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Synergistic antihyperglycemic effects of wheat bran insoluble dietary fiber–inulin complex via gut microbiota mediated short-chain fatty acid production

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ABSTRACT: The synergistic antihyperglycemic effects of wheat bran insoluble dietary fiber (WBIDF) complexed with inulin (IN) were investigated in this study. *In vitro* fecal fermentation demonstrated that WBIDF-IN significantly increased the production of short-chain fatty acids (SCFAs), particularly acetic and butyric acids, outperforming WBIDF and other WBIDF-soluble dietary fiber (SDF) complexes (including WBIDF-stachyose, WBIDF-fructo-oligosaccharides, and WBIDF-resistant dextrin). Subsequently, *db/db* mice received the optimized complex for 12 weeks. Compared with WBIDF or IN alone, WBIDF-IN supplementation significantly lowered fasting blood glucose, improved glucose tolerance, enhanced insulin sensitivity by reducing fasting insulin and HOMA-IR, and promoted GLP-1 secretion. HE staining revealed that WBIDF-IN markedly preserved islet morphology and cellular integrity, while immunofluorescence analysis confirmed the restoration of insulin expression to near-normal levels. 16S rRNA sequencing revealed that WBIDF-IN improved gut microbial diversity and shifted the microbial composition by enriching SCFAs-producing genera such as *Muribaculaceae*, *Ruminococcaceae*, and *Alloprevotella*, while reducing harmful taxa like *Romboutsia* and *Enterococcus*. Correlation analysis further revealed that the relative abundance of *Alloprevotella* was positively associated with colonic SCFAs and GLP-1 levels, but negatively correlated with fasting blood glucose. Collectively, these findings demonstrated that WBIDF-IN complexation exerts enhanced antihyperglycemic effects primarily through microbiota-mediated SCFAs production, which promoted GLP-1 secretion and pancreatic islet protection, providing a promising dietary strategy for type 2 diabetes management.

Keywords: Wheat bran insoluble dietary fiber, Inulin, SCFAs, Gut microbiota, Type 2 diabetes mellitus

1. Introduction

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder characterized by impaired insulin secretion or decreased insulin sensitivity. Globally, more than 415 million individuals are affected by diabetes, with over 100 million cases reported in China alone ^[1]. Chronic hyperglycemia is a vital risk factor for

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diseases including cardiovascular diseases, retinopathy, nephropathy. Among various management strategies, the International Diabetes Federation advises dietary interventions, particularly increased intake of dietary fiber (DF) could be a crucial aspect of a balanced diet or nutritional treatment therapy for the prevention and management of T2DM [2].

Dietary fibers from distinct botanical sources exert hypoglycemic effects through divergent mechanisms. Soluble dietary fibers, such as inulin and pectin, primarily ameliorate hyperglycemia by increasing chyme viscosity to retard glucose absorption and by serving as accessible substrates for microbial fermentation to yield short-chain fatty acids (SCFAs)^[3]. While, insoluble dietary fibers (IDF) (e.g., wheat bran) regulate glucose metabolism mainly through physical mechanisms, including delaying the adsorption of glucose, inhibition of carbohydrate-hydrolyzing enzymes, and acceleration of intestinal transit ^[4]. IDF, commonly cellulose, hemicellulose and lignin from plant cell wall, has traditionally been considered poorly fermentable, but contributes to gut motility, toxin elimination, and improved bowel health. Recent studies suggest that IDF can be partially fermented by gut microbiota and exert metabolic benefits. For instance, previous studies found that *Pyrus ussuriensis* Maxim pomace IDF modulated gut microbiota and alleviated high-fat diet-induced metabolic disturbances ^[5]. Similarly, kiwifruit IDF was reported to improve blood glucose and lipopolysaccharide (LPS) levels while reduce inflammation and insulin resistance ^[6]. These effects are attributed to enhanced SCFAs production, which may stimulate insulin sensitizing GLP-1 secretion, modulate appetite, and improve insulin secretion ^[7]. However, individual variation in gut microbiota composition significantly influences SCFAs-mediated effects, suggesting the need for personalized dietary strategies.

Wheat bran (WB), a major by-product of the milling industry, is rich in IDF but is often underutilized due to its coarse texture, resulting in substantial resource waste. Although the hypoglycemic potential of WB-derived IDF (WBIDF) has been previously reported, different types of dietary fiber exhibit variable effects on SCFAs production and gut microbiota modulation. We hypothesized that combining WBIDF with different types of SDFs (e.g., stachyose, fructo-oligosaccharides, inulin, and resistant dextrin) could enhance its functional properties through fiber–fiber synergy. These SDFs were selected based on their distinct degrees of polymerization and fermentation kinetics, representing diverse prebiotic properties. Here, we evaluate the SCFAs production potential of WBIDF-SDF complexes and identify the optimal DF combination by *in vitro* fermentation. Subsequently, the antihyperglycemic effects of the selected WBIDF-IN complex were investigated in *db/db* mice, with particular attention to glucose metabolism, insulin resistance, islet integrity, and gut microbiota modulation.

2. Materials and methods

2.1 Materials

Wheat bran (WB) was obtained from Binzhou Zhongyu Food Co., Ltd. (Shandong, China). Resistant dextrin (RD, degree of polymerization (DP) is 6-15) and fructo-oligosaccharides (FOS, DP 3-5) were provided by Bailong Chuangyuan Biotechnology Co., Ltd. (Shandong, China). Stachyose (ST) was purchased from Yien Chemical Technology Co., Ltd. (Shanghai, China). Inulin (IN, DP 10-30) was purchased from

Ningxia Vanilla Biotechnology Co., Ltd. (Ningxia, China). Thermostable α -amylase from *Bacillus licheniformis* (20000–60000 U/mL), amyloglucosidase from *Aspergillus niger* (120 U/mg), and protease from *Bacillus licheniformis* (2.4 AU/g) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Wheat bran insoluble dietary fiber (WBIDF) preparation

WBIDF was prepared following the method previously described with slight modifications [8]. Briefly, crushed WB was stirred with 90% ethanol at a ratio of 1:2 (w/v) for 30 min to remove lipids, followed by two washes with distilled water. Next, 10 times of distilled water was added to form a mixture. Then, WB was subjected to the enzyme hydrolyzation process as follows: First, 1.5% (v/v) thermostable α -amylase was added and incubated at 95 °C for 30 min with continuous stirring. Second, the pH was adjusted to 4.5 with 1M HCl and 1.5% (w/v) amyloglucosidase was added and reacted for 30 min at 60 °C with continuous stirring. Finally, the mixture was adjusted to pH 7.5 with 1 M NaOH then reacted with 3% (w/v) protease for 2 h at 50 °C. Then, the mixture was subjected to boiling water bath for 15 min for enzymes inactivation and centrifugated at $8000 \times g$ for 20 min. The precipitate was collected and washed with 95% ethanol and distilled water until the washings became clear. The washed residue was freeze-dried and ground to pass through a 177 μ m sieve to obtain WBIDF. According to the AACC Method 32–45.01 and 32–50.01, the purity of the final WBIDF was 94%, including cellulose (44%), hemicellulose (mainly arabinoxylans, 31%) and lignin (19%), along with minor amounts of resistant starch and protein residues (6%). The FT-IR spectra showed that WBIDF had a weaker C=O stretching vibration and a shift of C–O–C glycosidic bond vibrations to a higher wavenumber, which indicated a lower content of uronic acids or ester bonds and a more ordered glycosidic bond arrangement (Supplementary Fig.S1A). As the scanning electron microscope revealed, the WBIDF conforms to the typical features of high-crystallinity cellulose, whose surface was smooth and dense without obvious pores, appearing as irregular blocks with clear fracture edges (Supplementary Fig.S1B).

2.3 In vitro fermentation experiment

Four healthy volunteers (2 females and 2 males, aged 21–30) were recruited to provide fresh fecal samples. Only those who had not experienced antibiotics intervention or gastrointestinal disorders in the past three months were eligible. The study was approved by the Ethics Committee of Shandong Academy of Agricultural Sciences (Approval No. SAAS-2023-41), and informed consent was obtained from all donors. Equal amounts of fresh fecal samples (20 g) from each volunteer were pooled and homogenized with autoclaved culture medium to prepare 10% (w/v) suspensions, then filtered through sterilized gauze to obtain the gut microbiota suspension for fermentation, using an anaerobic chamber with an atmosphere of 85% N₂, 5% CO₂, and 10% H₂. A 1 mL aliquot of gut microbiota suspension was added to 9 mL of culture medium supplemented with 1% (w/v) of a 1:1 mixture of WBIDF and SDFs (IN, RD, FOS, and ST). The 1:1 ratio (w/w) was selected to provide an equal mass basis for evaluating the synergistic potential of the insoluble fiber matrix and different soluble substrates. The culture medium (per liter) contained 2g of trypticase peptone, 2 g of yeast extract, 0.04 g of potassium phosphate dibasic, 0.1g of sodium chloride, 0.01g of magnesium sulfate heptahydrate, 0.01g of calcium chloride dihydrate, 0.05g of cysteine hydrochloride, 10 μ L of vitamin K₁.

Fermentation was carried out at 37 ± 0.5 °C with continuous stirring at 80 rpm. Samples were collected at 6, 12, and 24 hours, and the concentrations of SCFAs including acetic, propionic, and butyric acids, were monitored throughout the fermentation period.

2.4 Analysis of SCFAs

The SCFAs, including acetic acid, propanoic acid, and butyric acid were analyzed by a previously reported method with slight modification^[9]. Briefly, 1 mL of fermentation medium was added to metaphosphoric acid (25%, 0.2 mL) in a 1.5 mL centrifuge tube and vortexed for 2 min. The mixture was centrifuged at 10000g for 15 min, and the supernatant was collected. After filtering through syringe filters (0.22 µm diameter), the supernatant was determined by gas chromatograph (Agilent 7890A) equipped with a DB-FFAP column (30 m × 0.53 mm × 0.35 µm) and flame ionization detector.

2.5 Animal experiment design

Male *db/db* mice and wild-type C57BL/6J mice (6 weeks old) were obtained from Huafukang Biotechnology Co., Ltd. (Beijing, China). The animals were housed individually and acclimated for one week under laboratory conditions (22 ± 2 °C, $60 \pm 5\%$ humidity, 12 h light/dark cycle). During this period, mice were provided with a standard AIN-93G diet (Trophic Animal Feed High-tech Co., Ltd., China) and had free access to water. All animal experiments were conducted strictly in compliance with protocols approved by the Animal Ethics Committee of Shandong Academy of Agricultural Sciences (Approval No. SAAS-2024-G65). After 1 week of acclimatization, *db/db* mice were randomly assigned to four groups ($n = 10$ per group) including diabetic control group (MD), WBIDF group, IN group and WBIDF-IN group. C57BL/6J mice ($n = 10$) were used as normal control group (NC). The MD group received a standard AIN-93G diet, while the intervention groups were fed modified AIN-93G diets supplemented with 8% WBIDF, IN, WBIDF-IN complex, respectively. The 8% dosage was selected as a moderate intervention level, higher than the standard 5% cellulose in AIN-93G but lower than high-dose interventions (e.g., $>10\%$) to avoid potential gastrointestinal side effects like diarrhea^[10].

2.6 Sample collection

After 12-week intervention, mice were euthanized under ether anesthesia via cervical dislocation. Blood was collected from inner canthus of the eyes, centrifuged at $3000 \times g$ for 10 min, and the serum was stored at -80 °C. The pancreas was immediately excised and fixed in 4% paraformaldehyde for histological examination. Cecal contents were harvested for SCFAs quantification. For gut microbiota profiling, fecal sample of randomly selected representative individual mice (4 mice each group) were collected.

2.7 Blood glucose levels and enzyme-linked immunosorbent assay (ELISA)

During the experimental period, the fasting blood glucose (FBG) levels were measured weekly. The FBG levels in the rats were determined by Accu-Chek Performa (Roche Diagnostics, Mannheim, Germany) from the tail vein after overnight fasting. An OGTT test was conducted after 12-week intervention. Glucose was

administered orally at a dose of 2 g/kg body weight, and blood samples were collected at 0, 15, 30, 60, and 120 minutes. The area under the curve (AUC) was calculated for FBG during the OGTT.

Serum insulin and GLP-1 content were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. Insulin resistance was assessed by calculating the homeostasis model assessment of insulin resistance (HOMA-IR), as described by Levy et al. (1998).

2.8 Histological study

Pancreatic tissues were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned at 5 μ m using a rotary microtome (RM 2235, Leica Biosystems, Germany). Sections were subjected to hematoxylin and eosin (H&E) staining and analyzed under an optical microscope (DM 2000, Leica Biosystems, Germany). For immunofluorescence, sections were stained with rabbit anti-insulin polyclonal antibody (ab63820, Abcam, USA) and imaged using a laser-scanning confocal microscope (LSM 710, ZEISS, Germany).

2.9 16S rDNA sequencing

Genomic DNA was extracted from faecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Amplification products were visualized on 2% agarose gels, purified using the AxyPrep DNA Gel Recovery Kit (Axygen, USA), and quantified with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, , USA). Equimolar amplicons were pooled and sequenced on an Illumina HiSeq 2500 platform (250-bp paired-end reads).

Raw Illumina HiSeq reads underwent preprocessing to remove adapter sequences and low-quality bases, generating clean reads. Overlapping paired-end reads were merged into contiguous sequences using Connecting Overlapped Pair-End software (COPE, v1.2.1). These tags were clustered into operational taxonomic units (OTUs) at 97% sequence similarity threshold using USEARCH (v7.0.1090). Representative sequences from each OTU were taxonomically classified against the Ribosomal Database Project (RDP) database using the RDP Classifier (v2.2). All analyses were conducted on the Majorbio I-Sanger Cloud Platform (www.i-sanger.com).

2.10 Statistical analysis

One-way ANOVA and repeated-measures ANOVA performed to exam the difference at $P < 0.05$ using SPSS (version 25.0, IBM, Chicago, USA). when necessary, Duncan's *post hoc* test was applied to identify specific group differences. Associations between significantly altered bacterial genera and matched metabolic indicators were evaluated using Spearman correlation in R software (v4.0.0) and visualized accordingly.

3. Results and discussion

3.1 Effects of WBIDF-SDF complexes on the SCFAs content in vitro fermentation

The production of acetic acid, propionic acid, and butyric acid were evaluated for different WBIDF-SDF complexes over a 24-hour *in vitro* fermentation period (Fig. 1A-C). All three SCFAs exhibited time-dependent increases. However, their production kinetics differed: acetic and propionic acids were rapidly generated during the first 12 h, whereas butyric acid production mainly occurred during the latter stage (12–24 h). Notably, the WBIDF-SDF complexes produced significantly higher amounts of SCFAs than WBIDF alone ($P < 0.05$). To further verify the fermentation extent, we monitored the changes in pH and total polysaccharide content. As shown in Supplementary Fig.S2, the pH values of the fermentation broth significantly decreased from around 8.0 (0 h) to 4.0–6.0 (24 h) across all groups, inversely correlating with SCFA production. Interestingly, the WBIDF-IN group exhibited the most substantial pH reduction (reaching 4.25 ± 0.15) and polysaccharide consumption (decreased by 72%), confirming that the complex was effectively utilized by the gut microbiota. Although oligosaccharides such as FOS were generally believed to be fermented more rapidly than polysaccharides [11, 12], among the tested WBIDF-SDF complexes, WBIDF-IN exhibited the highest levels of SCFAs. This outcome was likely driven by the inherent high fermentability of inulin, which serves as a superior substrate for butyrate producers [13]. In addition, the structural complexity of WBIDF matrix imposed physical constraints on substrate-microbiota interactions, thereby effectively modulating fermentation kinetics. Specifically, the smaller molecular size of oligosaccharides likely facilitated their deeper embedding within the dense three-dimensional fiber network of WBIDF, thereby restricting their accessibility to microbial enzymatic systems. In contrast, long-chain inulin may predominantly localize on the surface or within inter-fiber pores of the matrix, preserving greater bioavailability [14, 15]

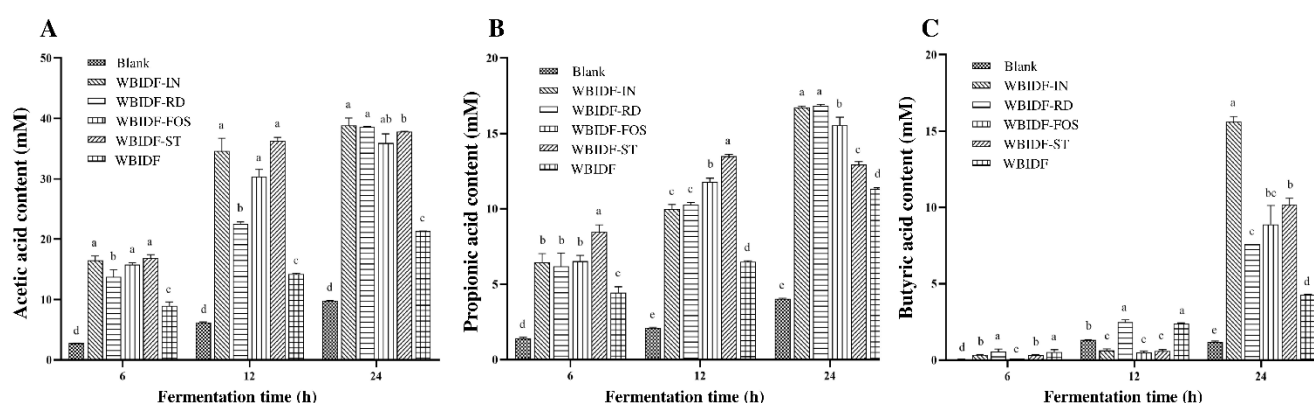


Fig. 1 SCFAs production during *in vitro* fermentation of WBIDF and WBIDF-SDF complexes. Production of acetic acid (A), propionic acid (B), and butyric acid (C) during 24-hour fermentation. Total SCFAs concentrations after 24 h for WBIDF-IN complexes with varying inulin ratios (0–100%) (D). Data are presented as mean $\pm$ SD ($n = 3$). Different letters indicate significant differences ($P < 0.05$).

3.2. Effects of WBIDF-IN complex on body weights and food intake of db/db mice

During the 12-week experimental period, it was observed that the body weight and food intake of MD groups (db/db mice) were significantly elevated than that of NC group ($P < 0.05$). However, starting from the 3rd week, the MD group showed significant decrease in body weight, which was consistent with previous findings that progressive weight loss could be attributed to the increased protein catabolism in muscle and

other tissues [16]. Interestingly, dietary fiber supplementation attenuated the body weight loss in *db/db* mice, suggesting that such intervention might improve glucose metabolism and thereby alleviate muscle protein degradation (Fig. 2).

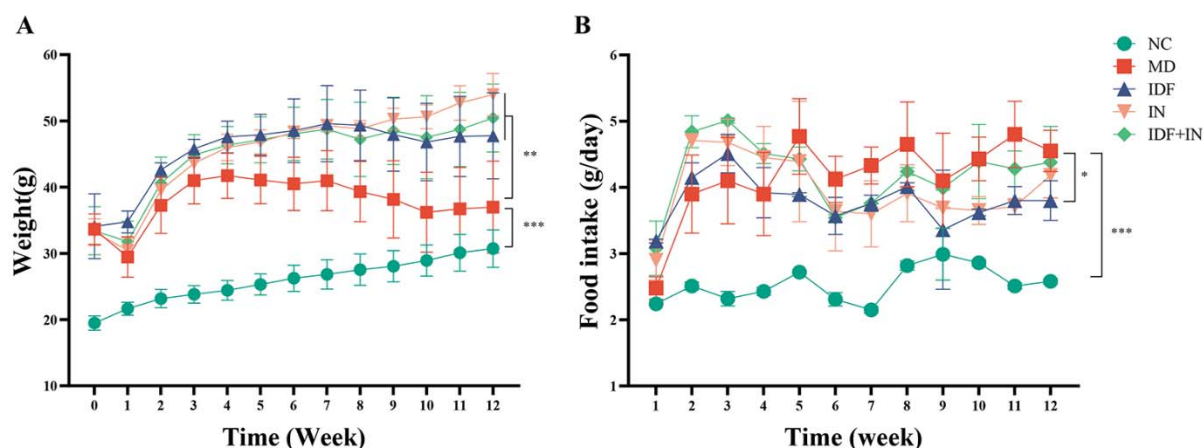


Fig. 2 The effect of WBIDF, IN and WBIDF-IN complex on body weight (A), food intake (B) in *db/db* mice. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared with the MD group.

3.3 Effects of WBIDF-IN complex on the fasting blood glucose and glucose tolerance of *db/db* mice

During the 12 weeks of intervention, the fasting blood glucose (FBG) levels of each group were monitored (Fig. 3A). Compared to the NC group, the FBG level in the diabetic control (MD) group remained significantly elevated ($P < 0.01$), confirming persistent hyperglycemia in *db/db* mice. Notably, dietary fiber interventions effectively reduced FBG levels to varying degrees. Among them, WBIDF-IN complexation showed the most pronounced hypoglycemic effect, reducing FBG by 38.7% compared to the MD group, which was greater than that achieved by either WBIDF (24.5%) or IN (31.2%) supplementation alone.

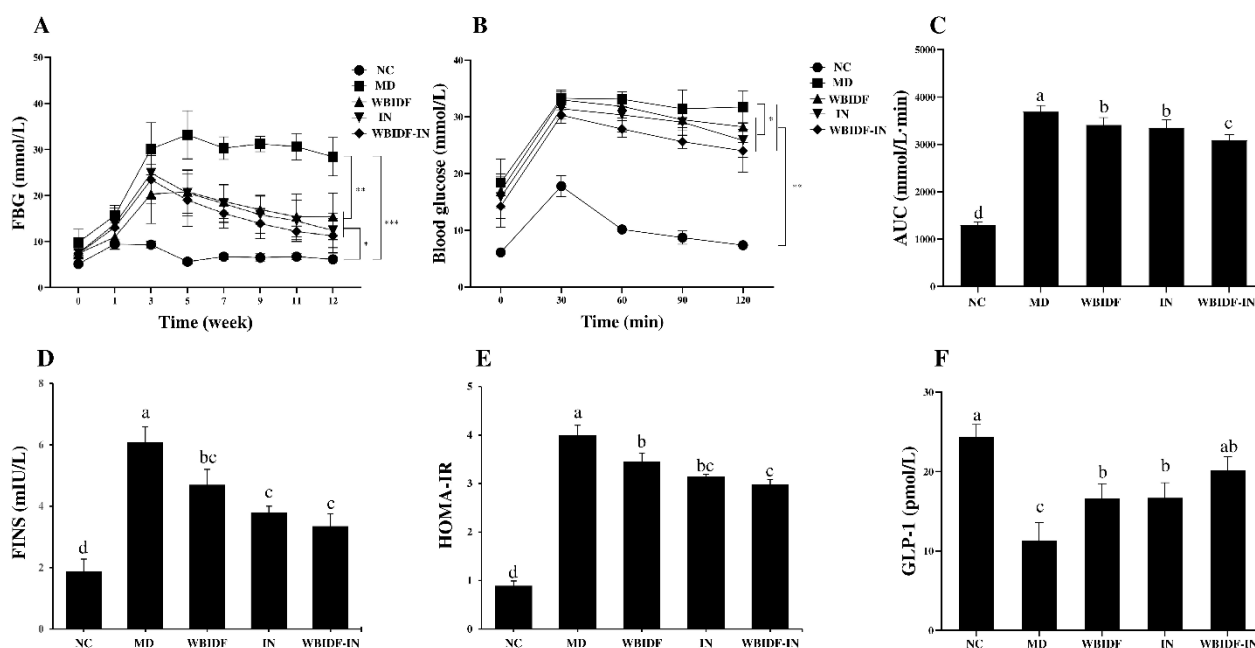


Fig. 3 The effect of WBIDF, IN and WBIDF-IN complex on the hyperglycemia, insulin resistance, glucose tolerance, and intestinal hormone levels in *db/db* mice. (A) Fasting blood glucose (FBG), (B) Oral glucose tolerance test (OGTT), (C) Area under the curve (AUC), (D) Fasting insulin (FINS), (E) Homeostasis index of insulin resistance (HOMA-IR) and (F) Serum Glucagon like peptide-1 (GLP-1). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared with the MD group. Data were presented as the mean $\pm$ SD ($n = 10$). Bars with different letters indicating significant differences ($P < 0.05$).

To further evaluate glucose regulation capacity, an oral glucose tolerance test (OGTT) was performed after dietary fiber intervention. As shown in Fig. 3C, following glucose administration, blood glucose levels in all groups peaked at 30 min. However, the MD group maintained elevated glucose levels throughout the test, indicating impaired glucose clearance. In contrast, WBIDF-IN significantly improved glucose tolerance, as evidenced by a sharper decline in blood glucose levels within 120 min post-load.

The area under the curve (AUC) of OGTT (Fig. 3D) quantitatively reflected these trends. Compared with the MD group, AUC values were reduced by 9.0%, 11.6%, and 15.1% in the WBIDF, inulin, and WBIDF-IN groups, respectively. The reduction in the WBIDF-IN group was statistically significant ($P < 0.01$), and greater than those observed in single-fiber groups ($P < 0.05$), suggesting a synergistic improvement in glycemic control.

3.4 Effects of WBIDF-IN complex on the insulin resistance and GLP-1 level of db/db mice

To further evaluate the metabolic effects of WBIDF-IN complexation beyond glycemic control, serum fasting insulin (FINS) and GLP-1 levels were measured after 12 weeks of intervention. As shown in Fig. 3 D and E, compared to the NC group, both FINS level and HOMA-IR were significantly elevated in the MD group ($P < 0.05$). Notably, WBIDF-IN complex intervention effectively reversed the increase of FINS level and HOMA-IR. This enhancement may be attributed to the elevated colonic production of SCFAs, particularly butyrate and acetate. Previous cross-sectional study has indicated that circulating SCFA levels are positively correlated with GLP-1 levels and insulin sensitivity^[17]. Furthermore, high dietary fiber intake has been confirmed to selectively increase the abundance of SCFA-producing bacteria and GLP-1 levels, thereby alleviating insulin resistance^[18]. Mechanistically, SCFAs act as ligands for G-protein-coupled receptors (GPR41 and GPR43) expressed on enteroendocrine L-cells. Activation of these receptors triggers a signaling cascade that increases intracellular Ca^{2+} concentrations, thereby stimulating the exocytosis of GLP-1^[19]. The enhanced GLP-1 secretion subsequently potentiates glucose-stimulated insulin secretion and inhibits glucagon release, contributing to improved glycemic control^[20].

3.5 Effects of WBIDF-IN complex on the injured islets of db/db mice

HE staining was conducted to assess the protective effect of WBIDF-IN complex on islet integrity in db/db mice (Fig. 4A). Islets of the MD group were severely atrophied, with distorted architecture, irregular cell distribution, and marked reduction in islet area and cell number. Dietary fiber interventions partially alleviated islet destruction, with the WBIDF-IN group exhibiting the most evident improvement.

Further evaluation via immunofluorescence staining of insulin and glucagon provided additional insight into islet cell function. Compared with NC group, the insulin fluorescence in MD group was markedly reduced, while dietary fiber treated group especially WBIDF-IN complex most effectively restored the typical spatial localization and fluorescence intensity of insulin (Fig. 4A). Quantitative analysis of fluorescence intensity (Fig. 4B) confirmed a significant increase in insulin expression in the WBIDF-IN group ($P < 0.01$ vs. MD), approaching levels observed in the NC group.

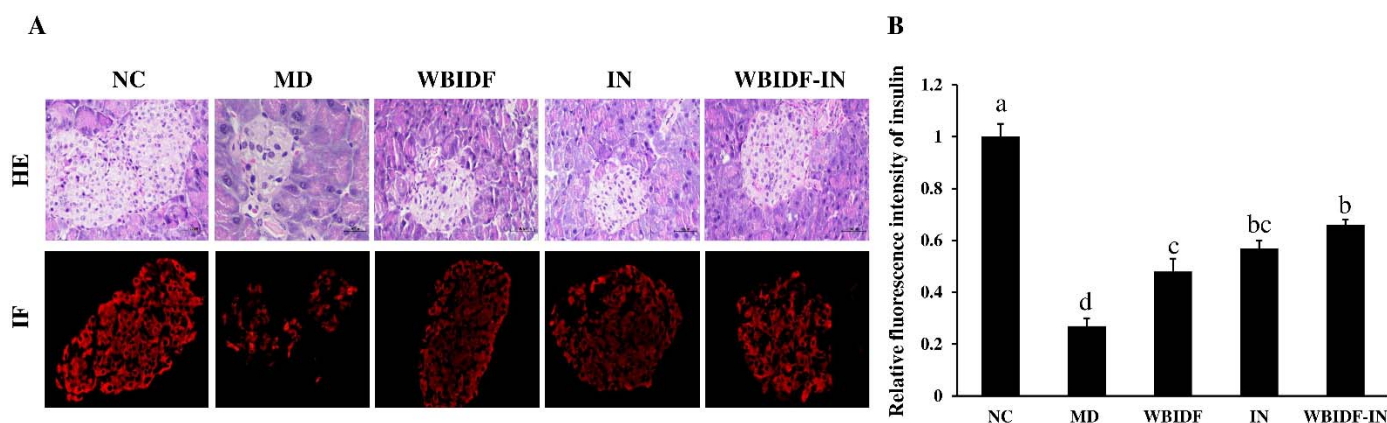


Fig.4 The effect of WBIDF, IN and WBIDF-IN complex on injured pancreatic islet cells in *db/db* mice. (A) Hematoxylin-eosin (HE) staining and immunofluorescence staining of insulin in pancreatic tissues. (B) Quantitative analysis of relative fluorescence intensity of insulin.

3.6 Effects of WBIDF-IN complex on cecal SCFAs concentration of *db/db* mice

Cecal SCFAs levels were analyzed after 12-week intervention to assess the *in vivo* fermentative capacity of dietary fibers. As shown in Table 1, acetic acid and butyric acid levels were significantly decreased in the MD group, indicating suppressed microbial fermentation in the diabetic state. In contrast, all dietary fiber intervention groups showed elevated SCFAs levels relative to the MD group, with the WBIDF-IN complex exhibiting the most pronounced effect. Specifically, the WBIDF-IN group showed significant increases in acetic acid and butyric acid levels ($P < 0.05$), outperforming either IDF or inulin alone. This suggests a synergistic interaction, whereby inulin promotes the growth of SCFAs-producing bacteria, while WBIDF may slow substrate degradation and prolong fermentation.

Table 1 The effect of WBIDF, IN and WBIDF-IN complex on fecal SCFAs levels ($\mu\text{mol/g}$).

Groups	Acetic acid	Propionic acid	Butyric acid
NC	2.89 ± 0.38^a	0.50 ± 0.04^a	0.30 ± 0.08^a
MD	1.24 ± 0.11^b	0.49 ± 0.06^a	0.16 ± 0.03^c
WBIDF	1.53 ± 0.15^b	0.44 ± 0.13^a	0.23 ± 0.09^{bc}
IN	2.34 ± 0.53^a	0.53 ± 0.19^a	0.20 ± 0.16^{bc}
WBIDF-IN	2.54 ± 0.34^a	0.47 ± 0.15^a	0.30 ± 0.15^a

Different letters indicate significant differences ($P < 0.05$).

3.7 Effects of WBIDF-IN complex on the gut microbial diversity in *db/db* mice

To assess the effects of WBIDF-IN supplementation on gut microbiota structure, 16S rRNA gene sequencing was performed. A total of 106,932 high-quality reads per sample were obtained and clustered into 2,876 ASVs at 97% similarity. Species richness and diversity were evaluated with α -indices including Chao1, Shannon and Simpson index. No significant difference in the alteration of α -diversity index was found among MD and dietary fiber intervention groups (Fig. 5 B-D), however, principal coordinate analysis (PCoA) revealed a distinct separation of microbial communities in the WBIDF-IN group from those in the MD, WBIDF, and IN groups (Fig. 5A), indicating a specific remodeling effect on gut microbiota structure.

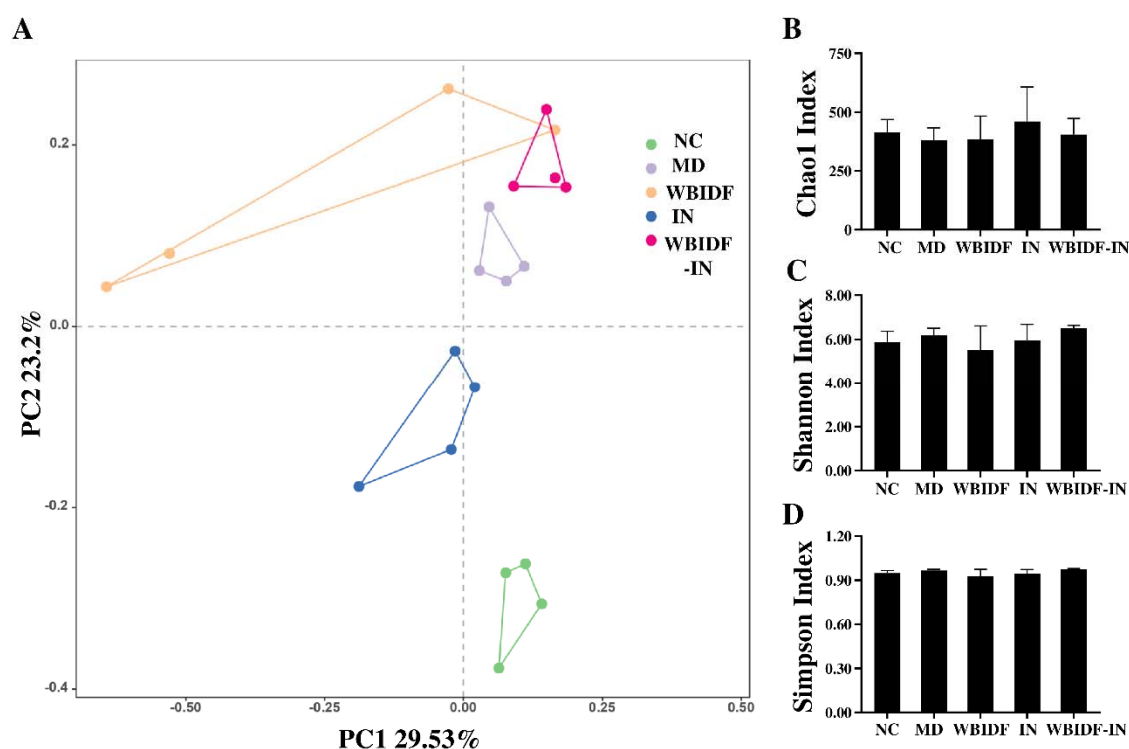


Fig.5 The effect of WBIDF-IN complex on the gut microbial diversity in *db/db* mice. (A) Principal coordinate analysis (PCoA) based on Bray–Curtis distance at the OTU level showing microbial community structure among groups. α -diversity indices including Chao1 (B), Shannon (C), and Simpson (D) indices were calculated to evaluate species richness and diversity.

3.8 Effects of WBIDF-IN complex on gut microbial composition in *db/db* mice

Firmicutes, *Bacteroidetes*, and *Proteobacteria* were the most abundant phyla in the gut microbiota across all groups. The MD group showed a notable decrease in *Firmicutes* and increases in *Bacteroidetes* and *Proteobacteria*, resulting in a dysregulated *Firmicutes/Bacteroidetes* ratio - a hallmark of diabetic dysbiosis (Fig. 6A & Supplementary Fig.S3). As shown in Supplementary Fig.S3A, the *Firmicutes/Bacteroidetes* ratio in the MD group was significantly lower than that in the NC group. Notably, WBIDF-IN intervention effectively restored this ratio to a level similar to the NC group. Regarding the phylum *Proteobacteria*, although its relative abundance increased following fiber intervention, the WBIDF-IN group exhibited significantly lower levels compared to the WBIDF group (Supplementary Fig.S3B).

At the family level, the abundance of *Erysipelotrichaceae*, a SCFA-producing group often associated with gut health, was significantly reduced in diabetic mice but selectively restored by IN supplementation, with limited response to WBIDF or other fibers (Fig. 6B). Interestingly, *Enterobacteriaceae* and *Morganellaceae*, two opportunistic pathogen families from the phylum *Proteobacteria*, were markedly increased in all fiber-treated diabetic groups, especially in the WBIDF group, indicating potential microbial shifts associated with WBIDF fermentation. Additionally, *Akkermansiaceae*, a mucin-degrading family linked to improved metabolic outcomes, was specifically enriched in the IN group, highlighting the selective prebiotic action of IN.

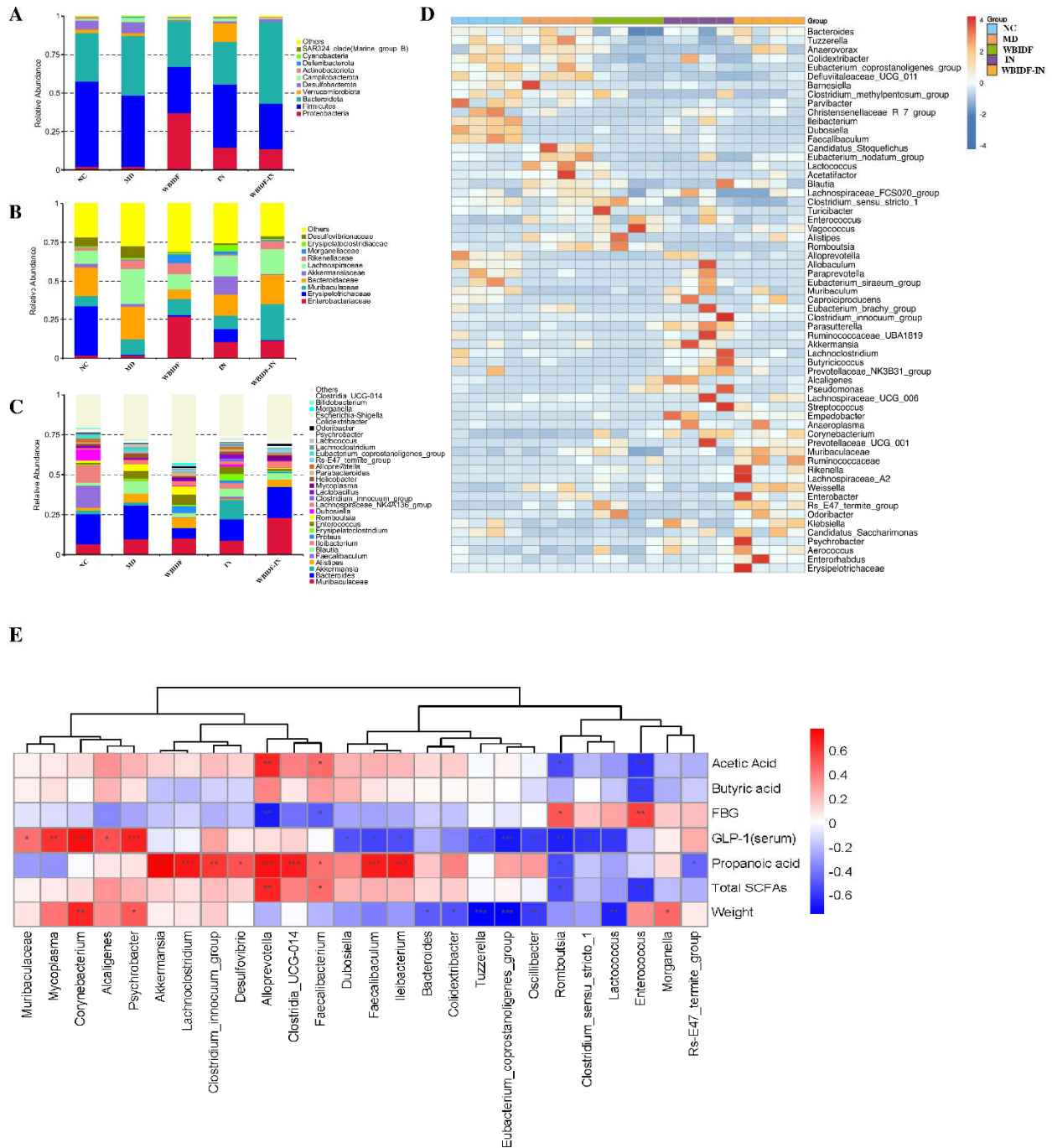


Fig.6 The effect of WBIDF-IN complex on microbial composition. Relative abundance of bacteria at phylum level (A), family level (B) and genus level (C). LEfSe analysis identified distinct microbial biomarkers enriched in each group (D). Correlation between gut microbiota and physicochemical parameters in *db/db* mice (E). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

At the genus level, *db/db* mice displayed enrichment of potentially pathogenic or pro-inflammatory taxa such as *Enterococcus*, *Romboutsia*, and *Alistipes*, along with a marked reduction in beneficial genera including *Faecalibaculum*, *Dubosiella*, and *Ileibacterium* (Fig. 6C). WBIDF-IN treatment selectively improved the relative abundance of *Muribaculaceae*, *Ruminococcaceae*, *Enterorhabdus*, and *Alloprevotella*, while suppressing the expansion of *Enterococcus* and *Romboutsia*. Compared to inulin or WBIDF alone, the combined complex more effectively restored the abundance of *Lachnospiraceae_NK4A136_group* and *Alloprevotella*, which are known to support metabolic and gut health. Previous study has shown that an

increase in *Lachnospiraceae_NK4A136_group* driven by whole grain consumption can alleviate gut dysbiosis and enhance SCFAs levels, particularly acetic and butyric acids, hence attenuating hyperglycemia in diabetic rodent models [21].

The discriminative microorganisms at genus level across groups were found out with LEfSe analysis. The abundance of these feature genus was exhibited with a heatmap (Fig. 6D). LEfSe analysis revealed that *Faecalibaculum*, *Dubosiella*, and *Ileibacterium* were strongly associated with the NC group, while *Bacteroides*, *Lactococcus*, and *Romboutsia* were dominant in the MD group. The WBIDF-IN group exhibited a unique microbial signature enriched in *Muribaculaceae* and *Lachnospiraceae* species. *Muribaculaceae* is a key constituent of the gut microbiome, responsible for producing enzymes that degrade complex carbohydrates, and synthesize beneficial SCFAs [22, 23]. Moreover, flourish of SCFAs-producing bacteria is widely recognized as an important indicator of restoring gut microbiota dysbiosis in diabetic mice [24]. It was confirmed that a high-fiber diet selectively increased the relative abundance of SCFAs-producing bacteria, including *Lachnospiraceae bacterium*, *Ruminococcus* and *Eubacterium rectale*, which contributed to the decrease of GHb level [18]. These results suggested a microbiota remodeling by WBIDF-IN supplementation toward a healthier composition.

The distinct hypoglycemic efficacy among groups is mirrored by their gut microbiota profiles and underlying mechanisms. WBIDF treatment improved glucose levels but induced the highest abundance of *Proteobacteria* and lower SCFA levels, suggesting that its hypoglycemic effect does not primarily rely on the gut microbiota-SCFA axis, but is possibly associated with physical adsorption, retarding the degradation of digestible carbohydrates, and delaying glucose absorption [4]. Inulin supplementation significantly enriched known prebiotic-degraders such as *Lachnospiraceae*, supporting a mechanism of rapid fermentation to produce SCFAs, particularly acetate. Notably, the WBIDF-IN complex exhibited the strongest antihyperglycemic effect, which may be attributed to the synergistic interaction between WBIDF and inulin. This synergy likely exerts a dual protective effect by retarding glucose absorption and ameliorating insulin resistance through enhanced SCFA production. This hypothesis is substantiated by the unique microbial signature observed in the WBIDF-IN group, which differed from both single-fiber treatments. The complex specifically enriched *Muribaculaceae* and *Alloprevotella* (key butyrate/propionate producers), while effectively preventing the expansion of *Proteobacteria* seen with WBIDF alone. This optimized microbial modulation creates a more favorable gut ecosystem for sustained SCFA production and GLP-1 stimulation than either fiber individually.

3.9 Correlation between gut microbiota and metabolic indicators

Spearman's correlation analysis was employed to elucidate the relationships between key gut microbial phylotypes and metabolic parameters (Fig. 6E). As expected, SCFA-producing bacteria enriched in the WBIDF-IN group, particularly *Muribaculaceae* and *Alloprevotella*, exhibited significant positive correlations with serum GLP-1, acetic acid and propionic acid levels, while showing strong negative correlations with FBG. In contrast, genera associated with metabolic dysbiosis, including *Romboutsia* and *Enterococcus*, were

positively correlated with FBG and negatively correlated with SCFAs and GLP-1 levels. These findings align with previous reports that *Alloprevotella* is a propionate-producing genus associated with improved glucose and lipid metabolism [25-27], while *Enterococcus* and *Romboutsia* are considered opportunistic pathogens linked to systemic inflammation and metabolic dysfunction [28, 29]. These distinct association patterns suggest that the specific modulation of these key phylotypes by the WBIDF-IN complex - promoting SCFA producers while displacing opportunistic pathogens - is a primary driver for the restoration of glucose homeostasis and insulin sensitivity.

4. Conclusions

This study demonstrated that WBIDF-IN complexation significantly enhanced SCFAs production *in vitro* and effectively alleviated hyperglycemia, improved insulin sensitivity, and promoted GLP-1 secretion in *db/db* mice. These improvements were accompanied by restored islet structure and enhanced insulin expression. Furthermore, WBIDF-IN supplementation modulated gut microbiota composition by enriching beneficial SCFAs-producing genera (e.g., *Alloprevotella*) and reducing potentially harmful taxa (e.g., *Romboutsia*, *Enterococcus*), suggesting that its antihyperglycemic effect is mediated through the gut microbiota-SCFAs-GLP-1 axis. These findings supported the potential of WBIDF-IN as a functional dietary component for type 2 diabetes management. Nevertheless, this study evaluated only a fixed 1:1 ratio of WBIDF to inulin, thus future investigations should optimize formulation ratios and explore dose-response relationships to maximize synergistic efficacy. In addition, well-designed clinical trials are necessary to confirm translational relevance in humans. From a nutritional perspective, future dietary recommendations for T2DM may benefit from considering not only total fiber intake but also the strategic combination of insoluble and soluble fibers to enhance gut microbiota-mediated metabolic benefits.

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Competing interests

None.

Ethical approval

The protocol for human fecal sample collection for *in vitro* fermentation was approved by the Ethics Committee of Shandong Academy of Agricultural Sciences (Approval No. SAAS-2023-41). All animal experiments were approved by the Animal Ethics Committee of Shandong Academy of Agricultural Sciences (Approval No. SAAS-2024-G65).

References

- [1] H. SUN, P. SAEEDI, S. KARURANGA, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045 [J]. Diabetes Res. Clin. Pract., 2022, 183: 109119.

- [2] M. M. KACZMARCZYK, M. J. MILLER, G. G. FREUND. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer [J]. *Metabolism*, 2012, 61(8): 1058-1066.
- [3] E. B. GIUNTINI, F. A. H. SARDÁ, E. W. DE MENEZES. The Effects of Soluble Dietary Fibers on Glycemic Response: An Overview and Futures Perspectives [J]. *Foods*, 2022, 11(23): 3934.
- [4] M. MAZHAR, Y. ZHU, L. QIN. The Interplay of Dietary Fibers and Intestinal Microbiota Affects Type 2 Diabetes by Generating Short-Chain Fatty Acids [J]. *Foods*, 2023, 12(5): 1023.
- [5] F. PENG, X. REN, B. DU, et al. Insoluble dietary fiber of pear fruit pomace (*Pyrus ussuriensis* Maxim) consumption ameliorates alterations of the obesity-related features and gut microbiota caused by high-fat diet [J]. *J. Funct. Foods*, 2022, 99: 105354.
- [6] K. WANG, Y. WANG, S. CHEN, et al. Insoluble and Soluble Dietary Fibers from Kiwifruit (*Actinidia deliciosa*) Modify Gut Microbiota to Alleviate High-Fat Diet and Streptozotocin-Induced TYPE 2 Diabetes in Rats [J]. *Nutrients*, 2022, 14(16): 3369.
- [7] K. J. O'RIORDAN, M. K. COLLINS, G. M. MOLONEY, et al. Short chain fatty acids: Microbial metabolites for gut-brain axis signalling [J]. *Mol. Cell. Endocrinol.*, 2022, 546: 111572.
- [8] J. WANG, B. SUN, Y. CAO, et al. In vitro fermentation of xylooligosaccharides from wheat bran insoluble dietary fiber by *Bifidobacteria* [J]. *Carbohydr. Polym.*, 2010, 82(2): 419-423.
- [9] J.-H. ZHU, Q. MAO, S.-Y. WANG, et al. Optimization and validation of direct gas chromatography-mass spectrometry method for simultaneous quantification of ten short-chain fatty acids in rat feces [J]. *J. Chromatogr. A*, 2022, 1669: 462958.
- [10] Z. ZHANG, X. CHEN, B. CUI. Modulation of the fecal microbiome and metabolome by resistant dextrin ameliorates hepatic steatosis and mitochondrial abnormalities in mice [J]. *Food Funct*, 2021, 12(10): 4504-4518.
- [11] M. L. STEWART, D. A. TIMM, J. L. SLAVIN. Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system [J]. *Nutrition research (New York, N.Y.)*, 2008, 28(5): 329-334.
- [12] M. P. H. VAN TRIJP, C. RÖSCH, R. AN, et al. Fermentation Kinetics of Selected Dietary Fibers by Human Small Intestinal Microbiota Depend on the Type of Fiber and Subject [J]. *Molecular nutrition & food research*, 2020, 64(20): e2000455.
- [13] L. OMARY, E. E. CANFORA, M.-L. PUHLMANN, et al. Intrinsic chicory root fibers modulate colonic microbial butyrate-producing pathways and improve insulin sensitivity in individuals with obesity [J]. *Cell Reports Medicine*, 2025, 6(7).
- [14] J. KANG, S. YIN, J. LIU, et al. Fermentation models of dietary fibre in vitro and in vivo - A review [J]. *Food Hydrocoll.*, 2022, 131: 107685.
- [15] J. XU, R. WANG, W. LIU, et al. The specificity of ten non-digestible carbohydrates to enhance butyrate-producing bacteria and butyrate production in vitro fermentation [J]. *Food Sci. Hum. well.*, 2023, 12(6): 2344-2354.
- [16] F. SURIANO, S. VIEIRA-SILVA, G. FALONY, et al. Novel insights into the genetically obese (ob/ob) and diabetic (db/db) mice: two sides of the same coin [J]. *Microbiome*, 2021, 9(1): 147.
- [17] M. MÜLLER, M. A. G. HERNÁNDEZ, G. H. GOOSSENS, et al. Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans [J]. *Scientific Reports*, 2019, 9(1): 12515.
- [18] L. ZHAO, F. ZHANG, X. DING, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes [J]. *Science*, 2018, 359(6380): 1151-1156.
- [19] G. TOLHURST, H. HEFFRON, Y. S. LAM, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2 [J]. *Diabetes*, 2012, 61(2): 364-371.
- [20] J. G. BARRERA, D. A. SANDOVAL, D. A. D'ALESSIO, et al. GLP-1 and energy balance: an integrated model of short-term and long-term control [J]. *Nature Reviews Endocrinology*, 2011, 7(9): 507-516.
- [21] C. KIM, J. LEE, M. B. KIM, et al. Hypoglycemic effect of whole grain diet in C57BL/KsJ-db/db mice by activating PI3K/Akt and AMPK pathways [J]. *Food Sci. Biotechnol.*, 2019, 28(3): 895-905.
- [22] B. WANG, Q. KONG, X. LI, et al. A High-Fat Diet Increases Gut Microbiota Biodiversity and Energy Expenditure Due to Nutrient Difference [J]. *Nutrients*, 2020, 12(10): 3197.
- [23] B. J. SMITH, R. A. MILLER, T. M. SCHMIDT. Muribaculaceae Genomes Assembled from Metagenomes Suggest Genetic Drivers of Differential Response to Acarbose Treatment in Mice [J]. *mSphere*, 2021, 6(6): e0085121.
- [24] H. LI, S. LIU, H. CHEN, et al. Gut dysbiosis contributes to SCFAs reduction-associated adipose tissue macrophage polarization in gestational diabetes mellitus [J]. *Life Sci.*, 2024, 350: 122744.

- [25] B. HAN, L. SHI, M.-Y. BAO, et al. Dietary ellagic acid therapy for CNS autoimmunity: Targeting on *Alloprevotella rava* and propionate metabolism [J]. *Microbiome*, 2024, 12(1): 114.
- [26] M. WU, S. YANG, S. WANG, et al. Effect of Berberine on Atherosclerosis and Gut Microbiota Modulation and Their Correlation in High-Fat Diet-Fed ApoE^{-/-} Mice [J]. *Front. Pharmacol.*, 2020, 11: 223.
- [27] T. MAO, C. ZHANG, S. YANG, et al. Semaglutide alters gut microbiota and improves NAFLD in db/db mice [J]. *Biochem. Biophys. Res. Commun.*, 2024, 710: 149882.
- [28] D. SEVIAR, K. NYUNT, C. SAYER, et al. Romboutsia-induced bone marrow necrosis at presentation of acute myeloid leukaemia [J]. *Br. J. Haematol.*, 2022, 196(2): 262.
- [29] T. LAN, T. TANG, Y. LI, et al. FTZ polysaccharides ameliorate kidney injury in diabetic mice by regulating gut-kidney axis [J]. *Phytomedicine*, 2023, 118: 154935.