the DC group than in the NC group, indicating that the DC mice developed glucose metabolism disorders and insulin

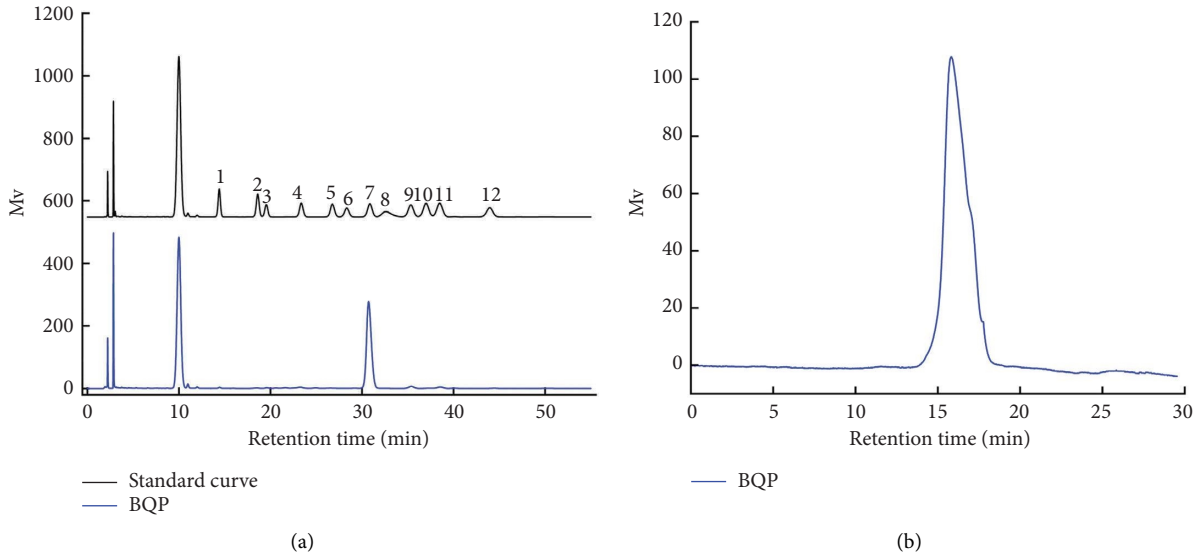


Figure 2: Chemical composition of BQP. (a) Standard curve of monosaccharide composition and monosaccharide composition of BQP: 1-mannose, 2-ribose, 3-rhamnose, 4-glucuronic acid, 5-galacturonic acid, 6-N-acetyl-glucosamine, 7-glucose, 8-N-acetyl-amino-galactose, 9-galactose, 10-xylose, 11-arabinose, and 12-fucose; (b) molecular weight distribution.

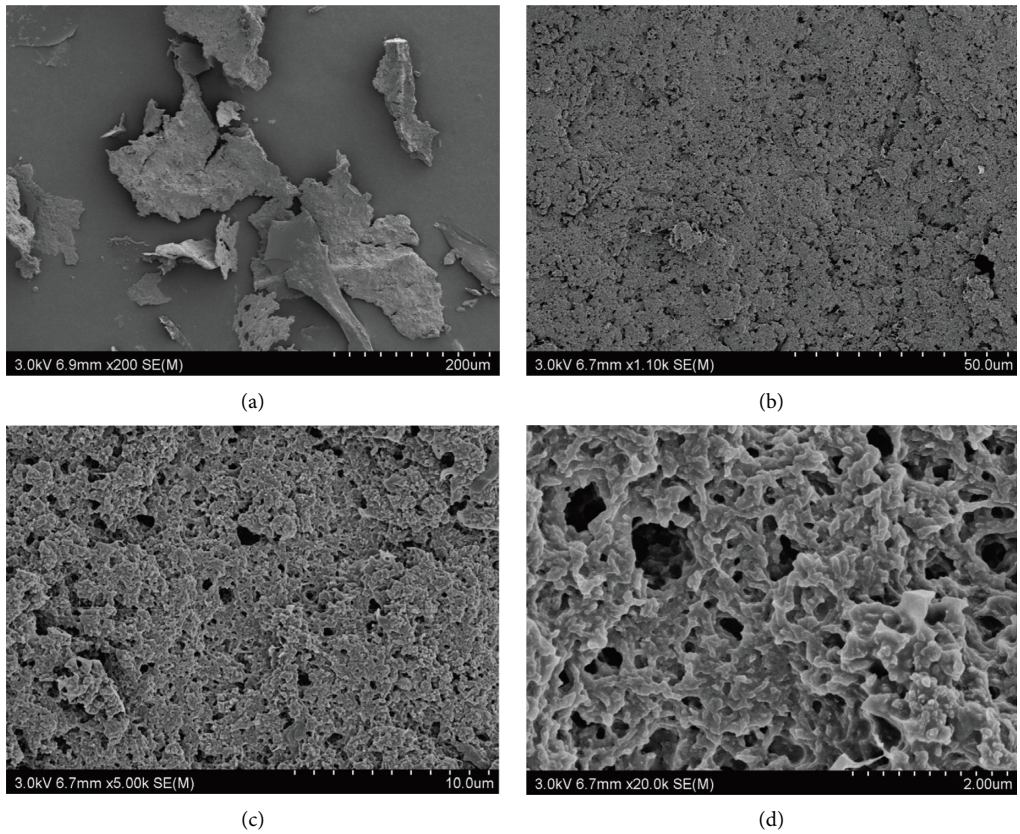


Figure 3: SEM images of BQP: (a) 200 μm, (b) 50 μm, (c) 10 μm, and (d) 2 μm.

resistance. After 4 weeks of BQP-L and BQP-H interventions, the levels of FBG, GHb, INS, and HOMA-IR were reversed (Figures 4(a)–4(d)). This indicates that BQP intervention was beneficial for restoring blood glucose levels and improving insulin resistance.

3.4. Effect of BQP on OGTT. OGTT is used to assess glucose tolerance in diabetes. After 30 min of glucose challenge, the blood glucose levels of normal and diabetic mice rose rapidly and then fell slowly. From 60 min onwards, the blood glucose in the BQP-L and BQP-H groups was significantly

Table 1: Effects of BQP on body weight and serum lipid levels in diabetic mice.

	NC	DC	BQP-L	BQP-H
Initial weight (g)	$25.83 \pm 0.91^{**}$	27.89 ± 0.97	27.15 ± 0.67	26.80 ± 0.61
Final weight (g)	$29.07 \pm 0.88^{**}$	32.50 ± 1.05	$30.57 \pm 0.62^{**}$	$30.05 \pm 0.40^{**}$
Weight gain (g)	$3.24 \pm 0.39^{**}$	4.61 ± 0.34	$3.41 \pm 0.42^{**}$	$3.26 \pm 0.30^{**}$
Food intake (g/mouse/day)	4.51 ± 0.28	4.65 ± 0.18	4.47 ± 0.13	4.38 ± 0.33
Water intake (mL/mouse/day)	$5.02 \pm 0.49^{**}$	8.24 ± 0.57	7.76 ± 0.28	$7.42 \pm 0.35^{*}$
Heart index (%)	$0.39 \pm 0.04^{*}$	0.48 ± 0.07	$0.39 \pm 0.02^{*}$	$0.38 \pm 0.04^{**}$
Liver index (%)	$3.50 \pm 0.14^{**}$	4.21 ± 0.23	$3.92 \pm 0.79^{*}$	$3.71 \pm 0.10^{**}$
TC (mmol/L)	$1.82 \pm 0.14^{**}$	3.37 ± 0.13	$2.78 \pm 0.13^{*}$	$2.63 \pm 0.19^{*}$
TG (mmol/L)	$0.64 \pm 0.09^{**}$	0.91 ± 0.12	$0.77 \pm 0.05^{**}$	$0.74 \pm 0.09^{**}$
LDL-C (mmol/L)	$3.57 \pm 0.27^{**}$	5.49 ± 0.98	$4.10 \pm 0.54^{**}$	$3.99 \pm 0.32^{**}$
HDL-C (mmol/L)	$1.20 \pm 0.11^{**}$	0.54 ± 0.14	$1.06 \pm 0.16^{**}$	$1.15 \pm 0.17^{**}$

Note. vs. DC group, $^{*}p < 0.05$; $^{**}p < 0.01$. vs. BQP-L group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

lower than that in the DC group. At 120 min, the blood glucose in the BQP-L and BQP-H groups was close to the initial blood glucose level and significantly lower than that in the DC group (Figure 4(e)), and the AUC of OGTT was also significantly lower than that in the DC group (Figure 4(f)), showing that BQP can regulate glucose tolerance in diabetic mice.

3.5. Effect of BQP on Serum Lipids. Serum TC, TG, and LDL-C were significantly higher, and HDL-C was significantly lower in the DC group than mice in the NC group, indicating the presence of abnormal lipid metabolism in the DC group. After BQP treatment, TC, TG, and LDL-C were significantly lower and HDL-C was significantly higher in the BQP-L and BQP-H groups than mice in the DC group (Table 1). It showed that BQP could improve abnormal lipid metabolisms in diabetic mice.

3.6. Effect of BQP on Antioxidative Stress Ability. Compared to the NC group, the DC group showed significantly lower ALT and GSH activities and higher MDA and NO levels. In contrast, after the intervention of BQP-L and BQP-H, CAT and GSH levels increased significantly, NO levels decreased and MDA levels decreased significantly (Figures 5(a)–5(d)), indicating that BQP could improve the antioxidant capacity in diabetic mice.

3.7. Effects of BQP on Inflammation and Liver Injury. The levels of IL-1β and ALT in the DC group were significantly higher than those in the NC group. Compared with the DC group, the levels of IL-1β and ALT in the BQP-L and BQP-H groups were reduced to different degrees (Figures 5(e) and 5(f)), indicating that BQP could relieve inflammation and liver injury in mice.

3.8. Determination of SCFAs in Gut. The contents of acetic acid, propionic acid, butyric acid, and total acid were significantly lower in the DC group than in the NC group. However, the contents of acetic acid, propionic acid, butyric acid, and total acid recovered to some extent after BQP-L and BQP-H intervention (Figure 6).

3.9. Diversity of the Gut Microbiota. The above experiments showed that BQP-H was more effective in the intervention of T2DM. Therefore, BQP-H was chosen for the determination of intestinal flora. The rarefaction curves show that the sequencing results cover almost all the sequences in the samples, indicating that the sequencing results could reflect the real situation of the microbial community in the samples (Figures 7(a) and 7(b)).

To assess the effect of BQP treatment on the diversity of the intestinal flora, we analyzed the alpha diversity and beta diversity of the intestinal flora after 4 weeks of BQP treatment. In alpha diversity, the Chao1 index and the ACE index were used to calculate the richness of bacterial communities, and the Shannon index was used to calculate the diversity of bacterial communities. The results showed that the richness and diversity of the intestinal flora decreased after BQP intervention (Figures 7(c)–7(e)). PCoA based on Bray–Curtis distance is used to analyze the beta diversity. DC and BQP occurred within group aggregation, indicating that each group was composed of similar microbial communities (Figure 7(f)).

3.10. Composition Analysis of the Gut Microbiota. The relative abundance of the dominant groups at the phylum level was analyzed for two groups (Figures 8(a)–8(c)). Compared to the DC group, the BQP group showed an increased abundance of the Firmicutes and decreased abundance of the Bacteroidota and the B/F ratio (Figure 8(d)). To further determine the enriched bacteria in two groups, LEfSe analysis was performed (Figures 9(a)–9(b)). In the BQP group, *Erysipelotrichaceae* and *Akkermansiaceae* were increased at the family level. Compared with the DC group, the abundance of *Dubosiella*, *Akkermansia*, *Faecalibaculum*, and *Allobaculum* was increased in the BQP group, while the abundance of *Helicobacter*, *Odoribacter*, and *Mucispirillum* was decreased.

3.11. Correlation of the Gut Microflora with Metabolic Parameters. To explore the associations of the gut microbiota with biochemical parameters, we specifically calculated Spearman's correlation coefficient between the top 40 most abundant genera and the biomarkers. *Helicobacter* and

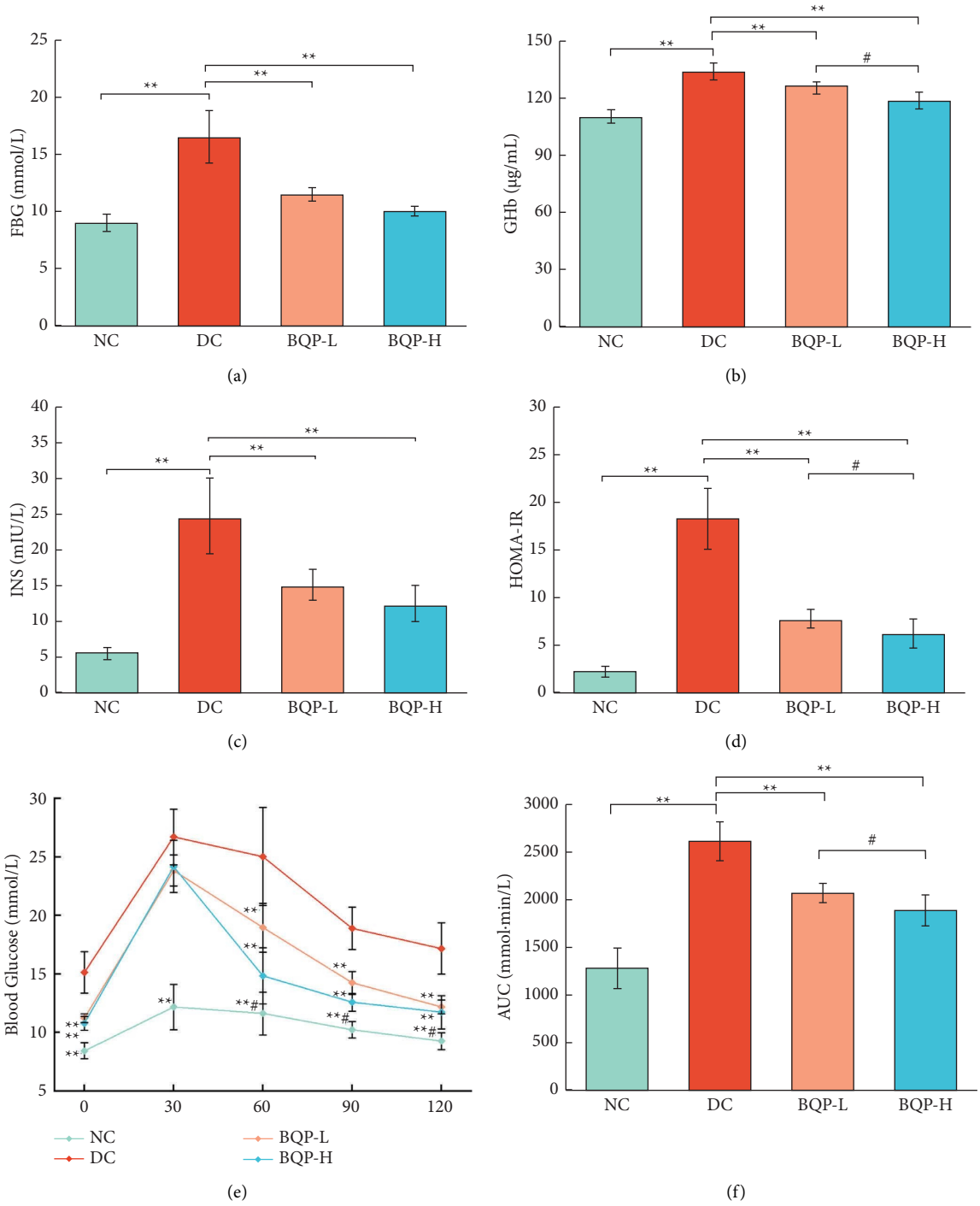


Figure 4: Effects of BQP on blood glucose levels and OGTT in diabetic mice. (a) FBG, (b) GHb, (c) INS, (d) HOMA-IR, (e) changes in oral glucose tolerance blood glucose concentration, and (f) area under the curve (AUC) in diabetic mice. vs. DC group, $^{*}p < 0.05$; $^{**}p < 0.01$. vs. BQP-L group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

Mucispirillum were enriched in the DC group, and they were positively correlated with the levels of IL-β, NO, LDL-C, TG, MDA, BW, ALT, FBG, HOMA-IR, INS, TC, and GHb but negatively correlated with CAT, GSH, HDL-C, and SCFAs levels. In addition, microorganisms enriched in the BQP group, such as *Allobaculum*, *Faecalibaculum*, *Dubosiella*, and *Akkermansia*, were negatively correlated with IL-β, NO, LDL-C, TG, MDA, BW, ALT, FBG, HOMA-IR, INS, TC, and GHb but positively correlated with CAT, GSH, HDL-C, and SCFAs (Figure 10).

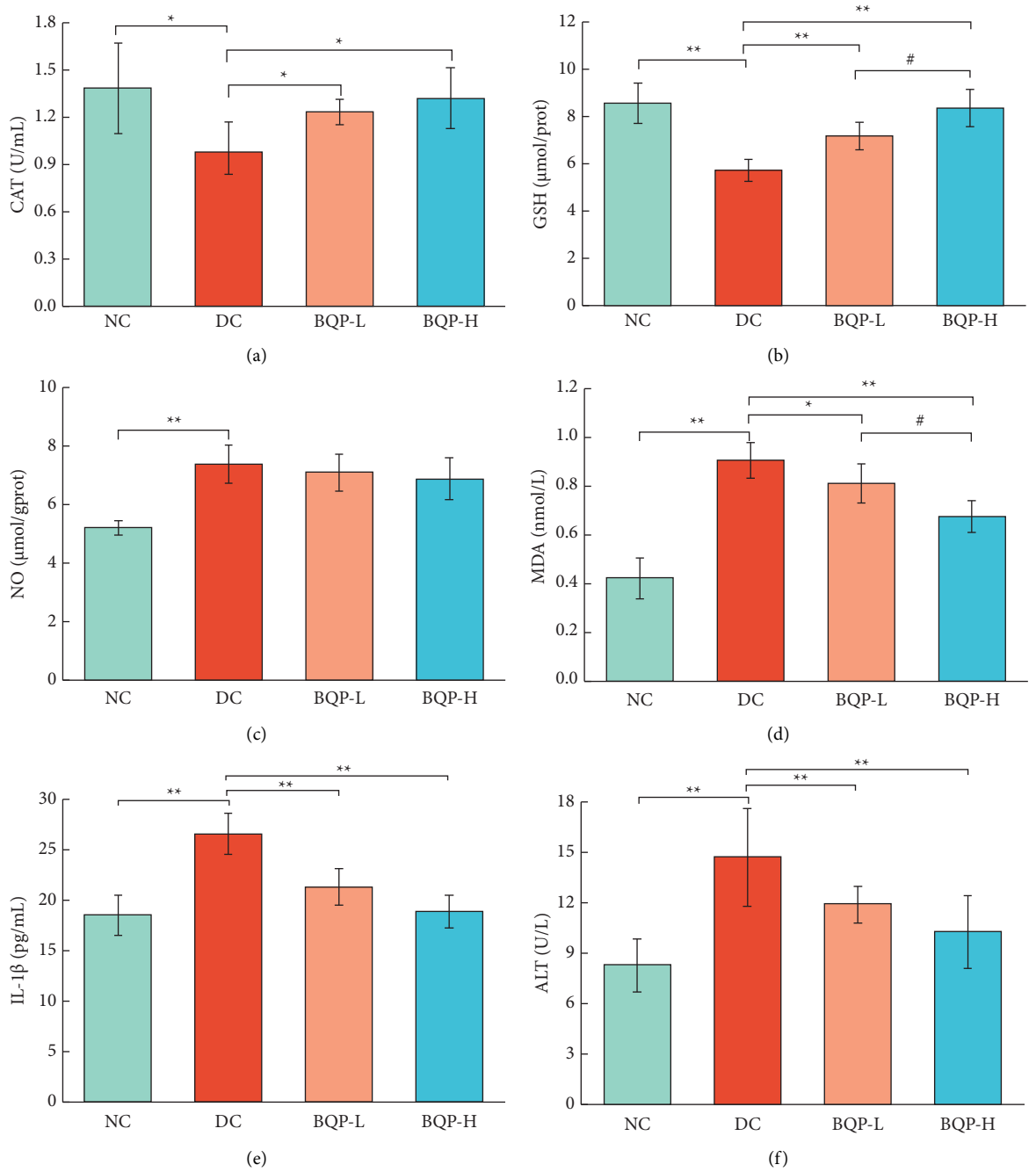


Figure 5: Effects of BQP on oxidative stress, inflammatory factors, and liver injury in diabetic mice. (a) MDA, (b) NO, (c) CAT, (d) GSH, (e) IL-1β, and (f) ALT. vs. DC group, $^{*}p < 0.05$; $^{**}p < 0.01$. vs. BQP-L group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

3.12. Functional Analysis. When studying the function of the microbiome, the analysis of the phenotype of the microbiome is also significant. The BugBase phenotype analysis (Figures 11(a)–11(f)) showed that the relative abundance of Gram-Positive and Contains-Mobile-Elements was significantly increased compared with the DC group, but the relative abundance of Forms-Biofilms, Gram-Negative, and Potentially-Pathogenic was significantly decreased. Therefore, the change in microbiome composition was accompanied by the change in bacterial phenotype, and the effect of BQP on intestinal flora of diabetic mice was also reflected in bacterial phenotypes.

We further predicted the microbial community function using the FAPROTAX software (Figure 12). Functional group chemoheterotrophy was the most abundant in the bacterial community, and their abundance decreased after BQP intervention. In addition, BQP improved animal parasites or symbionts, human pathogens, and human pathogen pneumonia that may have a negative impact on diabetes. Meanwhile, BQP could enhance the functional

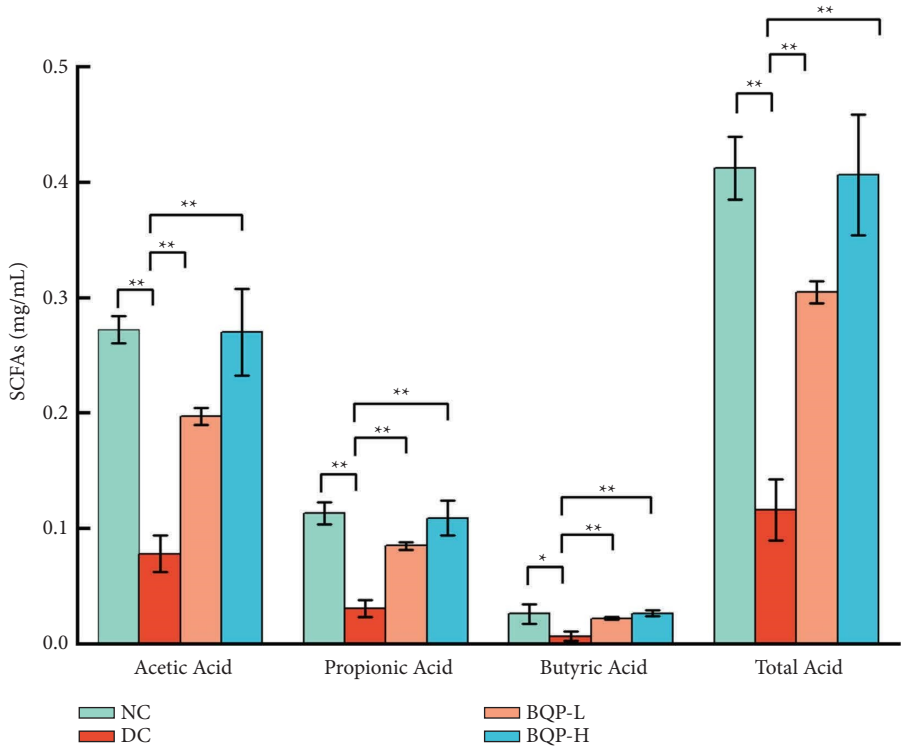


Figure 6: Effect of BQP on SCFAs in diabetic mice. vs. DC group, $^{*}p < 0.05$; $^{**}p < 0.01$. vs. BQP-L group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

groups related to the sulfur cycle of sulfate respiration and respiration of sulfur compounds and reduce the functional groups related to carbon cycles such as photoautotrophy and oxygenic photoautotrophy.

4. Discussion

In this study, black quinoa was used as a raw material to extract polysaccharide. SEM showed that the surface of BQP was rough and irregularly aggregated. BQP was mainly composed of glucose with a molecular weight of 8.087×10^3 Da. Low molecular weight polysaccharide (less than 10^4 Da) is conducive to human absorption and has high activity for human health [22].

Moreover, this study also revealed the antidiabetic effects and their mechanistic role of BQP in C57BL/6 mice. The mice are susceptible to diet-induced obesity and exhibit metabolic abnormalities, such as dyslipidemia and hyperglycemia [23]. After 4 weeks of administration, BW gain and an abnormal increase in food intake and water intake were modified in the BQP group mice. BQP treatment also decreased the levels of serum TC, TG, and LDL-C and increased the level of HDL-C. Furthermore, the levels of FBG, GHb, INS, and HOMA-IR were significantly reduced after 28 days in the BQP group mice, compared with the DC group mice. Obesity is a major deleterious factor affecting insulin sensitivity, and appropriate supplementation with bioactive plant extracts as a weight loss intervention has been shown to have a positive effect on glycemic control and prevention of diabetic complications [24]. The results showed that BQP intervention could modulate abnormal lipid metabolism and hyperglycemia and improve insulin resistance in diabetic mice.

Obesity-induced insulin resistance and disorders of glucose and lipid metabolism increase oxidative stress [25]. Oxidative stress produces excess oxygen species (ROS) and makes tissues susceptible to oxidative stress, which ultimately leads to the development and progression of most diabetic complications [7]. After BQP intervention, the concentration of CAT and GSH was increased, while the concentration of NO and MDA decreased. CAT and GSH are important antioxidants, scavenging free radicals in the organism, thus turning ROS into stable and harmless molecules [17, 26, 27]. Excess NO produces oxidative stress and eventually MDA, which leads to cellular aging or death [17, 28]. Besides, BQP intervention reduces the concentration of IL-1β and ALT. Oxidative stress produces proinflammatory cytokines, which can induce insulin resistance and promote the development of T2DM and cause liver injury [29]. Together, these results showed that BQP not only ameliorates oxidative stress but also inhibits the inflammatory response and the development of liver injury.

Intestinal flora is closely related to diseases such as obesity and diabetes [29]. In our study, microbial community abundance and distribution in diabetic mice were significantly altered by BQP intervention. Alpha diversity was reduced after 4 weeks of BQP treatment, which was consistent with a previous report [30]. It was mainly caused by the bacteriostatic effect of quinoa [31]. The beta diversity results also showed that BQP significantly altered the composition of the gut microbiota. Interestingly, a decrease in the B/F ratio was found in the BQP group mice, which

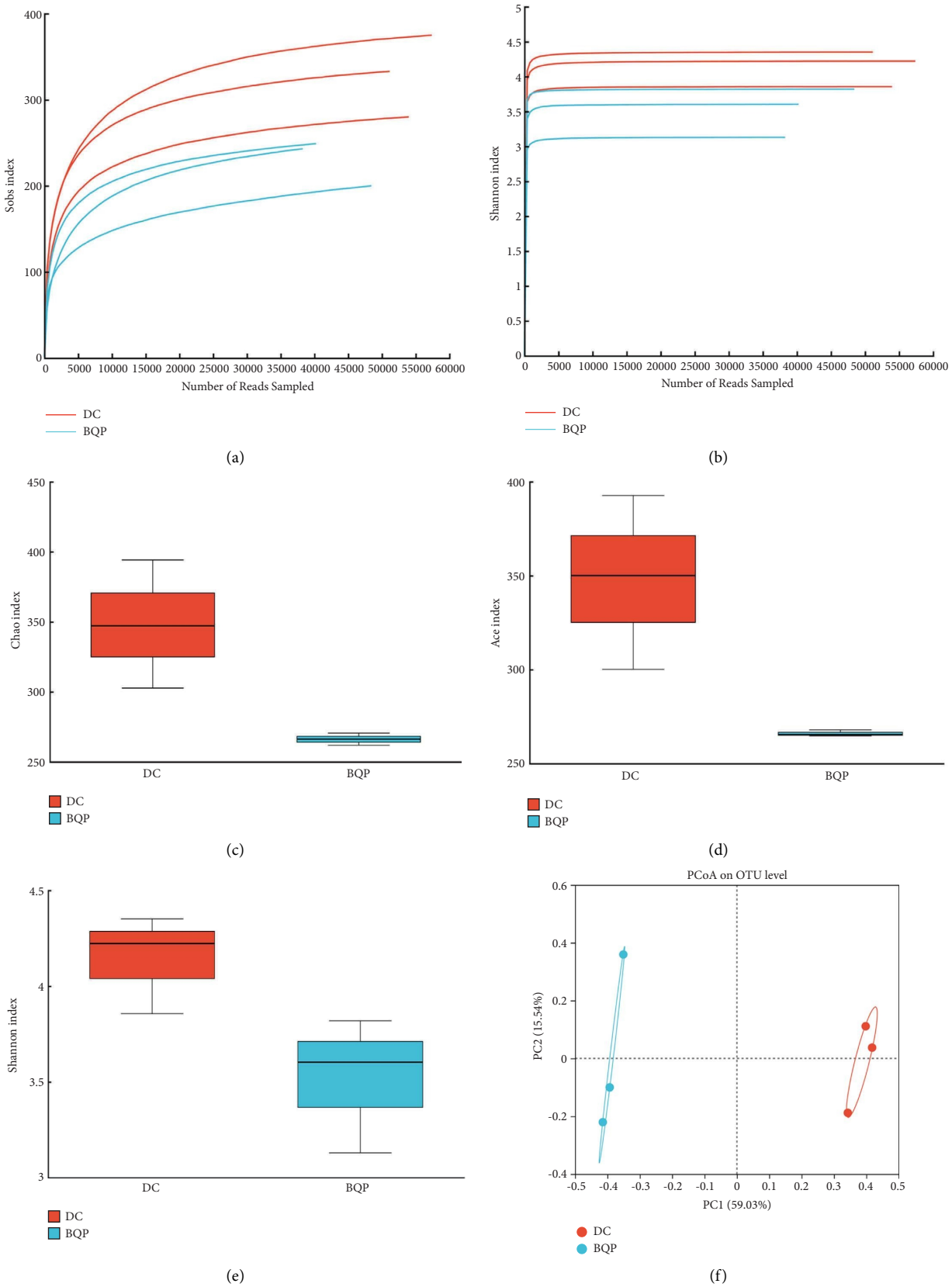


Figure 7: Effect of BQP on alpha diversity and beta diversity. (a) Sobs index curves, (b) shannon index curves, (c) chao index, (d) ACE index, (e) shannon index, (f) principal coordinates analysis (PCoA).

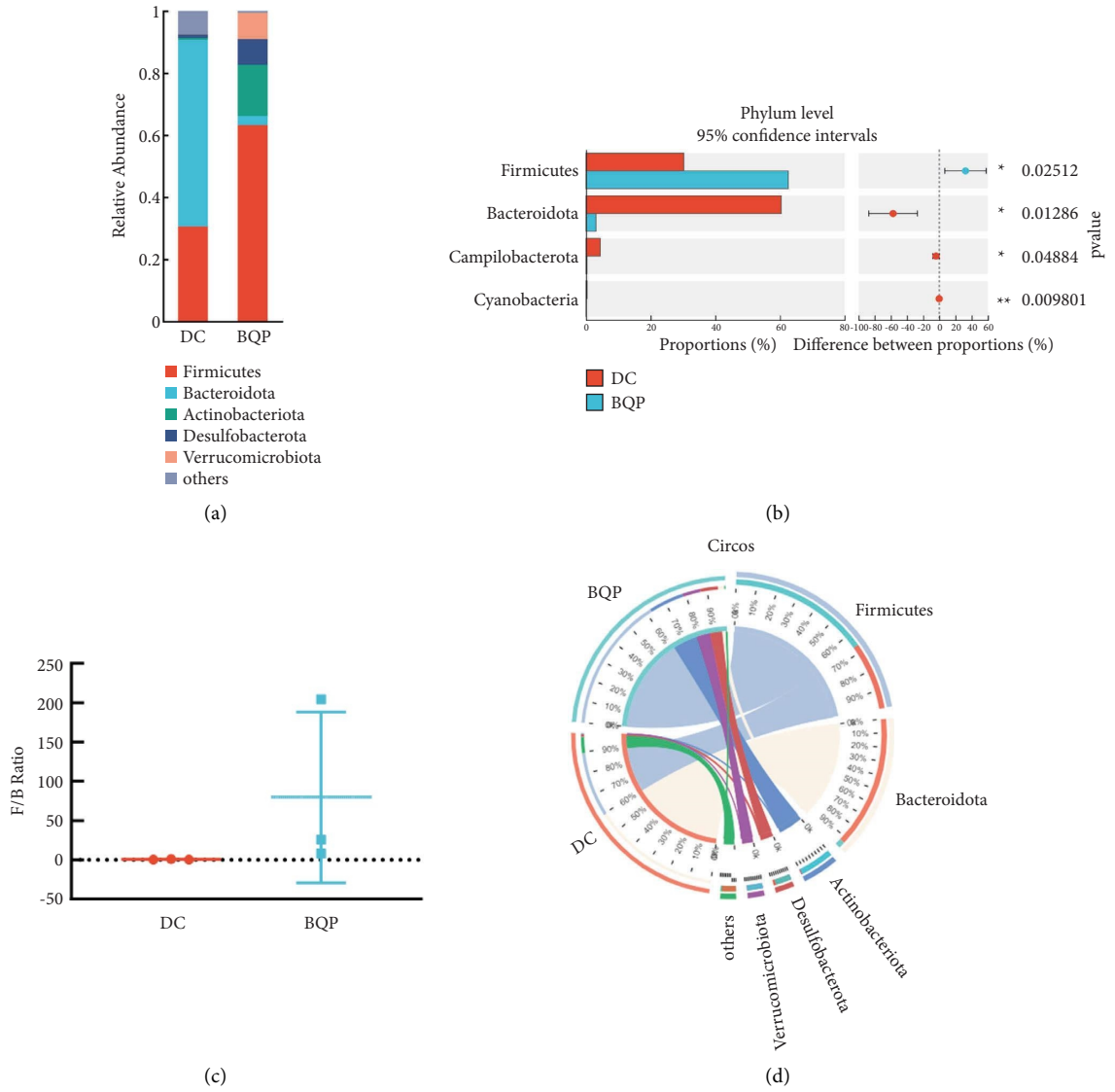
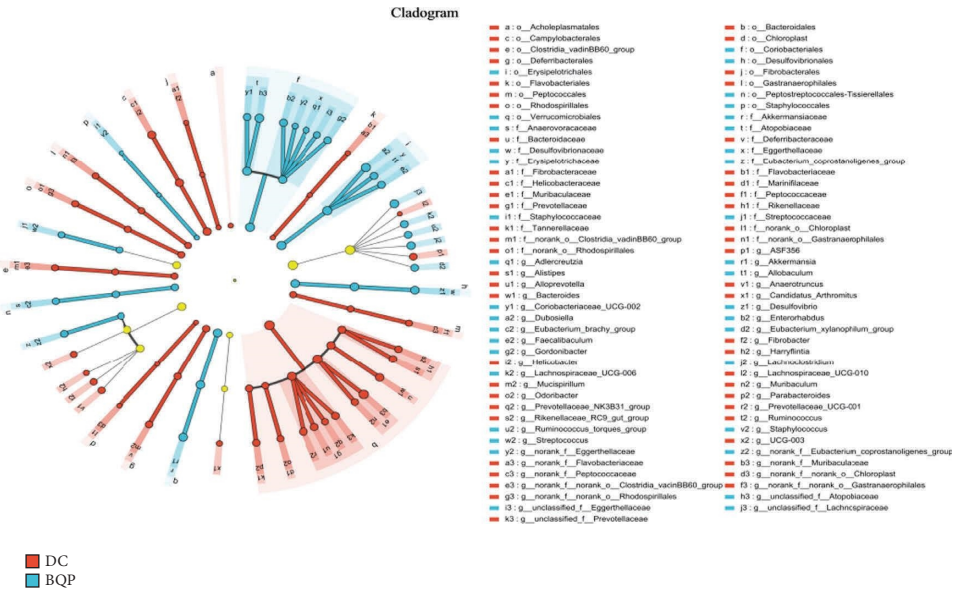
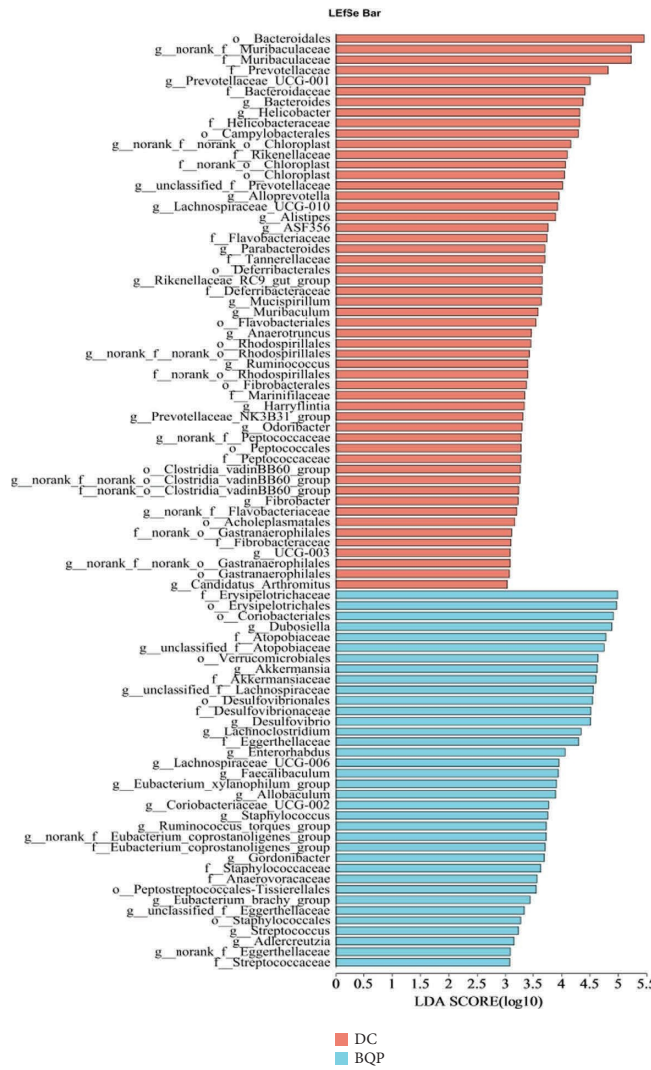


Figure 8: BQP altered the composition of the gut microbiota in diabetic mice after 4 weeks of treatment. (a) Compositional change at the phylum level, (b) significant differences in bacterial abundance at the phylum level, (c) comparison of Firmicutes to Bacteroidetes ratio, and (d) Circos plot displaying the relationship between samples and bacteria at the class level.

could be associated with an improvement in oral glucose loading [32]. Moreover, BQP reduced the abundance of *Helicobacter*, *Odoribacter*, and *Mucispirillum* and increased the abundance of *Allobaculum* in T2DM mice, which were thought to connect with the development of diabetic inflammation and damage to the intestinal barrier [33–36]. The significant decrease of IL-1β levels in the BQP group mice also proved this inference. In addition to the decrease in pathogenic bacteria, the increased abundance of *Dubosiella*, *Akkermansia*, and *Faecalibaculum* could be thought to lead to an increase in the content of SCFAs in BQP group mice, as the previous studies reported [37–40]. In the present study, BQP intervention significantly increased SCFA content in diabetic mice, which may lead to improvement of islet cell dysfunction and insulin resistance [41]. In SCFAs, acetic acid inhibits fat accumulation and propionic acid improves insulin sensitivity [29]. Acetic acid, propionic acid, and butyric acid can all improve glycolipid metabolisms through different pathways [29, 42]. The above results demonstrate that BQP could improve abnormal glycolipid metabolism in diabetic mice by reducing the number of pathogenic bacteria and increasing the number of SCFAs-producing bacteria. Meanwhile, Spearman correlation analysis was to determine whether the abundance of gut microbes was associated with characteristic indicators of T2DM. IL-β, NO, LDL-C, TG, MDA, BW, ALT, FBG, HOMA-IR, INS, TC, and GHb were positively correlated with *Allobaculum*, *Faecalibaculum*, *Dubosiella*, and



(a)



(b)

Figure 9: LEfse analysis of gut taxa from order levels to genus levels between two groups. (a) Cladogram; (b) linear discriminant analysis (LDA) score (LDA > 3).

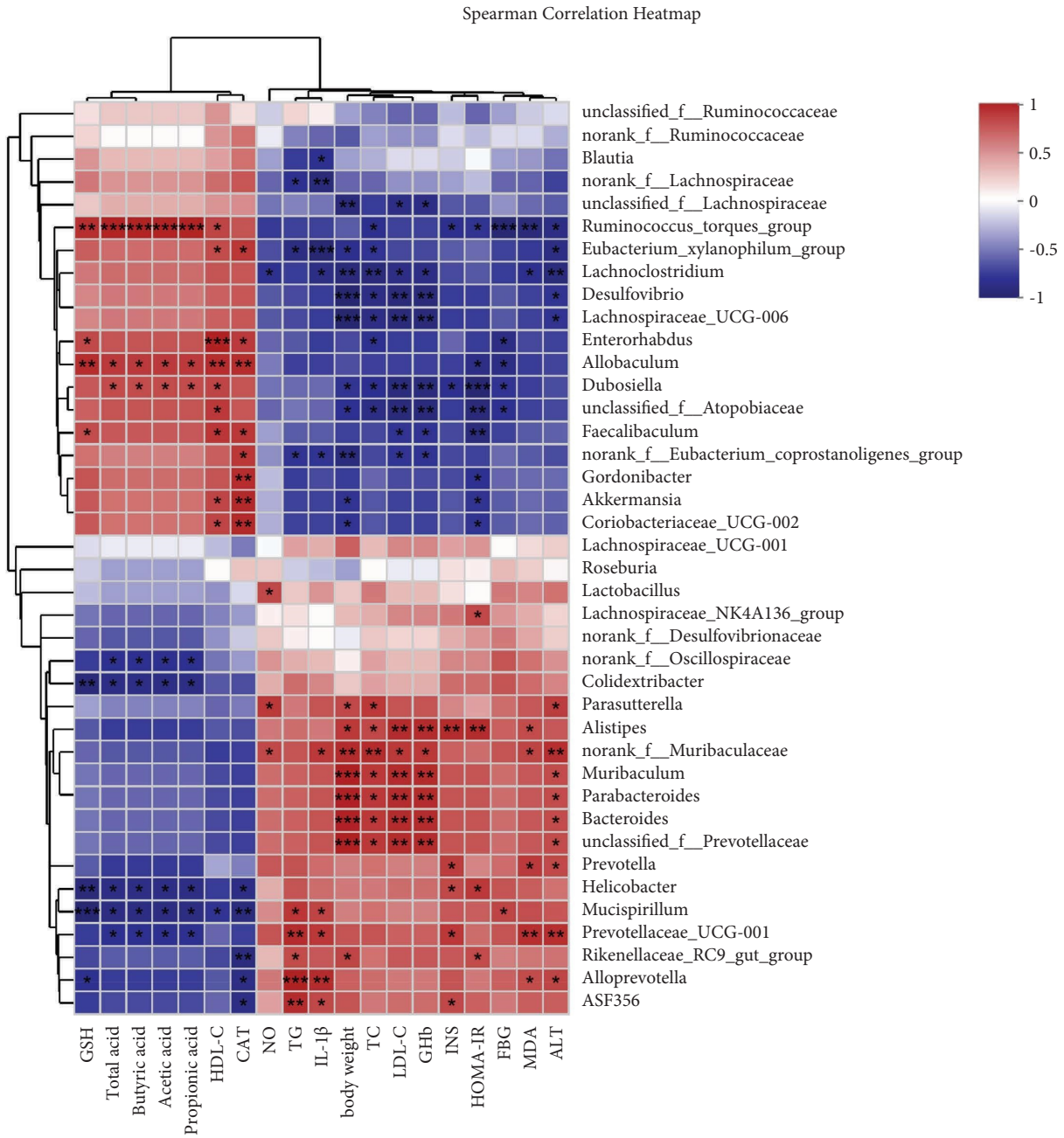


FIGURE 10: Spearman's correlations of the top 40 most abundant genera with biomarkers. Red indicates a positive correlation; blue indicates a negative correlation. Significant correlations are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ and marked with asterisks.

Akkermansia, and the above biochemical indicators were negatively correlated with *Helicobacter* and *Mucispirillum*. The results indicated that the changes in intestinal microorganisms were closely related to the biochemical parameters. It was further confirmed that intestinal flora is involved in host glycolipid metabolisms, oxidative stress, inflammation, and liver injury and plays an important role in the development of diabetes.

Furthermore, BugBase was used to predict potential pathogenicity, and FAPROTAX was used to predict the function of bacterial data. BQP altered the sulfur cycle. The interaction between the sulfur cycle and the gut microbiota produces hydrogen sulfide which has been shown to have the potential to interfere with diabetes and its associated complications [43]. In addition, BQP intervention reduced Gram-negative bacteria and potential pathogenicity in the

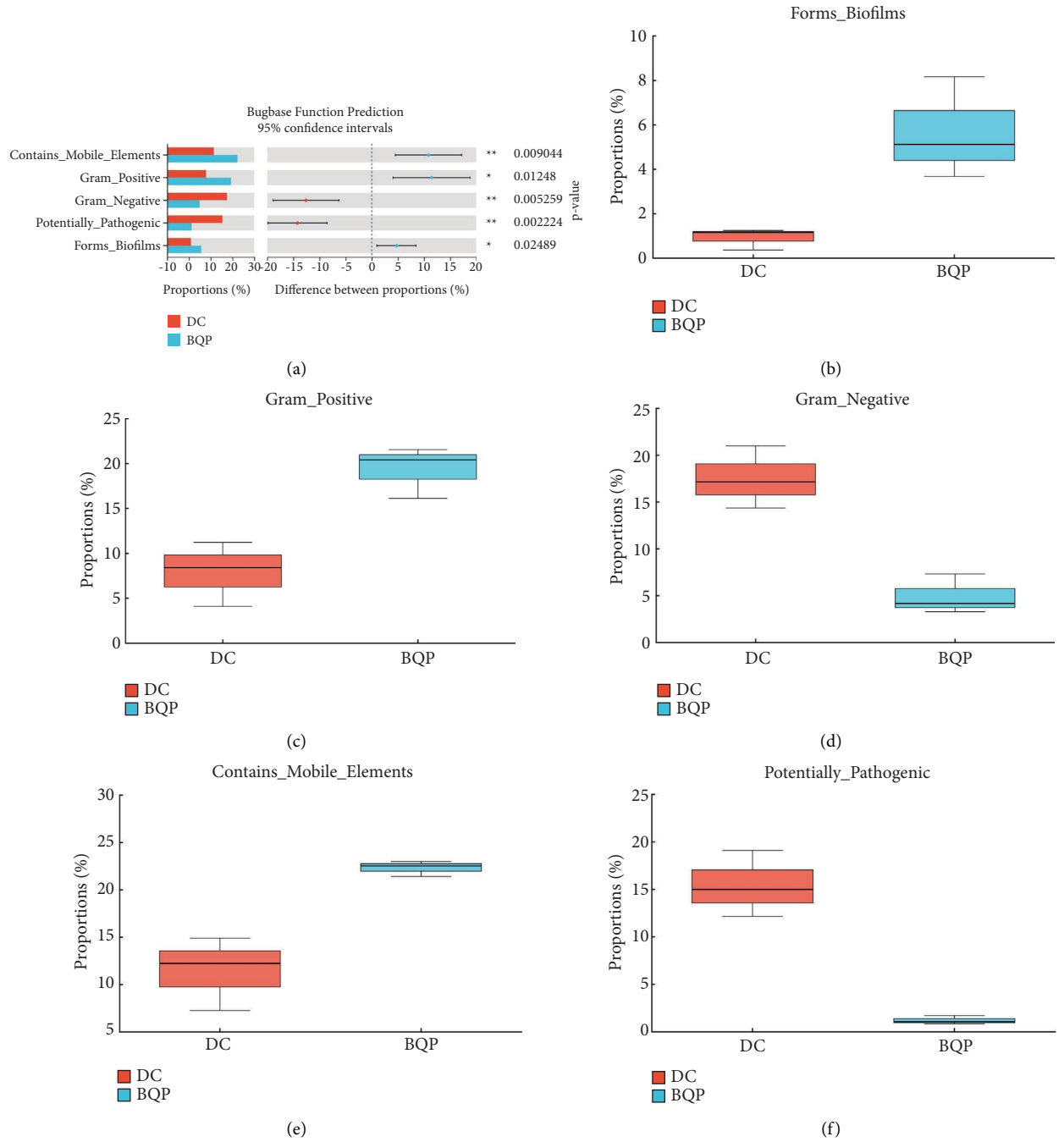


Figure 11: BugBase phenotype analysis on the intestinal bacteria. (a) Prediction of the ecological functions of gut microbiota in DC and BQP groups, (b) forms_biofilms, (c) gram_positive, (d) gram_negative, (e) contains_mobile_elements, and (f) potentially_pathogenic. $^{*}p < 0.05$; $^{**}p < 0.01$.

intestinal flora of diabetic mice. Lipopolysaccharides (LPS) produced by Gram-negative bacteria are implicated in the development of several diseases, and low-level inflammation caused by LPS damage to the intestinal mucosa can damage pancreatic islet β-cells [44, 45], suggesting that BQP may improve diabetes-induced immune dysfunction in mice, reduce the number of potential intestinal pathogens, and reduce coinfections.

This experiment preliminarily demonstrated the ameliorative effect of BQP on T2DM in mice with T2DM

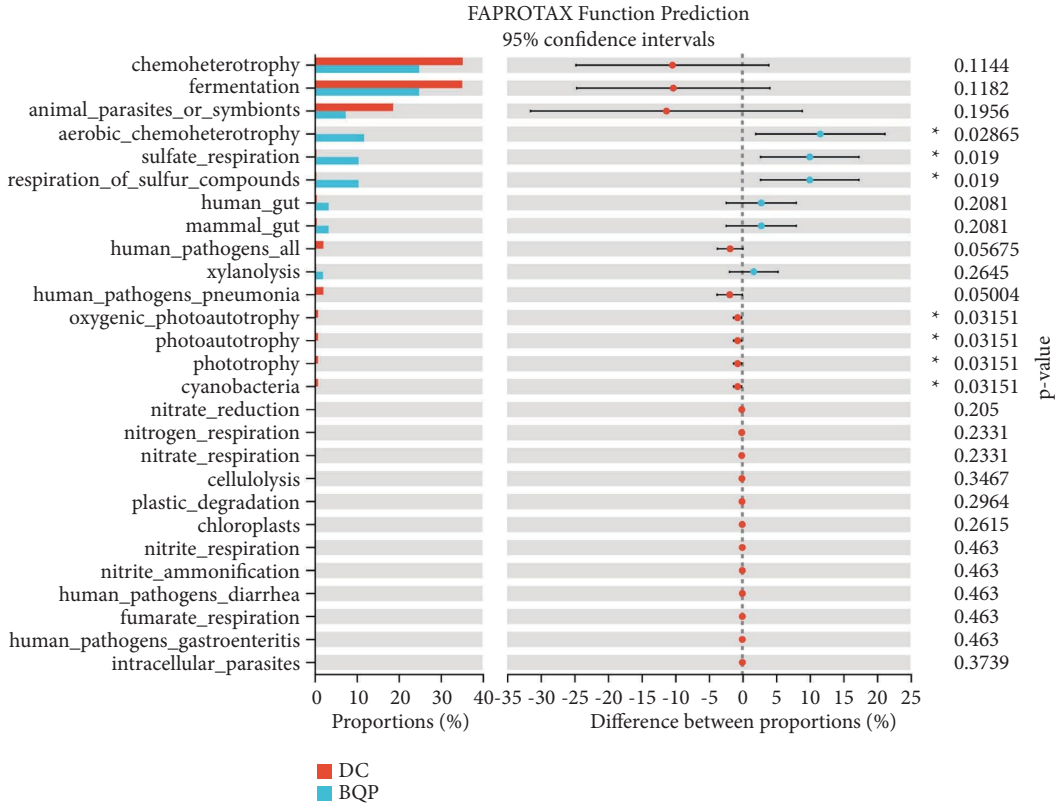


Figure 12: Bacterial functional groups using FAPROTAX. vs. DC group, $^{*}p < 0.05$; $^{**}p < 0.01$.

induced by the combination of HFD and STZ. However, animal experiments have some limitations. First, the sample size of the experiment was limited, and more samples would have provided a more solid foundation for this study. Second, there are some differences between mice and human individuals. Third, the effect of purified BQP on fecal microbiota and metabolites in T2DM mice was further investigated. Finally, *in vitro* fermentation experiments could also help to further characterize the antidiabetic effects of BQP as well as its effects on the intestinal flora. Therefore, it is worthwhile to conduct more studies on the effects of BQP on gut flora in the future. For example, the effects of BQP on T2DM can be further explored by performing fecal transplantation tests on T2DM patients.

5. Conclusion

In conclusion, our results suggest that BQP intervention can effectively improve obesity and lipid levels in T2DM mice and ultimately alleviate glycemic abnormalities and insulin resistance in T2DM mice. In addition, BQP can improve the antioxidant capacity, alleviate inflammatory response, and reduce liver injury in T2DM mice. In addition, BQP can increase the level of SCFAs in the cecum of diabetic mice and alter the intestinal flora, thereby effectively improving gastrointestinal health. The underlying mechanism by which BQP has a beneficial effect on T2DM may be through the modulation of obesity and gut microbiota, which provides a theoretical basis for the use of BQP as a potential prebiotic for food and pharmaceutical applications to ameliorate the development of T2DM.

Data Availability

The raw sequencing data have been deposited into the NCBI Sequence Read Archive (SRA) database under the Accession Number of PRJNA914117.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yanqing Zang was responsible for conceptualization, methodology, data curation, project administration, writing the review, and editing. Kaiming Wu was responsible for formal analysis, investigation, visualization, and writing the original draft. Jiaci Liu was responsible for investigation, writing the review, and editing. Yang Cao was responsible for investigation. Changyuan Wang was responsible for supervision and project administration. Yanqing Zang and Kaiming Wu contributed equally to this work and share first authorship.

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References

[1] H. J. Yang, T. Zhang, X. G. Wu et al., "Aqueous blackcurrant extract improves insulin sensitivity and secretion and modulates the gut microbiome in non-obese type 2 diabetic rats," *Antioxidants*, vol. 10, no. 5, p. 756, 2021.

[2] Y. Yao, L. Yan, H. Chen, N. Wu, W. Wang, and D. Wang, "Cyclocarya paliurus polysaccharides alleviate type 2 diabetic symptoms by modulating gut microbiota and short-chain fatty acids," *Phytomedicine*, vol. 77, Article ID 153268, 2020.

[3] D. M. Nathan, J. M. Lachin, A. Balasubramanyam et al., "Glycemia reduction in type 2 diabetes— glycemic outcomes-glycemic outcomes," *New England Journal of Medicine*, vol. 387, no. 12, pp. 1063–1074, 2022.

[4] F. Zhang, D. Chen, L. Zhang et al., "Diaphragma juglandis extracts modifies the gut microbiota during prevention of type 2 diabetes in rats," *Journal of Ethnopharmacology*, vol. 283, Article ID 114484, 2022.

[5] B. H. Wang, J. J. Cao, B. Zhang, and H. Q. Chen, "Structural characterization, physicochemical properties and α-glucosidase inhibitory activity of polysaccharide from the fruits of wax apple," *Carbohydrate Polymers*, vol. 211, pp. 227–236, 2019.

[6] S. Chen, H. Huang, and G. Huang, "Extraction, derivatization and antioxidant activity of cucumber polysaccharide," *International Journal of Biological Macromolecules*, vol. 140, pp. 1047–1053, 2019.

[7] Q. Zhang, O. J. Olatunji, H. Chen, A. J. Tola, and O. O. Oluwaniyi, "Evaluation of the anti-diabetic activity of polysaccharide from cordyceps cicadae in experimental diabetic rats," *Chemistry and Biodiversity*, vol. 15, no. 8, Article ID e1800219, 2018.

[8] J. Yang, H. Chen, Q. Nie, X. Huang, and S. Nie, "Dendrobium officinale polysaccharide ameliorates the liver metabolism disorders of type II diabetic rats," *International Journal of Biological Macromolecules*, vol. 164, pp. 1939–1948, 2020.

[9] K. Khairuddin, S. Sudirman, L. Huang, and Z. L. Kong, "Caulerpa lentillifera polysaccharides-rich extract reduces oxidative stress and proinflammatory cytokines levels associated with male reproductive functions in diabetic mice," *Applied Sciences*, vol. 10, no. 24, p. 8768, 2020.

[10] Y. Chai, J. Luo, and Y. Bao, "Effects of Polygonatum sibiricum saponin on hyperglycemia, gut microbiota composition and metabolic profiles in type 2 diabetes mice," *Biomedicine and Pharmacotherapy*, vol. 143, Article ID 112155, 2021.

[11] M. Zhang, L. Yang, M. Zhu et al., "Moutan Cortex polysaccharide ameliorates diabetic kidney disease via modulating gut microbiota dynamically in rats," *International Journal of Biological Macromolecules*, vol. 206, pp. 849–860, 2022.

[12] Z. R. Huang, Q. Z. Huang, K. W. Chen et al., "Sanghuangporus vaninii fruit body polysaccharide alleviates hyperglycemia and hyperlipidemia via modulating intestinal microflora in type 2 diabetic mice," *Frontiers in Nutrition*, vol. 9, Article ID 1013466, 2022.

[13] M. Tan, S. Chang, J. Liu et al., "Physicochemical properties, antioxidant and antidiabetic activities of polysaccharides from quinoa (Chenopodium quinoa willd.) seeds," *Molecules*, vol. 25, no. 17, p. 3840, 2020.

[14] H. Zeyneb, H. Pei, X. Cao, Y. Wang, Y. Win, and L. Gong, "In vitro study of the effect of quinoa and quinoa polysaccharides on human gut microbiota," *Food Science and Nutrition*, vol. 9, no. 10, pp. 5735–5745, 2021.

[15] M. Tan, Q. Zhao, and B. Zhao, "Physicochemical properties, structural characterization and biological activities of polysaccharides from quinoa (Chenopodium quinoa Willd.) seeds," *International Journal of Biological Macromolecules*, vol. 193, no. Pt B, pp. 1635–1644, 2021.

[16] Y. Ren and S. Liu, "Effects of separation and purification on structural characteristics of polysaccharide from quinoa (Chenopodium quinoa willd)," *Biochemical and Biophysical Research Communications*, vol. 522, no. 2, pp. 286–291, 2020.

[17] X. Dong, S. X. Zhao, X. L. Yin, H. Y. Wang, Z. G. Wei, and Y. Q. Zhang, "Silk sericin has significantly hypoglycaemic effect in type 2 diabetic mice via anti-oxidation and anti-inflammation," *International Journal of Biological Macromolecules*, vol. 150, pp. 1061–1071, 2020.

[18] W. H. Zhang, J. Wu, L. Weng, H. Zhang, J. Zhang, and A. Wu, "An improved phenol-sulfuric acid method for the determination of carbohydrates in the presence of persulfate," *Carbohydrate Polymers*, vol. 227, Article ID 115332, 2020.

[19] Y. Deng, L. Huang, C. Zhang et al., "Novel polysaccharide from Chaenomeles speciosa seeds: structural characterization, α-amylase and α-glucosidase inhibitory activity evaluation," *International Journal of Biological Macromolecules*, vol. 153, pp. 755–766, 2020.

[20] I. F. Olawuyi and W. Y. Lee, "Structural characterization, functional properties and antioxidant activities of polysaccharide extract obtained from okra leaves (Abelmoschus esculentus)," *Food Chemistry*, vol. 354, Article ID 129437, 2021.

[21] X. L. Yin, B. Q. Xu, and Y. Q. Zhang, "Gynura divaricata rich in 3, 5-/4, 5-dicaffeoylquinic acid and chlorogenic acid reduces islet cell apoptosis and improves pancreatic function in type 2 diabetic mice," *Nutrition and Metabolism*, vol. 15, no. 1, p. 73, 2018.

[22] X. Zhang, Y. Hu, C. Jin, and W. Wu, "Extraction and hypolipidemic activity of low molecular weight polysaccharides isolated from rosa laevigata fruits," *BioMedical Research International*, vol. 2020, Article ID 2043785, 20 pages, 2020.

[23] S. Jia, Z. Gao, S. Yan et al., "Anti-obesity and hypoglycemic effects of poncirus trifoliata L. Extracts in high-fat diet C57bl/6 mice," *Molecules*, vol. 21, no. 4, p. 453, 2016.

[24] X. Liu, X. Zeng, W. Liu, Y. Lu, J. Cheng, and Y. Chen, "An overview of dietary supplements on obesity and type 2 diabetes: efficacy and mechanisms," *Current Drug Metabolism*, vol. 22, no. 6, pp. 415–440, 2021.

[25] Y. Zang, J. Liu, A. Zhai et al., "Effects of highland barley β-glucan on blood glucose and gut microbiota in streptozotocin-induced, diabetic, C57BL/6 mice on a high-fat diet," *Nutrition*, vol. 107, Article ID 111882, 2023.

[26] Y. Zhao, M. Jayachandran, and B. Xu, "In vivo antioxidant and anti-inflammatory effects of soluble dietary fiber Konjac glucomannan in type-2 diabetic rats," *International Journal of Biological Macromolecules*, vol. 159, pp. 1186–1196, 2020.

[27] A. Martha Orendu Oche, J. Tamunotonye Watson, G. Sani Hyedima, and B. Sadiya Ufeli, "Leptadenia hastata Leaf Extract ameliorates oxidative stress and serum biochemical parameters in Streptozotocin-Induced diabetes in Wistar

rats," *Journal of Diabetes and Metabolic Disorders*, vol. 21, no. 2, pp. 1273–1281, 2022.

[28] M. Q. Man, J. S. Wakefield, T. M. Mauro, and P. M. Elias, "Role of nitric oxide in regulating epidermal permeability barrier function," *Experimental Dermatology*, vol. 31, no. 3, pp. 290–298, 2022.

[29] J. Dong, Q. Liang, Y. Niu et al., "Effects of Nigella sativa seed polysaccharides on type 2 diabetic mice and gut microbiota," *International Journal of Biological Macromolecules*, vol. 159, pp. 725–738, 2020.

[30] L. Su, C. Xin, J. Yang et al., "A polysaccharide from Inonotus obliquus ameliorates intestinal barrier dysfunction in mice with type 2 diabetes mellitus," *International Journal of Biological Macromolecules*, vol. 214, pp. 312–323, 2022.

[31] S. Pathan and R. A. Siddiqui, "Nutritional composition and bioactive components in quinoa (Chenopodium quinoa willd.) greens: a review," *Nutrients*, vol. 14, no. 3, p. 558, 2022.

[32] A. L. Cunningham, J. W. Stephens, and D. A. Harris, "Gut microbiota influence in type 2 diabetes mellitus (T2DM)," *Gut Pathogens*, vol. 13, no. 1, p. 50, 2021.

[33] A. Li, N. Wang, N. Li et al., "Modulation effect of chenpi extract on gut microbiota in high-fat diet-induced obese C57BL/6 mice," *Journal of Food Biochemistry*, vol. 45, no. 4, Article ID e13541, 2021.

[34] J. Hao, Y. Zhang, T. Wu et al., "The antidiabetic effects of Bifidobacterium longum subsp. longum BL21 through regulating gut microbiota structure in type 2 diabetic mice," *Food and Function*, vol. 13, no. 19, pp. 9947–9958, 2022.

[35] T. Wu, Y. Zhang, W. Li et al., "Lactobacillus rhamnosus LRa05 ameliorate hyperglycemia through a regulating glucagon-mediated signaling pathway and gut microbiota in type 2 diabetic mice," *Journal of Agricultural and Food Chemistry*, vol. 69, no. 31, pp. 8797–8806, 2021.

[36] Q. Ma, Y. Li, J. Wang et al., "Investigation of gut microbiome changes in type 1 diabetic mellitus rats based on high-throughput sequencing," *Biomedicine and Pharmacotherapy*, vol. 124, Article ID 109873, 2020.

[37] Y. Chen, Y. Ouyang, X. Chen et al., "Hypoglycaemic and anti-ageing activities of green alga Ulva lactuca polysaccharide via gut microbiota in ageing-associated diabetic mice," *International Journal of Biological Macromolecules*, vol. 212, pp. 97–110, 2022.

[38] Z. Huang, Y. Liu, X. Liu et al., "Sanghuangporus vaninii mixture ameliorated type 2 diabetes mellitus and altered intestinal microbiota in mice," *Food and Function*, vol. 13, no. 22, pp. 11758–11769, 2022.

[39] N. Liu, M. Chen, J. Song, Y. Zhao, P. Gong, and X. Chen, "Effects of auricularia auricula polysaccharides on gut microbiota composition in type 2 diabetic mice," *Molecules*, vol. 27, no. 18, p. 6061, 2022.

[40] X. Pan, J. Meng, L. Xu et al., "In-depth investigation of the hypoglycemic mechanism of Morchella importuna polysaccharide via metabonomics combined with 16S rRNA sequencing," *International Journal of Biological Macromolecules*, vol. 220, pp. 659–670, 2022.

[41] Q. Song, Y. Wang, L. Huang et al., "Review of the relationships among polysaccharides, gut microbiota, and human health," *Food Research International*, vol. 140, Article ID 109858, 2021.

[42] H. Gao, J. J. Wen, J. L. Hu et al., "Polysaccharide from fermented Momordica charantia L. with Lactobacillus plantarum NCU116 ameliorates type 2 diabetes in rats," *Carbohydrate Polymers*, vol. 201, pp. 624–633, 2018.

[43] Y. Wang, H. Zhang, G. Ma, Z. Tian, and B. Wang, "The contribution of intestinal Streptococcus to the pathogenesis of diabetic foot ulcers: an analysis based on 16S rRNA sequencing," *International Wound Journal*, vol. 19, no. 7, pp. 1658–1668, 2022.

[44] S. Xu, Y. Wang, J. Wang, and W. Geng, "Kombucha reduces hyperglycemia in type 2 diabetes of mice by regulating gut microbiota and its metabolites," *Foods*, vol. 11, no. 5, p. 754, 2022.

[45] M. V. Salguero, M. A. I. Al-Obaide, R. Singh, T. Siepmann, and T. L. Vasylyeva, "Dysbiosis of Gram-negative gut microbiota and the associated serum lipopolysaccharide exacerbates inflammation in type 2 diabetic patients with chronic kidney disease," *Experimental and Therapeutic Medicine*, vol. 18, no. 5, pp. 3461–3469, 2019.