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Extraction, characterization, and impact of Tartary buckwheat husk dietary fiber on metabolic functions and gut microbiota composition in obese mice

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Abstract

Tartary buckwheat dietary fiber (TBDF) was extracted from Tartary buckwheat husks via enzymatic hydrolysis, and the extraction process was optimized by response surface methodology. The optimal conditions were as follows: pH of 4.5, 16% enzyme concentration, and 10.5-h hydrolysis. To study its effects on metabolic health, different TBDF concentrations were added to mice's drinking water for 5 weeks. Results showed that TBDF significantly inhibited weight gain in obese mice, reduced liver index and fat-to-body ratio, alleviated hepatic steatosis, and prevented excessive fat accumulation. Higher-concentration TBDF had more hypoglycemic effects. After the intervention, Illumina MiSeq high-throughput sequencing was used to analyze gut microbiota. Comparing fecal samples from four groups, TBDF supplementation was found to enrich gut microbiota, increasing the relative abundance of *Bacteroidetes*. At the genus level, beneficial bacteria, such as *Roseburia*, *Enterococcus*, and *Lactobacillus*, were promoted. In conclusion, TBDF improves metabolic parameters and gut microbiota, offering a new dietary fiber-based strategy for obesity and metabolic disorders.

Keywords: Tartary buckwheat dietary fiber (TBDF); high-fat diet; *in vivo* lipid reduction; gut microbiota

Introduction

Obesity has emerged as a global health challenge with far-reaching implications. In recent years, the issue has reached alarming proportions, especially among adolescents. According to the latest research, the global population of obese individuals has exceeded one billion, with approximately 159 million being children and adolescents aged 5–19 years, highlighting the prevalence and severity of adolescent obesity (Alnuaimi *et al.*, 2023; Cirulli *et al.*, 2019; Di Bonito *et al.*, 2018; Hariri and Thibault, 2010; Hoyt *et al.*, 2014; Osborn and Olefsky, 2012; Wang *et al.*, 2019; Yoshimoto *et al.*, 2013; Yousef *et al.*, 2022). From 1990 to 2022, the obesity rate among children and adolescents aged 5–19 years has more than tripled. If this trend continues, it is predicted that by 2022, the number of obese adolescents will exceed the number of those with normal weight. The World Health Organization (WHO) and other international organizations have called for immediate measures, including providing healthier and more nutritious diets and increasing physical exercise, to prevent and reduce the risk of diseases, such as heart disease, diabetes, and cancer in adulthood (Chen, 2018).

The occurrence of obesity is closely related to gut microbiota imbalance. In a healthy state, the gut microbiota is in a stable state and plays a key role in metabolism. However, various internal and external factors can disrupt this balance, often leading to abnormalities in glucose and lipid metabolism as well as obesity (Zhang and Sun, 2021). The composition of gut microbiota is influenced not only by the host's genetic genes but also by dietary structure, which is a major factor affecting gut microbiota composition and metabolism. Currently, nine phyla of gut microbiota have been identified, with *Bacteroidetes* and *Firmicutes* being the dominant groups, occupying the vast majority of gut microbiota. *Firmicutes* and *Bacteroidetes* play a crucial role in maintaining human health. Holscher *et al.* (2015) suggested that changes in the abundance of *Firmicutes* and *Bacteroidetes* influence the body's metabolism, thereby controlling weight gain. When the proportion of *Bacteroidetes* is lower than *Firmicutes*, the host absorbs more nutrients. Additionally, studies have shown that an increase in the ratio of *Firmicutes* to *Bacteroidetes* may lead to obesity (Suheiyan *et al.*, 2023).

Buckwheat bran dietary fiber is used in the food and pharmaceutical industries. It can be added to food or used as a raw material/excipient in pharmaceuticals. The author should clarify its intake limit, toxicity and standards. Its application types are diverse, such as powder, granules, etc. In addition, its bioavailability needs consideration, that is, its digestion, absorption, and metabolism in the human body. Adjusting dietary structure and types, reducing the intake of high-calorie foods, and replacing high-sugar foods with high-fiber fruits and vegetables are currently effective ways to prevent and treat obesity (Emerging Risk Factors Collaboration, 2011). Adding dietary fiber to high-fat diets can also effectively prevent obesity. Research has shown that consuming fiber from grains reduces the risk of disease, with grains being an important source of dietary fiber (Chou *et al.*, 2021; Fitrasyah *et al.*, 2023). Tartary buckwheat belongs to the cereal family and has a much higher dietary fiber content than in rice, barley, and other grains. It is an excellent famine-relief crop and also a food and medicinal crop in China. It has significant effects in delaying the increase of postprandial blood glucose, stabilizing blood glucose levels, and exhibiting antioxidant properties. Dietary fiber has benefits such as promoting bowel movement, lowering postprandial blood glucose and insulin responses, reducing cholesterol, regulating gut microbiota, and exhibiting prebiotic effects (Meng, 2019). However, the mechanism by which Tartary buckwheat dietary fiber (TBDF) improves obesity is not well understood. Therefore, it is necessary to study the effects of TBDF on the composition, diversity, and host metabolism of gut microbiota.

Materials and Methods

Experimental reagents and materials

The following reagents were used in the study: 95% ethanol; acetone; sodium hydroxide; glacial acetic acid; concentrated hydrochloric acid; concentrated sulfuric acid; petroleum ether (boiling range: 30–60°C); maleic acid; protease, 7 U/mg; pancreatic α-amylase, 40 U/mg; amylase-glucosidase, 30 U/mg; neutral protease; potassium bromide (chromatographic grade); etc.

The following materials were procured: tartary buckwheat husks (purchased from Hangfei Tartary Buckwheat Co. Ltd., China); mice (140 in number, KM genetic lineage, 10–12 weeks old males, weighing 26±2 g); standard diet (comprising crude protein 18.8%; crude fat 5.2%; crude fiber 3%; crude ash 5.4%; calcium 1.1%; total phosphorus 0.7%; fat 4.3%; carbohydrates 67.3%; and protein 19.2%); high-fat diet (comprising crude protein 22.5%, crude fat 24.2%, crude fiber 3.2%, crude ash 5.6%, calcium 1.2%, total phosphorus 0.8%, fat 23.6%, carbohydrates 41.4%, and protein 23.7%). Both types of feed were purchased from Papai (Shandong) Pet Products Co. Ltd., with license No. SCXK (Xiang) 2011-0003.

Instruments and equipment

The experimental instruments used in the study are shown in Table 1.

Preparation of TBDF

The extraction process mainly referred to the National Food Safety Standard ID. GB5009.88–2023 (National Health Commission of the People's Republic of China, 2023). The raw material was crunched, sieved through an 80-mesh screen, and dried at 105°C for 4 h to remove moisture. It was defatted thrice with petroleum ether, and then immersed in ethanol to remove impurities, followed by filtration and ethanol evaporation. After removal of sugar, the sample was dried at 40°C overnight. Maleic acid was added, and enzyme solutions (pancreatic α–amylase, 400 U/mL, and amylase–glucosidase, 30 U/mL) were prepared. After cooling, pH was adjusted and protease was added to remove proteins. After reaction, the liquid was filtered. The filtrate was concentrated, 95% ethanol was added, and vacuum filtration was conducted.

Table 1. Experimental equipment.

Instrument/equipment	Model	Manufacturer	Countries and regions
Crusher	HR2168	Philips Electronics	The Netherlands
Electric hot air drying oven	101	Taiyu Oven Equipment Co. Ltd., Suzhou City	China
Fourier transform infrared spectrometer	FTIR-1500	JOSVOK (Tianjin) Technology Development Co. Ltd.	China
High-throughput sequencer	-	Biomarker Biotechnology Co. Ltd.	China
HiSeq sequencing platform	-	Illumina Inc.	USA
Scanning electron microscope	TM3000	Hitachi, Japan	Japan
Ion sputter coater	SBC-12	KYKY Technology Co. Ltd., Beijing	China
Ultra-pure water system	Milli-Q	Millipore, USA	USA
Electronic analytical balance	XS105	Mettler-Toledo Instrument Co. Ltd.	Switzerland
Blood glucose meter	GS550	Tencent Computer Systems, Shenzhen	China
Blood lipid meter	PFS-30A	Guilin URIT Medical Electronics Co. Ltd.	China
Pipette	1 mL, 200 µL, 50 µL	Eppendorf, Nederland B.V.	The Netherlands

Mixed enzymes and protease were added for secondary enzymatic hydrolysis and alcohol precipitation. The residue was washed with ethanol and acetone, and dried at 105°C overnight to remove proteins and ash. The final product was Tartary buckwheat husk dietary fiber.

Establishment of mouse model

The experiment was reviewed and approved by the Animal Welfare and Ethics Committee of Xichang University, confirming that it adheres to the ethical principles of animal experimentation. In all, 120 mice were selected. The housing environment had a humidity of 55±5%, a temperature of 23±4°C, with a 12-h light–dark cycle, and the mice had a free access to food and water. After 1-week acclimation period, all animals were divided into six groups, with 20 mice in each group.

According to the recommendations of the Food and Agriculture Organization (FAO), the recommended dietary fiber intake for a normal human population is 27 g/day. The daily water intake of KM mice was 4–6 mL, and different doses of TBDF were added to animals' drinking water. The dietary fiber intake in the drinking water was adjusted to 0 g/100 mL, 1 g/100 mL, and 2 g/100 mL. The experimental period lasted for 5 weeks, during which the mice had a free access to food and water.

Physiological index measurements

Body weight, body length, and Lee's index

Throughout the experiment, the mice were observed on daily basis. Every week, the body weight and body length of the mice were measured and recorded, and Lee's index was calculated for each animal using the following formula:

$$\text{Lee's index} = \frac{\sqrt[3]{\text{body weight(g)} \times 1}}{\text{body length(cm)}} \quad \text{(Fang, 2009)}$$

Organ index measurement

At the end of the experiment, the mice were immediately euthanized by cervical dislocation, and their liver, heart, and abdominal fat were separated and weighed. The liver index, heart index, and fat-to-body ratio were calculated for each group of mice using the following formulas:

$$\text{Liver index (\%)} = \frac{\text{Liver weight}}{\text{Mouse weight}} \times 100\%$$

$$\text{Heart index (\%)} = \frac{\text{Heart weight}}{\text{Mouse weight}} \times 100\%$$

$$\text{Fat-to-body ratio (\%)} = \frac{\text{Fat weight}}{\text{Mouse weight}} \times 100\%$$

Blood glucose measurement

During the experiment, blood samples were taken from the tail of each mouse on every 7th day to measure blood glucose levels using a blood glucose meter. The blood glucose levels of each group were monitored on weekly basis to track changes.

Blood lipid biochemical analysis

A PFS-30A blood lipid analyzer was used to measure total cholesterol (TC), low-density lipoprotein cholesterol (LDL), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and the TC–HDL ratio. At the end of the experimental period, blood was collected from the abdominal cavity of each animal, and the blood lipid levels (TC, TG, LDL, HDL, and TC–HDL ratio) were analyzed. The blood lipid levels were compared across the groups.

Mouse fecal DNA extraction and high-throughput sequencing

At the end of the 5th week of feeding, fecal samples from the normal dose (ND) group, model group, and two high-fat experimental dose (HFD) groups were collected. Five samples were collected from each group, resulting in 20 samples. The samples were rapidly placed on dry ice and sent to Biomarker Biotechnology Co. Ltd. for analysis. Based on conserved regions, primers were designed with sequences F: ACTCCTACGGGAGGCAGC and R: GGACTACHVGGGTWTCTAAT, with the adapter sequences attached to the ends of the primers. After purification, the samples were quantified and normalized to construct sequencing libraries. The libraries were quality-checked, and if qualified, high-throughput sequencing was performed on the Illumina NovaSeq 6000 platform.

The raw reads obtained from sequencing were filtered, and primer sequences were identified and removed using software, resulting in clean reads. The sequences were clustered into Operational Taxonomic Units (OTUs) at a 97% similarity level. The samples were analyzed on the basis of OTU classification results.

Gene function prediction

Based on the 16S rRNA sequencing results, the PICRUSt2 software was used to predict gene functions or phenotypes of the samples and calculate the abundance of functional genes or phenotypes.

Results and Analysis

Enzymatic extraction process optimization of TBDF

The interaction of various factors on the purity of total dietary fiber extracted from Tartary buckwheat husks is shown in Figure 1C. With the enzyme hydrolysis time fixed, increasing the pH value and the addition of mixed enzymes led to a trend where the purity of TBDF first increased and then decreased. At a mixed enzyme addition of 15-17% and a pH range of 4.4–4.6, the extraction purity of the total dietary fiber from Tartary buckwheat husks remained at a high level.

Using the Design-Expert 10 software for analysis, the optimal extraction conditions were determined, which were as follows: pH of 4.6, mixed enzyme addition of 16%, and an enzymatic hydrolysis time of 10.9 h. To verify reliability of the results, the extraction parameters were adjusted at pH 4.5, 16% mixed enzyme addition, and 10.5 h of enzymatic hydrolysis, considering practical conditions. After multiple parallel experiments, the actual extraction purity of TBDF was found as 84.17%, which was very close to the theoretical value predicted by the model (81.534%), indicating that the model could reasonably optimize the extraction conditions of TBDF, as shown in Figure 1A.

The treated and untreated groups were observed under magnifications of ×250, ×500, and ×1,500, as shown in Figure 1D. The untreated Tartary buckwheat husk surface appeared compact and well-preserved, with presence of a large amount of starch granules. After treatment, the surface of dietary fiber was curled, which could be due to disruption in cell wall structure during extraction, leading to the removal of hemicellulose and lignin, thus exposing dietary fiber and causing it to aggregate into a wrinkled structure (Deng, 2018).

Moreover, the enzyme solution caused the partial detachment of outer surface, leaving dietary fiber with a loose structure and free from other starch granule impurities, indicating that large molecular impurities, such as starch, were completely removed. After treatment, the surface of Tartary buckwheat husks was smooth and compact.

In the FTIR spectra, a broad peak near 3,387 cm^{-1} represented the –OH stretching vibration of dietary fiber, while the absorption peaks at 2,928 cm^{-1} and 1,050 cm^{-1} matched to the C–H stretching vibration of the methylene group and the C–O–C stretching vibration in pyranose ring, respectively (Liu *et al.*, 2019). The absorption peak at 892 cm^{-1} was related to the bending vibration of β-glycosidic bond (Xue *et al.*, 2021). These absorption peaks appeared in all sample spectra, indicating that the prepared TBDF retained dietary fiber structure, similar to the untreated Tartary buckwheat husks.

Absorption peak near 1,535 cm^{-1}, representing the N–H bending vibration in the protein amide structure and the C=C stretching vibration of the lignin aromatic ring, weakened significantly or disappeared. The peak at 1,737 cm^{-1}, corresponding to the C=O stretching vibration of xylan acetyl groups in hemicellulose, almost disappeared (Zhao, 2021), indicating that during the preparation process, non-cellulose substances, such as proteins, lignin, and hemicellulose, were removed, as shown in Figure 1B.

Effects of TBDF feeding on mouse body weight, body length, and Lee's index

Throughout the experiment, the body weight of all groups of mice showed an upward trend. The HFD group

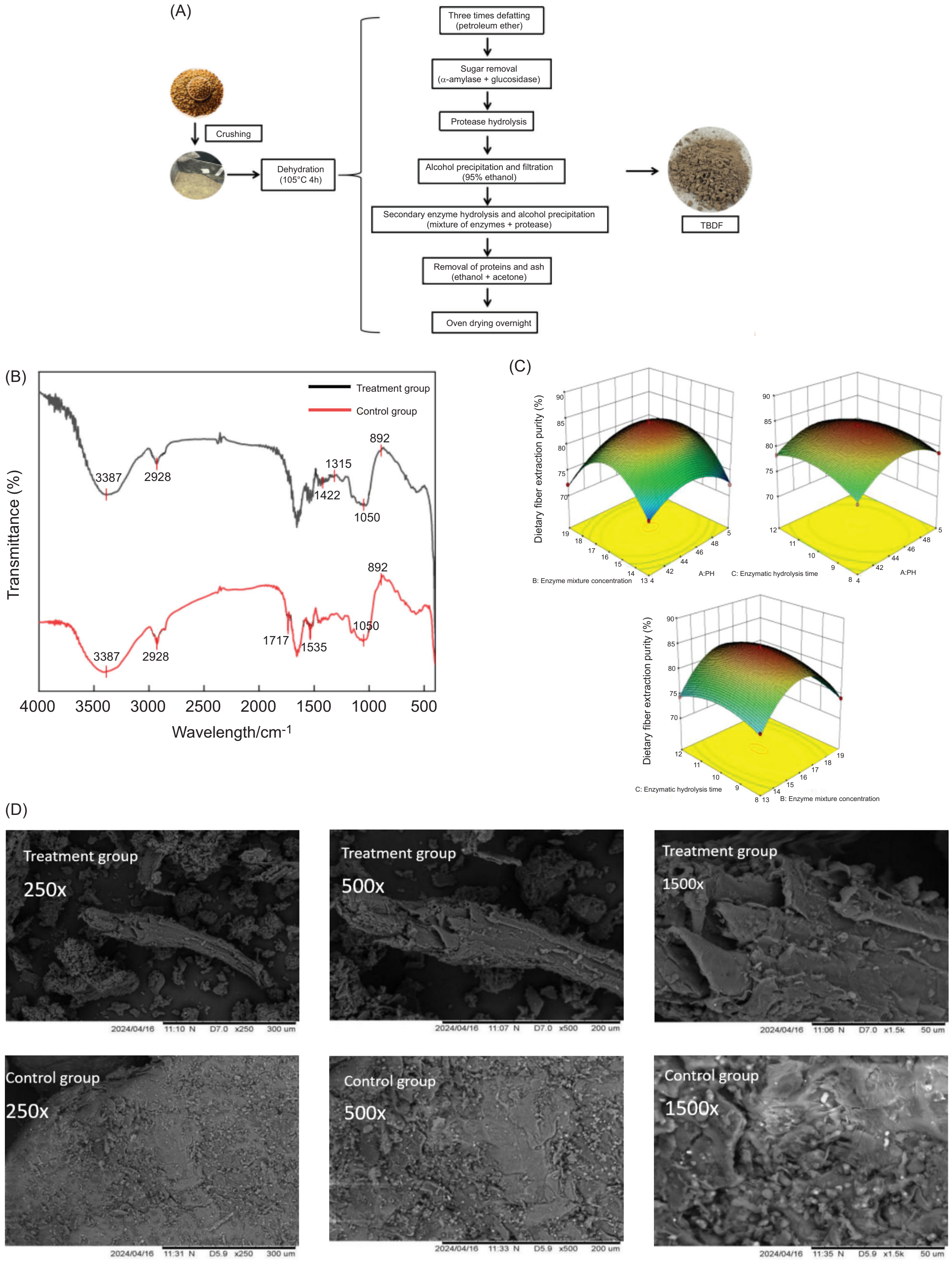

Figure 1. Enzymatic extraction process optimization results for TBDF. (A) Extraction process; (B) Fourier-transform infrared (FTIR) spectra of tartary buckwheat husks and TBDF; (C) response surface interaction experimental results; (D) Scanning electron microscopy (SEM) images of tartary buckwheat husk residue and TBDF.

showed maximum weight increase, with an average of 10.6 g, 22.2% higher than the ND group, which showed a significant difference from the ND group, confirming the successful establishment of obesity model. All TBDF-treated experimental groups showed a trend of suppressed weight gain. Significant differences were observed between ND group and ND+TBDFA group ($P < 0.05$), with the ND+TBDFA group demonstrating the smallest weight increase (3.03 g). However, no correlation was observed between TBDF concentration and weight gain in the mice. Significant differences were discovered between HFD group and HFD+TBDFB group ($P < 0.05$), with the HFD+TBDFB group showing the smallest weight gain, 3.7 g lower than the HFD group. This indicated that the higher the TBDF concentration during high-fat feeding, the more effectively it reduced fat in mice.

During the experimental period, the body length of the mice in all groups increased. However, under the induction of high-fat feed, no significant difference in body length gain was observed among the groups, suggesting that TBDF had no significant effect on the body length of mice, and no correlation was observed between TBDF concentration and body length growth.

The Lee's index of the ND group remained at the same level, while the Lee's index of the HFD group showed an upward trend. The Lee's index of other experimental groups decreased in general and was lower than that of the ND group. In the 5th week, the Lee's index of the HFD group was significantly higher than that of other groups. Analyzing the data with SPSS one-way ANOVA showed significant differences ($P < 0.05$) in the Lee's index increment between the HFD group and other experimental groups, except for the ND group. In the group of mice fed with regular diet, TBDF had a nonsignificant impact on Lee's index, and no correlation was determined between TBDF concentration and Lee's index increment. However, in the group of mice fed with high-fat feed, TBDF had a significant effect on Lee's index. Compared to the HFD group, the Lee's index increment in the HFD+TBDFB group decreased by an average of 27 g/cm^3. A negative correlation was observed between Lee's index and TBDF concentration.

Blood glucose levels were measured on every 7th day by collecting blood from the tail tips of mice. Compared to the ND group, the HFD group had a significant increase in blood glucose ($P < 0.05$), showing symptoms of hyperglycemia, indicating that continuous high-fat feeding lead to an increase in blood glucose levels in mice, as shown in Figure 2.

In mice fed with regular diet and supplemented with different concentrations of TBDF, blood glucose remained at normal levels, with no significant difference from the ND group. In mice fed with high-fat diet, supplementation with different concentrations of TBDF significantly inhibited the increase in blood glucose ($P < 0.05$), indicating that TBDF could effectively lower blood glucose levels in obese mice. A negative correlation was observed between TBDF concentration and blood glucose levels.

Effects of TBDF feeding on mouse blood lipid profile

Analysis of blood lipid profile (TC, TG, and HDL) showed that compared to the ND group, the HFD group had elevated TC, HDL, and TG values, with significant increase in TC ($P < 0.05$), further confirming the successful establishment of obesity model. After the administration of different doses of TBDF, all three lipid indicators in the HFD experimental groups decreased, indicating that continuous high-fat feeding increased blood lipid levels and presented health risks. Notably, many studies on diabetic mice reported lower HDL levels in the HFD group, compared to normal groups, a result of severe glucose-lipid metabolic disturbances (Chen *et al.*, 2019a, 2019b; Gao *et al.*, 2018). However, the findings of this experiment were diametrical, with higher HDL levels in HFD group. This could be explained by the primary function of HDL, which is to transport cholesterol from peripheral tissues to the liver for metabolism and excretion via bile. High-fat feeding increases cholesterol levels in the blood, stimulating the liver to synthesize more HDL to maintain metabolic homeostasis, thereby elevating HDL levels in the serum (Wojczynski *et al.*, 2011; Wolf, 1996). Further investigation is needed to explore the underlying mechanisms. No significant changes were observed in the TC/HDL and LDL levels, indicating that different concentrations of TBDF had no effect on these two indicators in mice, as shown in Figure 3.

Effects of TBDF feeding on mouse organ indices

Under the induction of high-fat diet, liver index in the HFD group was significantly higher than in the ND group ($P < 0.05$), confirming the successful establishment of obesity model. With TBDF intervention, liver index in the high-fat diet group decreased significantly ($P < 0.05$). A negative correlation was observed between liver index and TBDF concentration.

Heart index in the HFD group showed no significant difference, indicating that TBDF did not have a significant effect on heart index, and no correlation was determined between TBDF concentration and heart index.

The fat-to-body ratio in HFD group was significantly higher than in ND group ($P < 0.05$), indicating that high-fat feeding induced a large accumulation of adipose

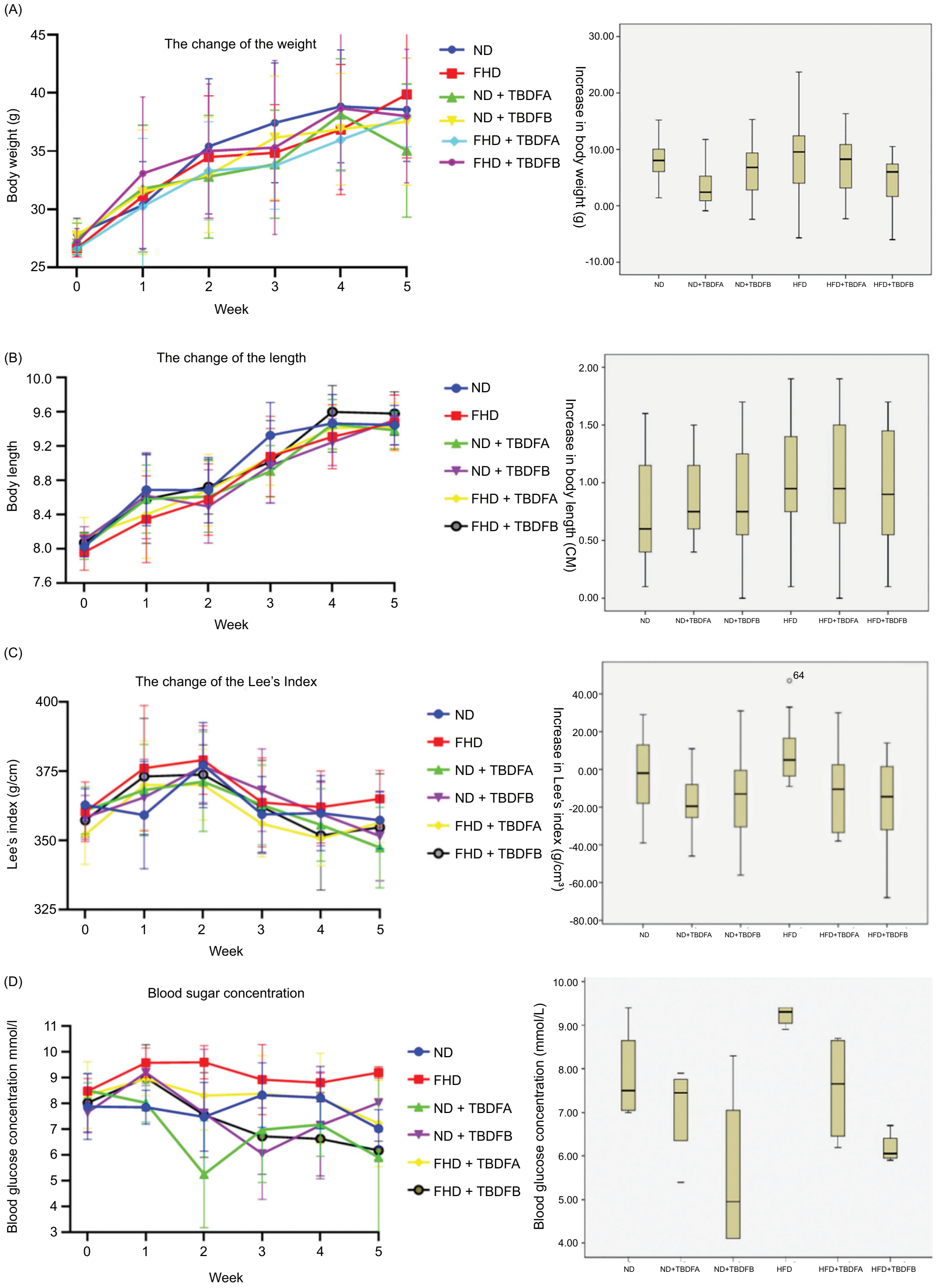

Figure 2. Effects of TBDF feeding on mice's body weight, body length, and Lee's index. (A) Body weight changes and incremental mean; (B) body length changes and incremental mean; (C) Lee's index changes and incremental mean; (D) Effect of TBDF on blood glucose.

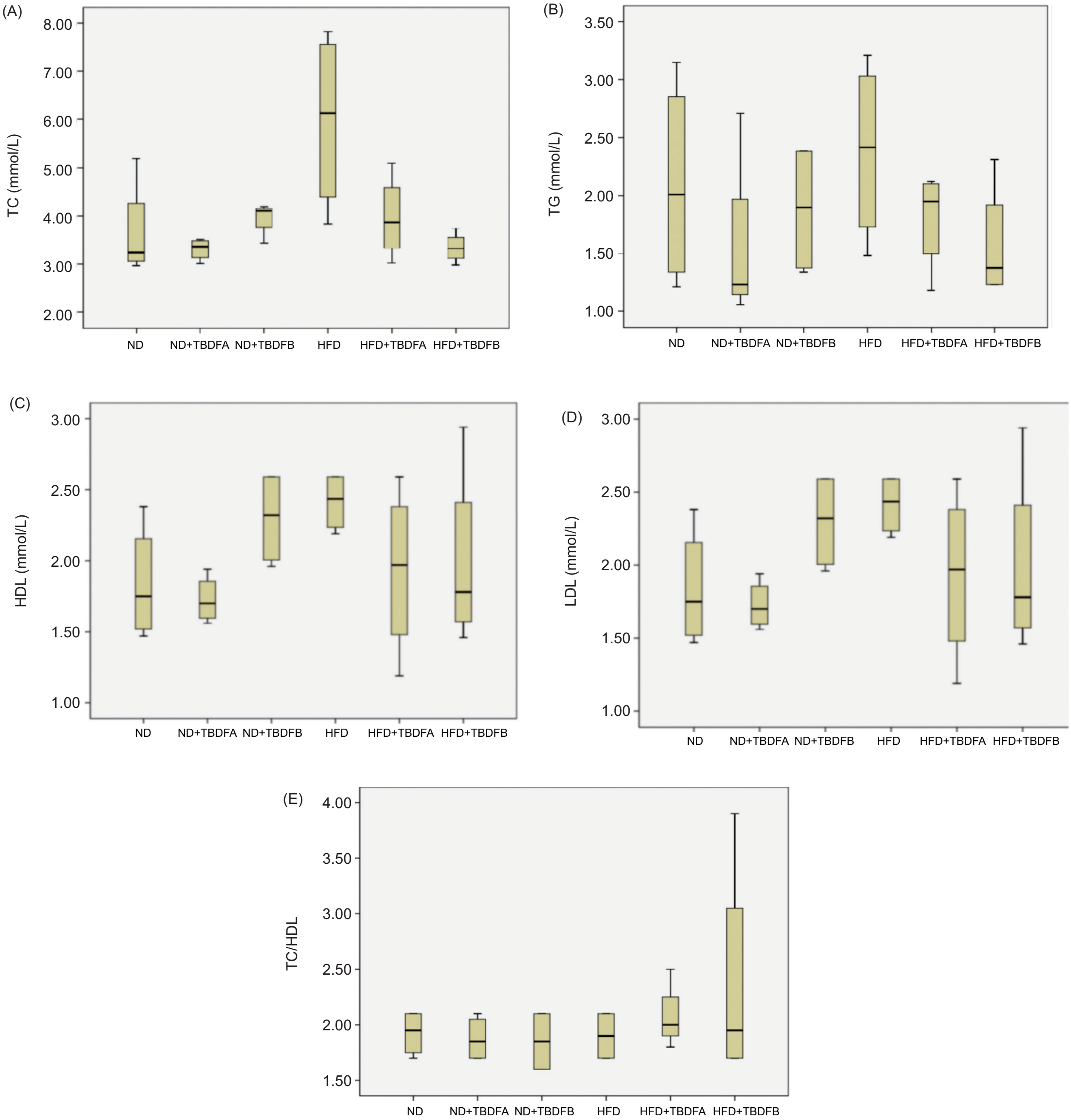

Figure 3. Effects of TBDF feeding on mice's blood lipid profile. (A) Average TC levels in each group; (B) average TG levels in each group; (C) average HDL levels in each group; (D) average LDL levels in each group; (E) average TC/HDL ratio in each group.

tissue, a typical characteristic of obesity (Liu, 2020). After the administration of TBDF, the fat-to-body ratio decreased, but the increment in fat-to-body ratio did not decrease with increasing TBDF concentration, showing no correlation between the two, as shown in Figure 4.

rRNA V3–V4 region sequencing

A total of 2,533,785 raw reads were generated from the sequencing of 20 samples. After quality control and assembly, 1,051,587 clean reads were obtained, with an average of 52,579 clean reads per sample and a minimum of 8,835 clean reads per sample.

Clustering of OTU was performed to determine OTU count for each sample. OTU refers to a unit of classification representing a species in population genetics studies, where sequences with over 97% similarity are grouped together. The total number of OTUs was ranked as follows: HFD+TBDFB > HFD+TBDFA > HFD > ND.

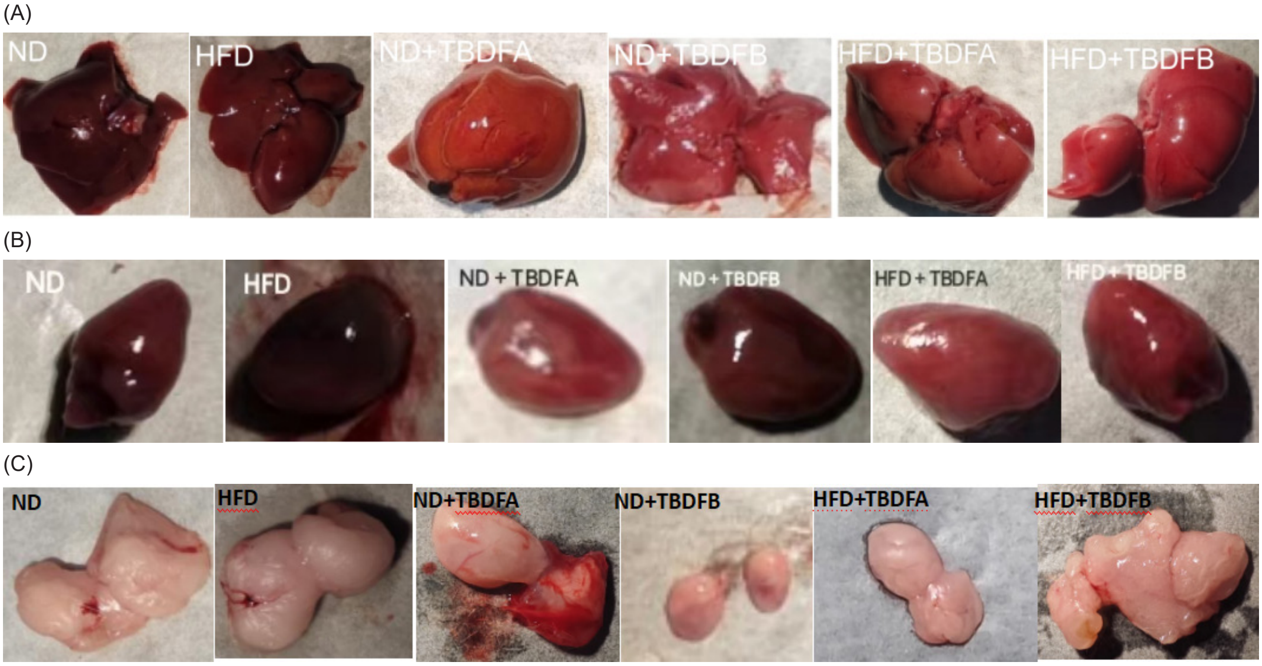

Figure 4. Effects of TBDF feeding on mice's organ indices. (A) Liver size, color comparison; (B) heart size, color comparison; (C) fat size, color comparison.

Among the four feeding groups, 214 OTUs were shared across all groups, while unique OTUs in the NC, HFD+TBDFA, HFD+TBDFB, and TFD groups were 1,183, 1,150, 1,046, and 770, respectively. The HFD+TBDFB group exhibited a 36% increase in unique bacteria, compared to the TFD group. This proposed that the inclusion of TBDF in the diet could alter the diversity of gut microbiota.

Alpha-diversity is commonly used to measure the diversity and richness of microbiota within a group, with indices such as the Shannon index (community diversity), Abundance-based Coverage Estimator (ACE) index (community richness), and Chao1 index (species richness) used frequently (Zhao *et al.*, 2019). A higher value indicates greater diversity in the composition of microbiota. Coverage parameter, which reflects sequencing depth, was more than 99.9% in all samples, ensuring that the sequencing depth was sufficient to capture the vast majority of microbial species information, making the data representative for subsequent analysis.

After the intervention with TBDF, both HFD+TBDFA and HFD+TBDFB groups showed significant increase in ACE indices, compared to the TFD group, with statistically significant differences ($P < 0.05$). This indicates that the inclusion of TBDF in diet facilitates better utilization by gut microbiota, thus promoting microbial growth.

Furthermore, after the consumption of TBDF, the Shannon index of gut microbiota increased in all TBDF-fed groups, with a positive correlation to TBDF concentration. The HFD+TBDF groups exhibited higher Shannon index values, compared to the control group. This suggests that while a high-fat diet tends to reduce the variety of gut microbiota, the intake of TBDF significantly increased the number and diversity of gut microbiota species, enhancing microbial diversity. This finding provides new insights into the potential of TBDF in regulating the balance of gut microbiota.

Beta-diversity is used to assess the similarity of microbial community composition between different samples. β-diversity analysis in this study was based on methods such as binary Jaccard, Bray–Curtis, weighted UniFrac (bacteria only), and unweighted UniFrac (bacteria only), with main analyses including principal coordinate analysis (PCoA), principal component analysis (PCA), and unweighted pair group method with arithmetic mean (UPGMA) to observe differences between individuals or groups.

According to PCoA and PCA analyses, the HFD+TBDFA and HFD+TBDFB groups were closely clustered, with a significant overlap, while both were separated from the control groups (ND), indicating that TBDF significantly alters gut microbiota composition. The high- and low-fat diets have considerable impact on mice gut microbiota.

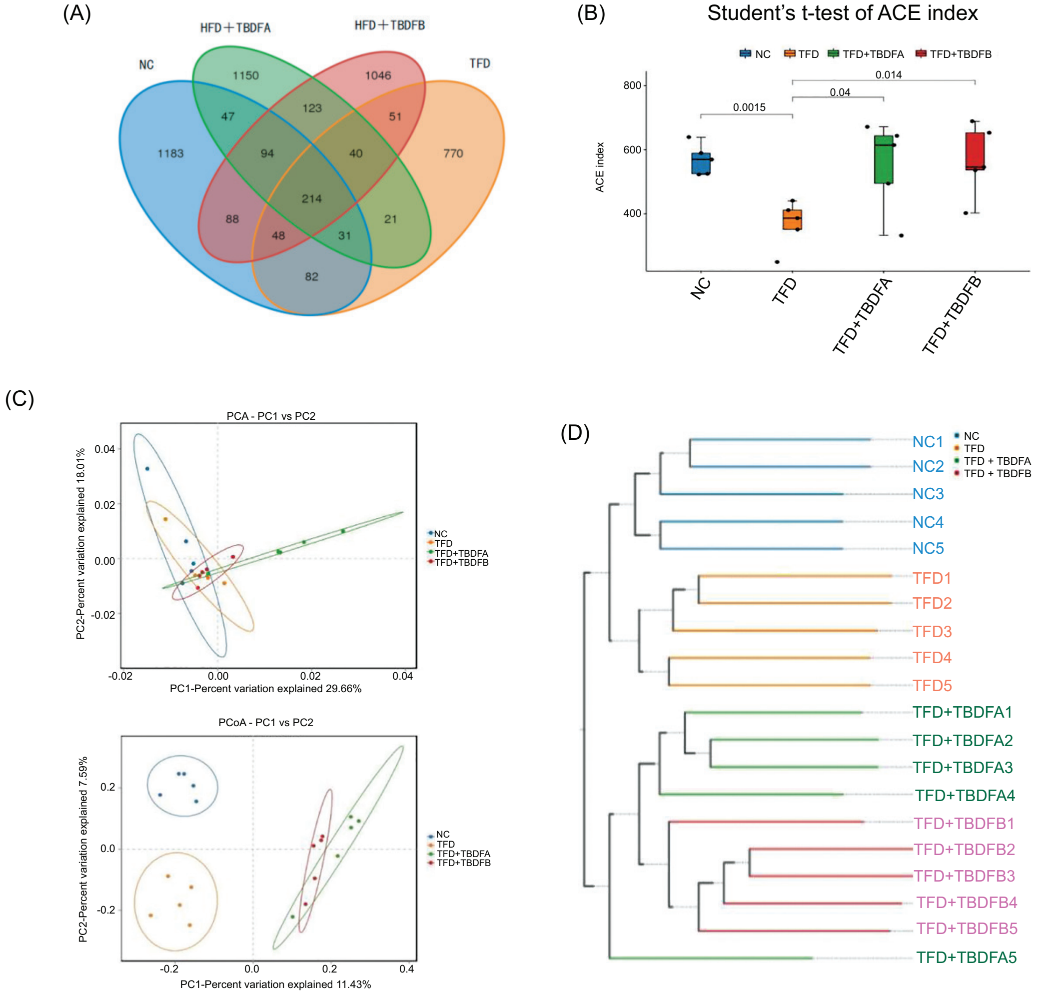

Figure 5. Analysis of α- and β-diversity of mice's gut microbiota after high-fat diet fiber (wheat dietary fiber [WDF]) intervention. (A) Venn diagram of features; (B) ACE index plot; (C) PCA plot; (D) PCoA plot; and (E) UPGMA clustering of samples.

The UPGMA clustering results showed that NC, HFD, and HFD+TBDF groups formed distinct clusters. Each of NC and HFD group clustered with their five samples, while the HFD+TBDF group formed its own cluster. This indicates that there were clear differences in community composition across groups, and TBDF supplementation clearly altered the microbiota structure in mice gut, as shown in Figure 5.

Analysis of gut microbiota structure

Of the 20 samples, the top 10 species were listed in terms of abundance, with the remaining species grouped as "others." At the phylum level, the dominant microbiota include *Bacteroidota* (48.84%, 43.02%, 57.74%, and 51.96%), *Firmicutes* (44.35%, 51.49%, 39.06%, and 40.77%) as well as *Proteobacteria* and *Campylobacteria*, which also rank among the top phyla. Addition of TBDF to a high-fat diet promotes the relative abundance of *Bacteroidota*, *Proteobacteria*, and *Actinobacteria*. *Bacteroidota* is rich in carbohydrate-active enzymes, which degrade and utilize polysaccharides and effectively break down organic acids, such as acetate and propionate. Many carbohydrate-utilizing microorganisms belong to *Firmicutes*, such as probiotics and lactobacilli (Wang *et al.*, 2023). A study conducted by Chen *et al.* (2018) showed that the abundance of *Proteobacteria* increased in obese mice. Holscher *et al.* (2015) demonstrated that the abundance of *Firmicutes* and *Bacteroidota* influence body's metabolism, thereby controlling the rate of weight gain. If *Firmicutes* are in abundance than *Bacteroidota*, then the body effectively absorbs nutrients, leading to weight gain. Research conducted by Dominianni *et al.* (2015) confirmed that dietary fiber from vegetables, fruits, and legumes plays a significant role in maintaining gastric and intestinal health, as it increases the abundance of *Actinobacteria*.

These results suggested that TBDF intake improved abundance and diversity of gut microbiota in mice, in line with previous findings (Schnorr *et al.*, 2014), where increased diversity and abundance were noted in populations consuming whole grains or fiber-rich diets. However, level of *Bacteroidota* was reduced, while *Firmicutes* and *Proteobacteria* levels were elevated.

The analysis focused on specific microbial genera, with vertical clustering representing the similarity of genera. The length of branches and the proximity between them reflect the degree of similarity in the abundance of species between the two samples (Zhang, 2020). Supplementation with 2 g/100 mL TBDF significantly increased the relative abundance of genera such as *Flavobacteriaceae*, *Rhodobacteriaceae*, and *Micrococcaceae*. *Flavobacteriaceae* belongs to the phylum *Bacteroidota* and is a common bacterium in gut microbiota. It plays an important role in maintaining gut microecology, balancing the immune system, and supporting the overall health. *Rhodobacteriaceae* and *Micrococcaceae* belong to the phylum *Actinobacteria*, with *Escherichia coli* being one of the most well-known members of *Rhodobacteriaceae*. This bacterium is one of the most common in the gut of humans and other animals. *Micrococcaceae* is also part of the phylum *Actinobacteria*, and the bacteria within this genus participate in the decomposition and absorption of food residues in the intestines, as shown in Figure 6.

Analysis of functional gene prediction

Unlike metagenomic sequencing, 16S sequencing does not provide direct gene information. However, PICRUSt2 was used to further explore functional gene predictions based on 16S data. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of functional genes in the microbiota revealed significant differences in metabolic pathways among the four groups comprising 20 samples.

The five most contributing microorganisms in relevant metabolic pathways included *Actinobacteria*, *Bacteroidota*, *Proteobacteria*, *Cyanobacteria*, and *Desulfobacteria* (see Figure 7). The KEGG pathway analysis at phylum level showed high relative abundance in global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, and cell growth and death. These findings indicated that TBDF intervention influenced microbial pathways related to energy metabolism, amino acid processing, and the overall metabolic processes, contributing to improvements in metabolic regulation and energy balance in the mice.

Metabolism involves a variety of substances that must be coordinated to ensure the proper functioning of cells and organism as a whole. When changes occur in internal or external environments, the metabolic changes of these substances also need to be harmonized. Alterations in the gut microbiota structure affect its physiological functioning, resulting in further changes in metabolic activities (Cai and Li, 2021). It was found through analysis that compared to the control group, the whole body dietary fiber and biomarkers (WDFB) group showed significant changes in the proportion of beneficial bacteria and opportunistic pathogens. The analysis of KEGG pathway predicted functional genes and their associated metabolic pathways in the WDFB group.

As shown in Figure 8, secondary pathways involved in lipid metabolism and the digestive system were significantly upregulated ($P < 0.05$). This suggests that high-dose WDF might serve as a nutritional or energy source for gut cells, which can be utilized by gut microbiota. This, in turn, impacts the metabolic pathways of mice, promoting the overall metabolism and participating in other metabolic activities. Combining bioinformatic techniques with disease models enable a deeper understanding of metabolic regulation mechanisms within the body.

Conclusions

Tartary buckwheat dietary fiber was extracted by enzymatic hydrolysis. An obesity model was established with male KM mice by inducing high-fat diet, and different concentrations of TBDF were prepared in drinking water. After 5 weeks of free feeding, the effects of TBDF on various physiological indicators of obese mice were analyzed systematically. Paired-end sequencing was then applied, using the Illumina NovaSeq sequencing platform, with high-fat mice without TBDF as control, to study the impact of TBDF on gut microbiota.

Scanning electron microscopy and FTIR analysis: SEM results showed that enzymatic hydrolysis effectively removed proteins, hemicellulose, and lignin, exposing and aggregating dietary fiber into folds. FTIR analysis confirmed that this enzymatic method efficiently removed proteins, hemicellulose, and lignin. The optimal extraction conditions were found to be pH 4.5, 16% enzyme concentration, and an enzyme hydrolysis time of 10.5 h, yielding a dietary fiber content of 84.17%.

Effects on obese mice: TBDF significantly improved multiple physiological indicators, such as weight, body fat, blood sugar, blood lipids, liver, and fat levels, in obese mice. TBDF inhibited weight gain, reduced liver index, and decreased the likelihood of fatty liver disease. It also prevented excessive fat accumulation, with different

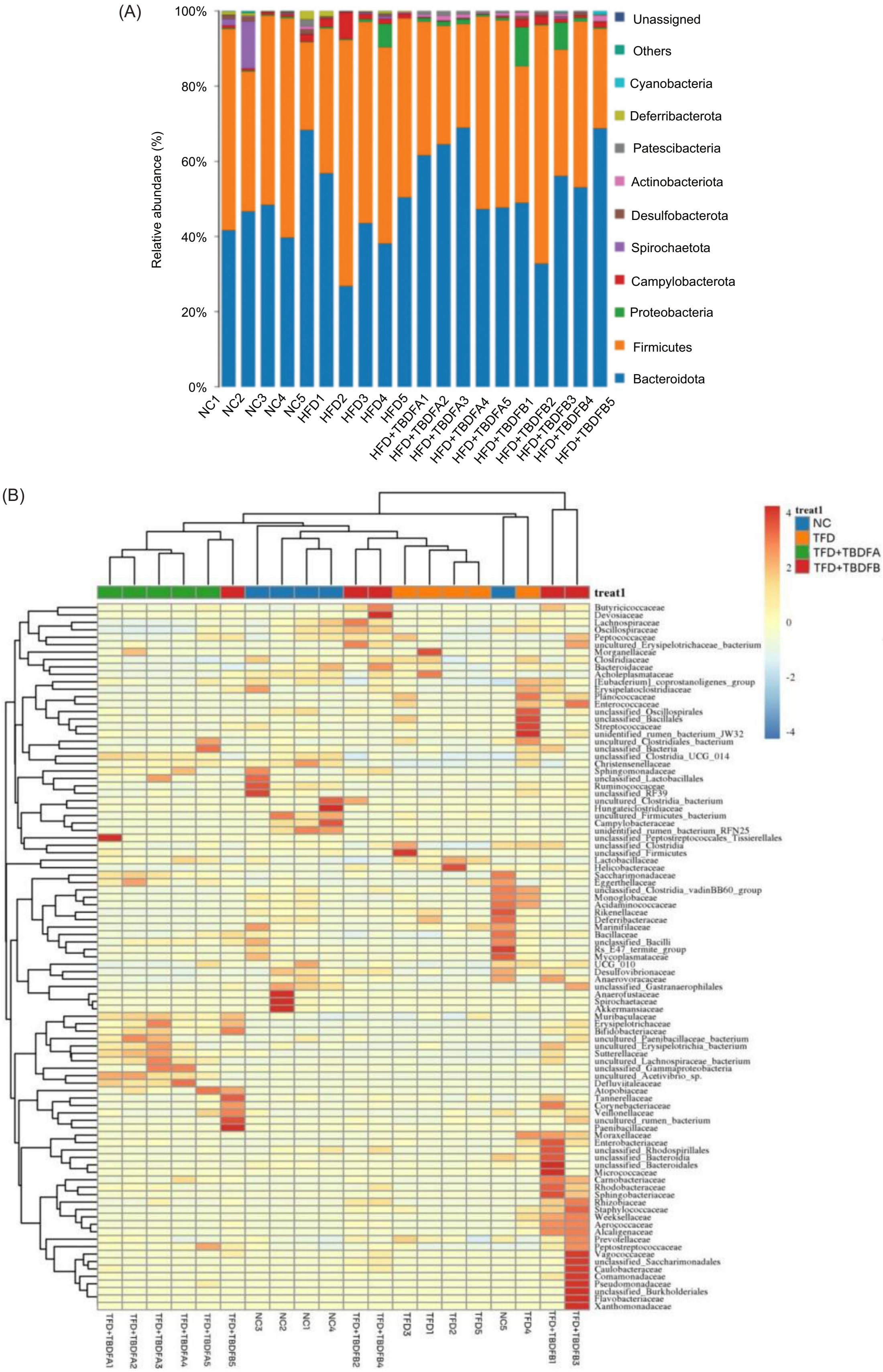

Figure 6. Gut microbiota structure analysis. (A) Bacterial distribution at phylum level. (B) Species abundance heatmap at genus level.

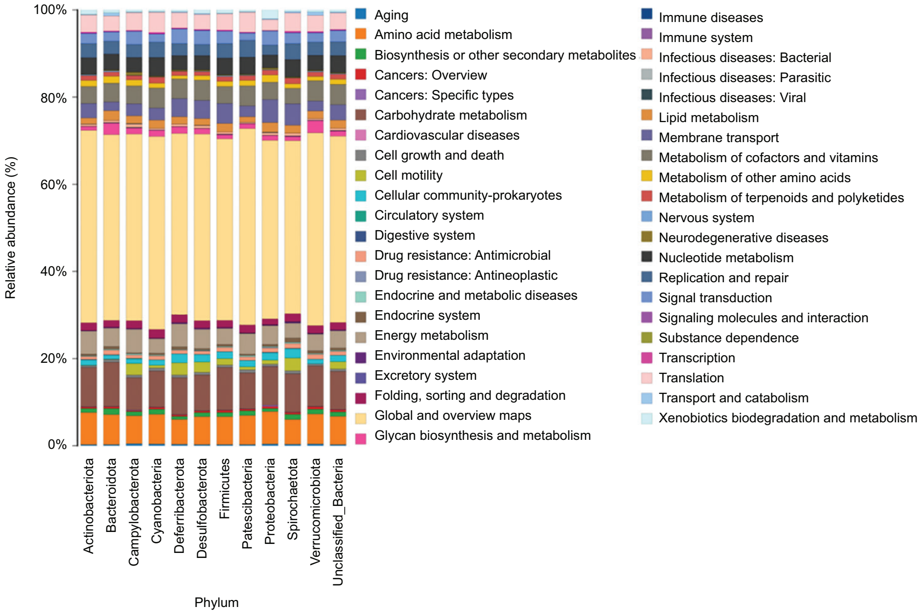

Figure 7. KEGG metabolic pathways at phylum level.

TBDF concentrations showing varying degrees of effectiveness in lowering blood sugar and lipids.

Gut microbiota regulation: TBDF helped to increase the relative abundance of gut microbiota in the HFD+TBDF group, resulting in a significantly different structure composition from the control group. At the phylum level, TBDF increased the relative abundance of *Actinobacteria*, *Proteobacteria*, and *Bacteroidota* while reducing the abundance of *Firmicutes*. At the genus level, high concentrations of TBDF promoted the growth of genera, such as *Flavobacteriaceae*, *Rhodobacteriaceae*, *Micrococcaceae*, and *Bifidobacteriaceae*. The KEGG pathway analysis indicated that TBDF was utilized by mice's gut microbiota to generate various metabolites. These metabolites interact and coordinate their biological functions, affecting metabolic pathways in mice. Following WDF intervention, secondary pathways in lipid metabolism and the digestive system were upregulated significantly.

The impact of dietary fibers on gut microbiota varies depending on their source. TBDF has a regulatory effect on gut microbiota. The findings in this study provide theoretical support for the research and application of TBDF.

Author Contributions

Conceptualization: Qiao Lin and Chen Ji; methodology: Qiao Lin, Chen Ji, Xin Yao, Xubin Hua, Yiran Chen, and Ju Qiu; investigation: Xin Yao, Xubin Hua, Yiran Chen, and Ju Qiu; resources: Yijia Zhu, Yousheng Deng, and Ruohan Liu; data curation: Xin Yao, Xubin Hua, Yijia Zhu, Yousheng Deng, Yiran Chen, Ruohan Liu, and Ju Qiu; writing—original draft preparation: Qiao Lin, Chen Ji, Xin Yao, Xubin Hua, Yijia Zhu, Yousheng Deng, Yiran Chen, Ruohan Liu, and Ju Qiu; writing—review and editing: Qiao Lin, Chen Ji, Xin Yao, Xubin Hua, Yijia Zhu, Yousheng Deng, Yiran Chen, and Ju Qiu; supervision: Qiao Lin; project administration: Qiao Lin; and funding acquisition: Qiao Lin. All authors read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declared no conflict of interest.

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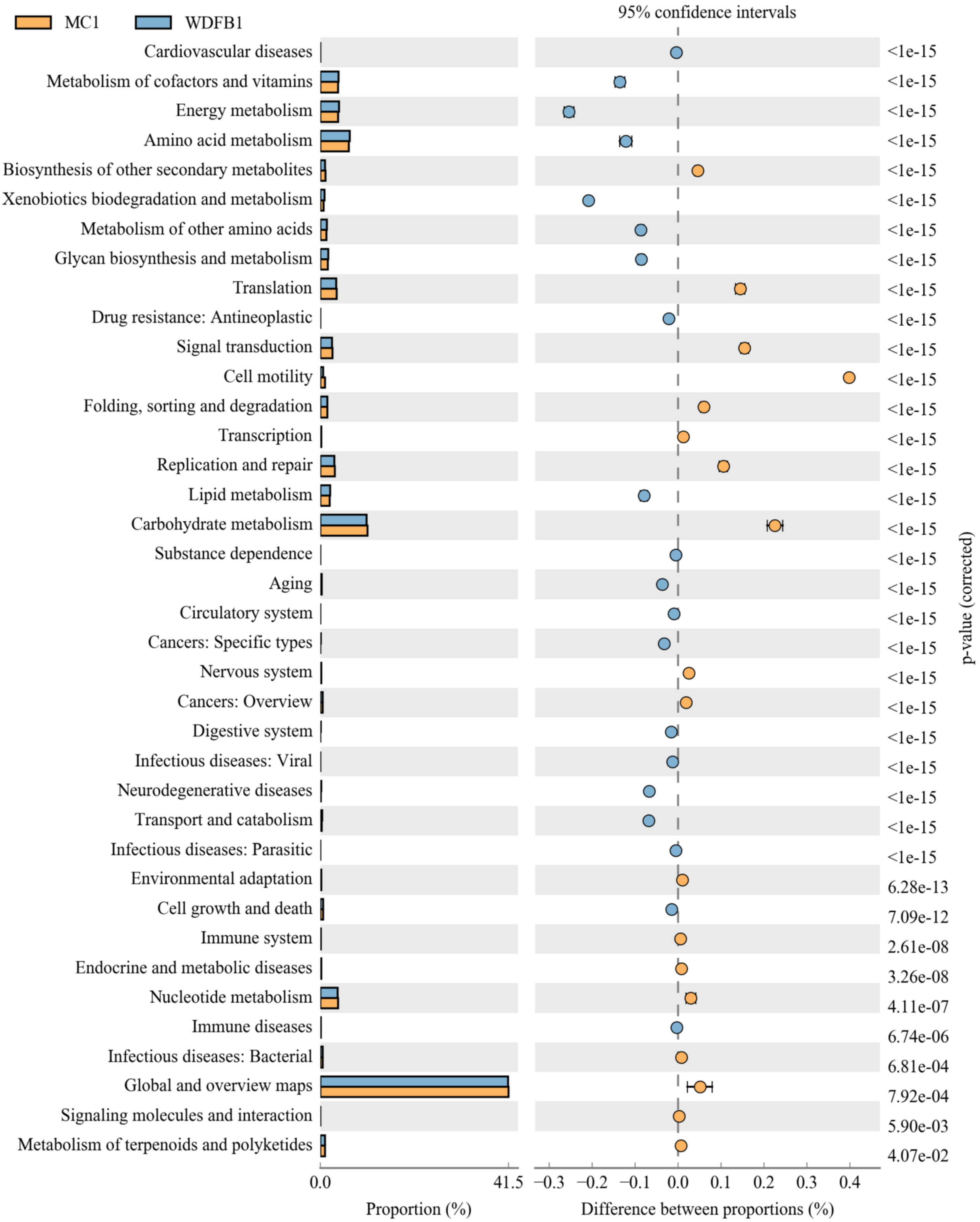

Figure 8. KEGG biological metabolic functional gene map.

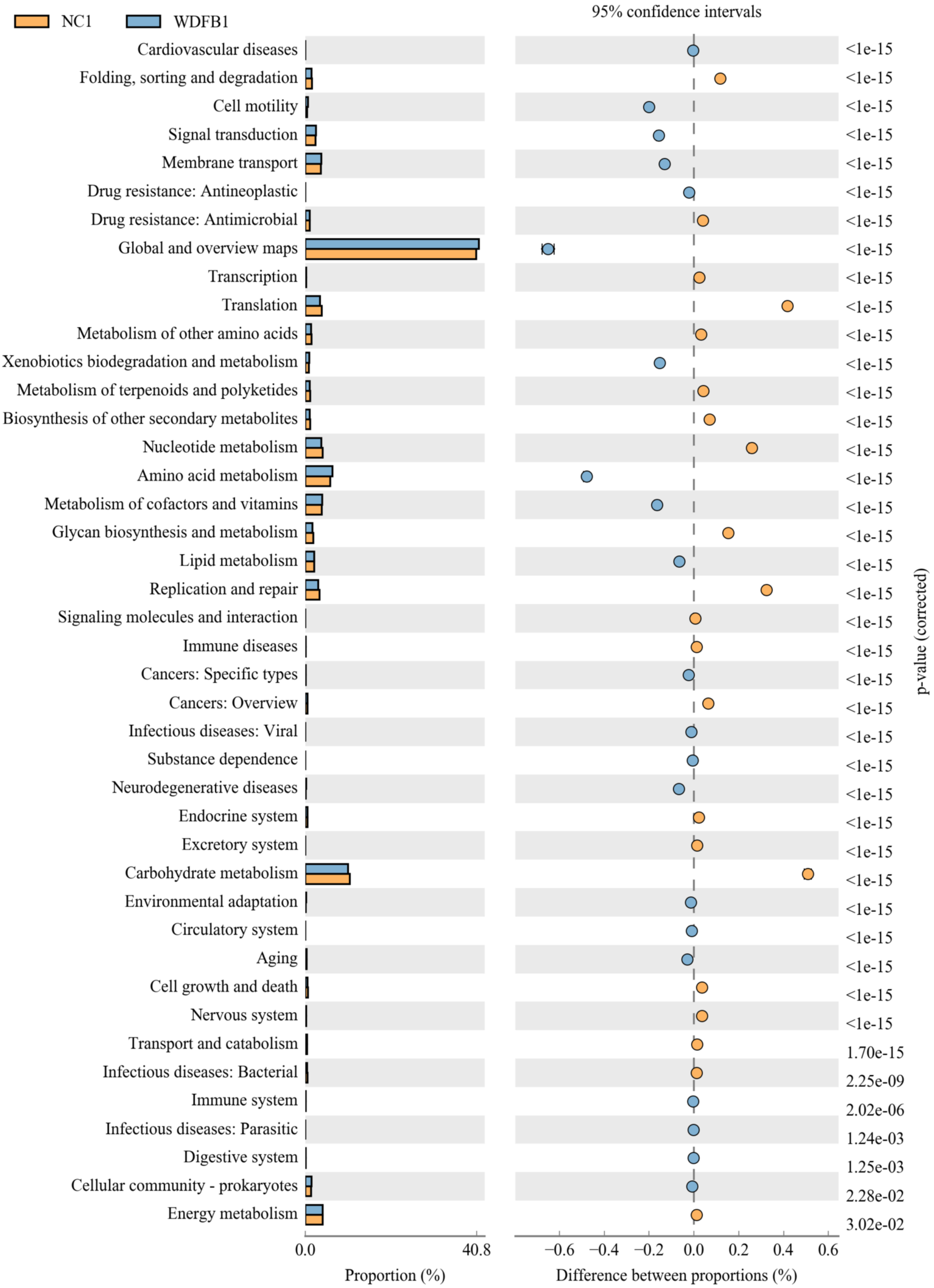

Figure 8. **Continued.**

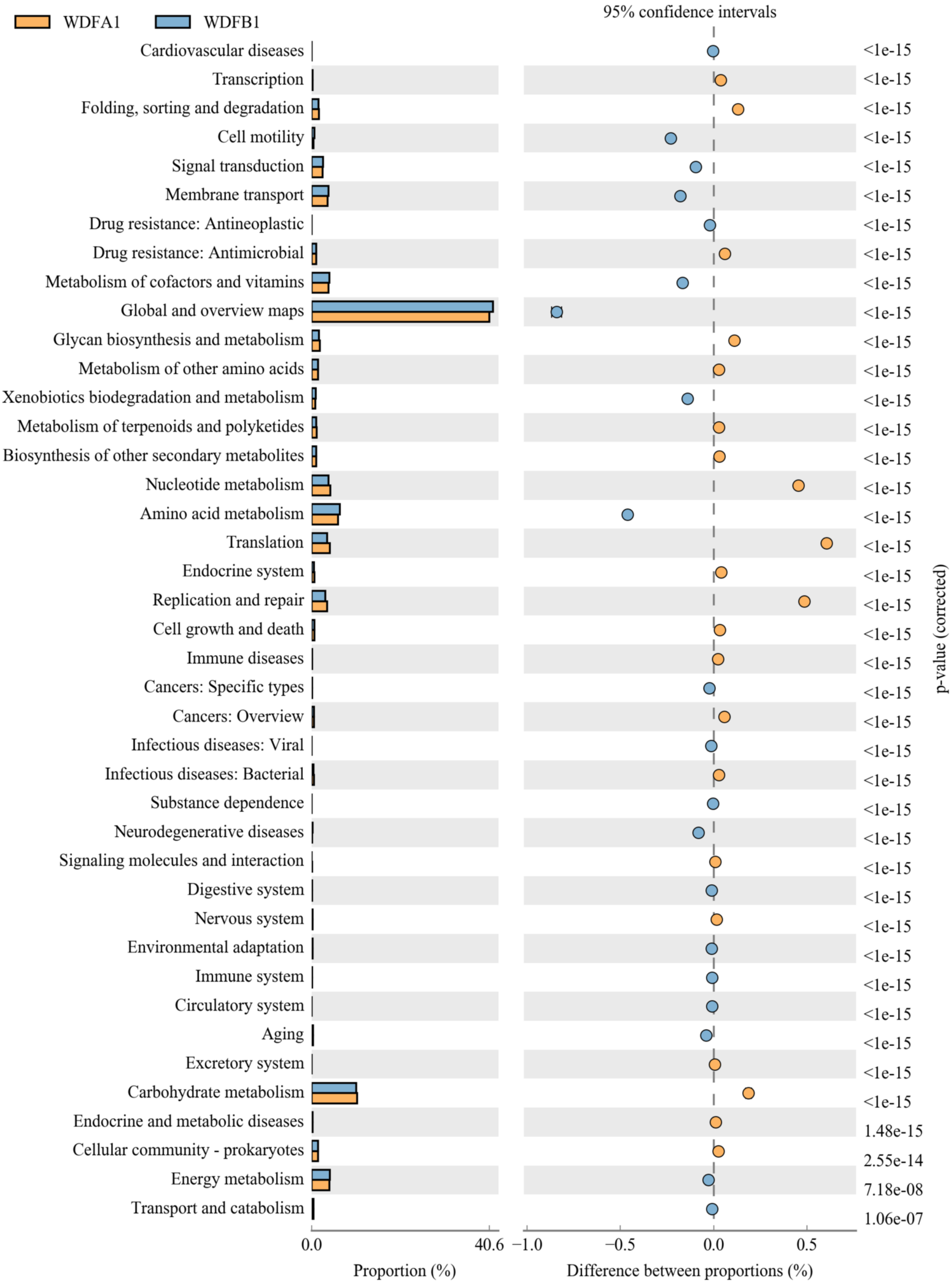

Figure 8. **Continued.**

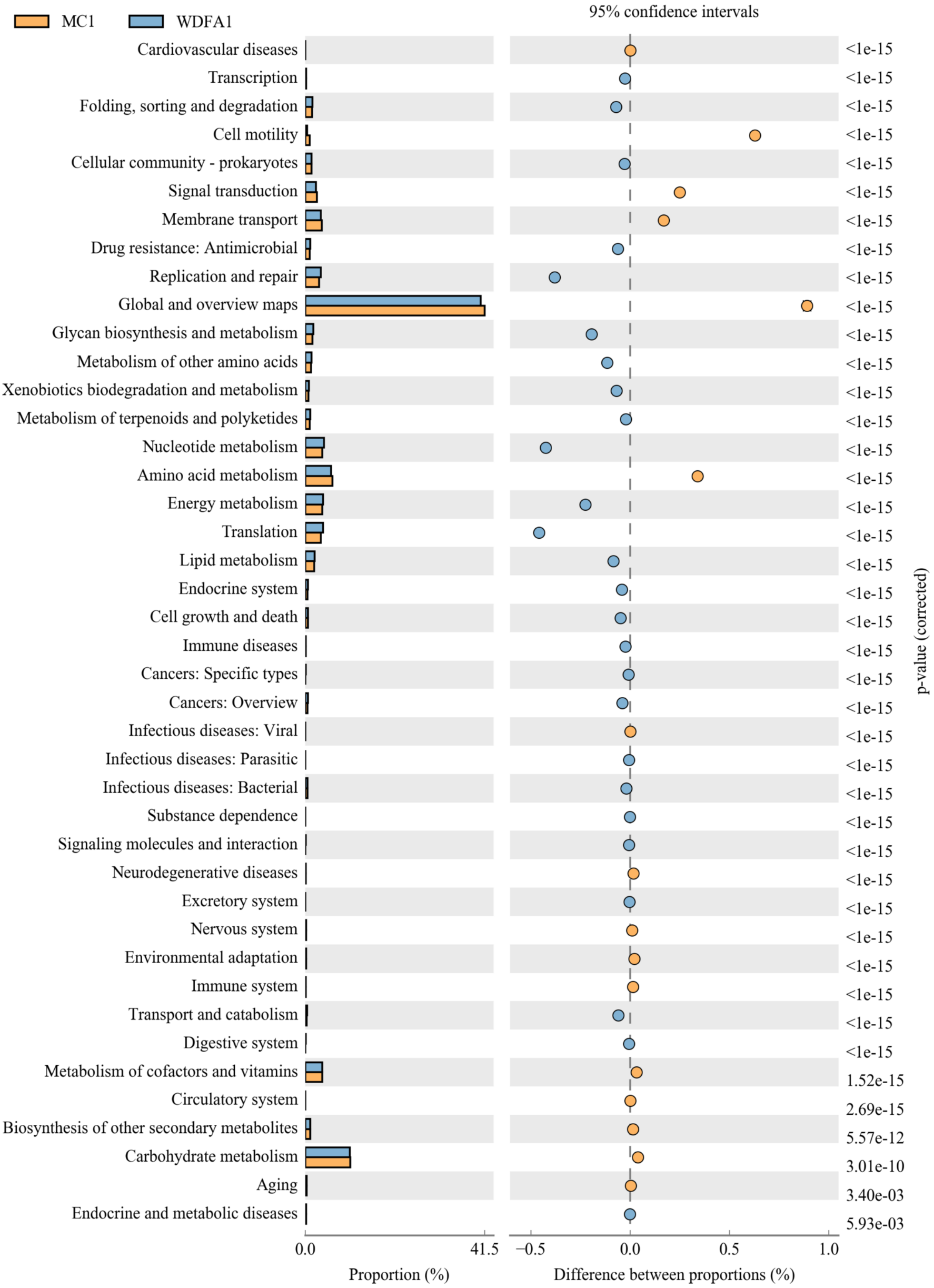

Figure 8. Continued.

Task Force Project of Liangshan Yi Autonomous Prefecture: Technology Research and Development of Low-GI Healthy Grain Products & Demonstration of Primary, Secondary and Tertiary Industries Integration (Project Code: LZKJ-2025-02).

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