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Posted Date: 30 March 2026

doi: 10.20944/preprints202603.2345.v1

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 doi:10.20944/preprints202603.2345.v1

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Synergistic Effect of *Bifidobacterium longum* Postbiotics and Dietary Herbs on Improving Obesity and Cognitive Impairment in Hyperlipidemic Mice

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Abstract

Hyperlipidemia-associated obesity is frequently accompanied by hepatic injury, bile acid dysregulation, gut microbial remodeling, and cognitive impairment. Although postbiotics have shown considerable cholesterol-lowering potential, the most effective postbiotic form and its compatibility with dietary herbs remain unclear. In this study, FB3-14-derived postbiotics were first screened in vitro for cholesterol micellar binding. Inactivated bacterial cells (Postcell) exhibited the strongest cholesterol-binding capacity and were therefore selected for in vivo validation, alone or in combination with dietary herbs (DH), in a high-fat, high-cholesterol (HFHC) mouse model. Consistently, Postcell showed superior efficacy in attenuating body weight gain, jejunal triglyceride accumulation, and hepatic dysfunction compared with other postbiotic forms. Importantly, Postcell_DH exerted broader metabolic benefits, including reductions in weight gain, food efficiency, bile acid dysregulation, and neuroinflammation. Multi-omics analysis further indicated that these effects may be mediated through remodeling of the gut microbiota and metabolome, particularly pathways involved in bile acid and tryptophan metabolism. Notably, *Clostridioides* and taurochenodeoxycholate-7-sulfate were negatively associated with total cholesterol (TC) and leptin, whereas *Clostridium_sensu_stricto_1* and 3-Hydroxyindolin-2-1-sulfate were negatively correlated with brain inflammatory level, lipid, and bile acid-related index. This study provides a practical strategy for developing postbiotic–herbal combination interventions against hyperlipidemia and related cognitive disorders.

Keywords: postbiotics; dietaty herbs; hyperlipidemia; bile acids; gut-brain axis

1. Introduction

Hyperlipidemia is a metabolic abnormality characterized by abnormally elevated circulating lipids or lipoproteins, including total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL), and is generally considered to be a major risk factor for cardiovascular diseases [1]. The intestine functions as a critical site for dietary cholesterol absorption and bile acid recycling, forming a functional axis that connects intestinal lipid handling with hepatic metabolism [2]. Importantly, bile acids, as cholesterol-derived metabolites, serve dual roles in lipid emulsification and metabolic signaling. Dysregulation of bile acid synthesis or circulation can therefore disrupt cholesterol homeostasis, intensify lipid accumulation, and accelerate metabolic dysfunction and liver injury [3]. Increasing evidence further indicates that a high-fat, high-cholesterol (HFHC) diet causes not only hepatic steatosis but also neuroinflammation, neuronal injury, and cognitive dysfunction through disruption of gut microbial diversity and composition [4].

 doi:10.20944/preprints202603.2345.v1

In recent years, postbiotics have attracted increasing attention worldwide as promising interventions for metabolic disorders [5,6]. Compared with live probiotics, postbiotics offer several advantages, including improved safety, particularly for immunocompromised individuals, as well as greater stability, longer shelf life, and lower costs for handling, storage, and transportation [7]. Postbiotics are generally defined as preparations derived from probiotic microorganisms that contain non-viable cells, their structural components, and/or metabolites while retaining biological activity [5]. Generally, postbiotics comprise a complex mixture of bioactive substances, including extracellular polysaccharides (EPS), cell wall components, cell-free supernatants, short-chain fatty acids (SCFAs), organic acids, vitamins, enzymes, teichoic acids, secreted proteins, bacteriocins, biosurfactants, neurotransmitters, and peptides [7]. Accumulating evidence indicates that postbiotics possess considerable lipid-lowering potential, as they promote cholesterol clearance in vitro and improve lipid metabolism, hepatic steatosis, and gut dysbiosis in vivo [8–10]. Additionally, postbiotics have been suggested to exert broad signaling effects across multiple organs and tissues, further supporting their potential in systemic metabolic regulation [11]. However, these postbiotic forms are not necessarily functionally equivalent [12]. Different postbiotic forms, such as whole inactivated cells, total postbiotic preparations, and cell-free supernatants, may differ substantially in their capacities to bind cholesterol, interact with bile salts, and regulate inflammatory responses or gut–brain communication. Therefore, the respective advantages and limitations of different postbiotic forms in host metabolic regulation remain to be systematically clarified.

Dietary herbs (DH) are increasingly recognized as promising interventions for chronic metabolic disorders because of their beneficial effects on obesity, type 2 diabetes, and oxidative stress. In addition, many dietary herbs also exhibit neuroprotective activity and are increasingly considered potential modulators of the gut–brain axis. Among these herbs, ginseng has been widely reported to exert anti-obesity effects, partly through ginsenosides and polysaccharides that interact with the gut microbiota [13]. In parallel, ginseng has also shown protective effects against brain injury and neuroinflammation, including alleviation of neuronal damage and inflammatory responses in neurodegenerative diseases [14]. Longan fruit is another representative dietary herb with multifunctional bioactivity. It has been reported to regulate lipid metabolism and improve metabolic disorders such as nonalcoholic fatty liver disease, while also affecting neurotransmitter-related processes involved in mood regulation and exhibiting anxiolytic and antioxidant properties [15,16].

These findings suggest that dietary herbs may complement postbiotics by providing benefits beyond conventional metabolic regulation, particularly in the context of liver–gut–brain interactions. Recently, increasing attention has been given to postbiotics generated through probiotic fermentation of dietary herbs, which have shown promising health benefits [17,18]. In contrast, studies investigating the direct combination of heat-inactivated bacterial cells and herbal components remain relatively limited. Notably, this combination strategy offers several practical advantages, including improved quality control, precise dose standardization, greater formulation flexibility, and preservation of the native bioactive components from both sources [19].

In the present study, we aimed to identify the most effective lipid-lowering form among FB 3-14 derived postbiotics and to investigate whether its combination with DH could provide broader protective effects against hyperlipidemia-associated metabolic and neurological disturbances. Different postbiotic forms were compared through in vitro and in vivo screening, after which Postcell, showing the strongest cholesterol-binding activity, was selected for combined intervention with DH. The synergistic effects of this combination were comprehensively evaluated in a hyperlipidemia mouse model by examining adiposity, lipid accumulation, liver function and histopathology, bile acid homeostasis, behavioral alterations, and brain inflammation. Moreover, 16S rRNA sequencing and untargeted metabolomics were employed to characterize the changes in gut microbiota and cecal metabolites. This study provides a theoretical and practical basis for the development of postbiotic–herbal combination strategies for the prevention and management of hyperlipidemia and its related cognitive impairment.

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2. Materials and Methods

2.1. Postbiotics and Dietary Herb Preparation

The probiotic strain *Bifidobacterium longum* subsp. *infantis* FB 3-14 (FB 3-14) used in this study was originally isolated from the feces of healthy breastfed infants and preserved at the General Microbiology Center of the China General Microbiological Culture Collection Center (CGMCC; No. 25762). The strain was cultured strictly anaerobically at 37 °C in an anaerobic workstation using BS broth/agar medium. After 24 h of incubation, the bacterial suspension was adjusted to 1×10^9 CFU/mL and designated as LiveBL. The PostBL was prepared by heat treatment in a water bath at 80 °C for 30 min, with minor modifications from a previously described method [8]. Subsequently, the cell-free supernatant (PostCFS) and inactivated bacterial cells (Postcell) were separated by centrifugation at 8000 g for 15 min. The absence of viable bacteria in all postbiotic preparations was confirmed by plate culture.

The DH formula, prepared based on previous studies [15,20,21] with minor modifications, and consisted of ginseng, longan fruit, lotus leaf, *Poria cocos*, Chinese yam, coix seed, *Polygonatum odoratum*, malt, hyacinth bean, dried tangerine peel, and hawthorn at a weight ratio of 3:3:3:3:2:2:2:2:1:1:1. All herbal materials were purchased from Kangmei Pharmaceutical Co., Ltd. (Guangdong, China). The herbs were decocted or extracted according to the previously described protocol [15,16], and the final preparation was physically mixed with Postcell at a 1:1 (v/v) ratio immediately before administration.

2.2. In Vitro Determination of Cholesterol-Binding and Antioxidant Activity

The cholesterol micelle solution consisted of 10 mmol/L sodium taurocholate, 10 mM cholesterol, 5 mM oleic acid, and 132 mM NaCl dissolved in PBS [22]. The solution was sonicated for 1 h to aid dissolution and incubated at 37 °C for 24 h. Different forms of FB 3-14's postbiotics and LiveBL were separately added to 5 mL of cholesterol micelle solution and incubated at 37 °C for 2 h. Following centrifugation, the cholesterol content in the supernatant was determined using a commercial assay kit. The cholesterol adsorption rate was calculated using the following formula: cholesterol micelle solubility inhibition rate (%) = (sample cholesterol content−control cholesterol content)/sample cholesterol content × 100%.

The DPPH free radical scavenging activity was assessed according to a previously reported method [23]. Briefly, 1 mL of the DPPH solution (0.2 mmol/L) was separately mixed with each postbiotic sample. The mixtures were homogenized and incubated at 37 °C in the dark for 30 min. The samples were then centrifuged at 4000 g for 5 min, and the absorbance of the supernatant was measured at 517 nm.

2.3. Animals and Experimental Design

Specific-pathogen-free C57BL/6J mice (6-8 weeks, male) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed under controlled conditions with a temperature of 20–25 °C and relative humidity of 50–60%. After a 1-week acclimatization period, the mice were randomly divided into 8 groups (n = 10 per group): Control, HFHC, LiveBL, PostBL, PostCFS, Postcell, DH (1 g/kg), and Postcell_DH (1×10^9 CFU/mL inactivated cells + 1 g/kg DH). The Control group received a standard diet, whereas the HFHC and treatment groups were fed an HFHC diet supplemented with different FB 3-14-derived forms or DH, either alone or in combination. The standard diet (D12450J, 3.85 Kcal/g, 10% fat) and the HFHC (D12109C, 4.51 Kcal/g, 40% fat, 1,25% cholesterol, 0.5% sodium cholate) were purchased from Xietong Co., Ltd. (Jiangsu, China). Mice had free access to food and water throughout the study. Body weight was recorded weekly, and food intake was monitored regularly. After 12-week treatment, mice were anesthetized and sacrificed to collect serum, liver, epididymal fat, brain, cecal contents, and ileum. All animal

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experiments were approved by the Institutional Animal Care and Use Committee of Nankai University (animal ethics test approval number: 2025-SYDWLL-000454).

2.4. Behavioral Assessment

Behavioral performance was assessed using the open-field (OF) and the elevated maze (EPM) test. In the OF test, mice were placed individually in the center of a square arena (50 cm × 50 cm) enclosed by 45-cm-high walls and allowed to explore freely for 5 min. Exploratory activity in the center zone, including center time and center distance, was recorded to reflect anxiety-like behavior and exploratory capacity. The apparatus was cleaned with 75% ethanol to remove any smell cues before testing. In the EPM test, each mouse was placed on the central platform facing a closed arm and allowed to explore the maze for 5 min. The percentage of time spent in the open arms and the number of open-arm entries were recorded to assess anxiety-related behavior.

2.5. Tissue Biochemical Analysis and Histological Examination

At the end of the intervention, serum, liver, intestine, adipose tissue, cecal contents, and brain tissues were harvested. Serum TC, TG, LDL-C, hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST), jejunal TG, and hepatic, ileal, and serum bile acid levels were quantified using commercial kits. Leptin, CYP7A1 activity, and brain inflammatory cytokines (IL-1β and TNF-α) were measured using ELISA kits according to the manufacturers' protocols.

Adipose tissue and liver were collected immediately after sacrifice, fixed in 4% paraformaldehyde, and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (H&E) according to standard procedures. Liver sections were also stained with Oil Red O to evaluate lipid droplet accumulation. For Nissl staining, brain sections were immersed in Nissl staining solution for 2–5 min, followed by rinsing with running water, drying at 65 °C, and mounting with neutral resin for microscopic analysis.

2.6. Gut Microbiota Analysis in Mice Fecal Samples

Fecal gut microbiota analysis was performed as previously described [24]. Briefly, total microbial DNA was extracted from mouse fecal samples and subjected to quality assessment. The V3–V4 regions of the bacterial 16S rRNA genes were amplified by PCR using the primer pair 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Sequencing libraries were constructed from the extracted DNA and sequenced using the Illumina NovaSeq platform (Illumina, San Diego, CA, USA). After sequencing, raw reads were processed through quality filtering, trimming, merging, denoising, and clustering. Taxonomic classification was then conducted to characterize the microbial composition and relative abundance of each sample. Alpha diversity, beta diversity, and PCoA were analyzed using QIIME 2, and Spearman correlation analysis was performed to assess the relationships between gut microbiota and metabolic parameters.

2.7. Untargeted Metabolomics Analysis in Mice Cecal Contents

UHPLC-MS/MS analysis was conducted at Novogene Co., Ltd. (Beijing, China) using a Vanquish UHPLC system (Thermo Fisher, Germany) coupled to an Orbitrap Q Exactive™ HF or Orbitrap Q Exactive™ HF-X mass spectrometer (Thermo Fisher, Germany). Samples were separated on a Hypersil Gold column (100 × 2.1 mm, 1.9 μm) with a 12 min linear gradient at a flow rate of 0.2 mL/min. For both positive and negative ion modes, the mobile phases consisted of eluent A (0.1% formic acid in water) and eluent B (methanol). The gradient program was as follows: 2% B at 1.5 min, 2%–85% B from 1.5 to 3.0 min, 85%–100% B from 3.0 to 10.0 min, 100% B from 10.0 to 10.1 min, and re-equilibration to 2% B until 12.0 min. The mass spectrometer was operated in both positive and negative electrospray ionization modes under the following conditions: spray voltage, 3.5 kV; capillary temperature, 320 °C; sheath gas flow rate, 35 psi; auxiliary gas flow rate, 10 L/min; S-lens RF level, 60; and auxiliary gas heater temperature, 350 °C. Raw UHPLC-MS/MS data were processed

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using XCMS for peak alignment, peak detection, and metabolite quantification. Peak intensities were first normalized to the total spectral intensity based on the first QC sample. Metabolite identification was then performed by matching the data against a high-quality secondary spectral database with a mass tolerance of 10 ppm and consideration of adduct ions, yielding qualitative and relative quantitative results. Statistical analyses were conducted using R (v.4.4.2), Python (version 2.7.6), and CentOS (release 6.6). For data that were not normally distributed, relative peak areas were normalized according to the following formula: sample raw quantitation value / (sum of metabolite quantitation values in the sample/sum of metabolite quantitation values in the QC1 sample). Compounds with coefficients of variation greater than 30% in QC samples were excluded from further analysis.

2.8. Statistical Analysis

All statistical analyses and data integration were performed in R (v4.4.2). Data are expressed as mean ± SEM. Group differences were analyzed using the nonparametric Wilcoxon signed-rank test, and *P*-values were adjusted for multiple testing using the Benjamini–Hochberg FDR method. Gut microbial richness and evenness were assessed by α-diversity indices, while differences in community structure were visualized by PCoA. For metabolomics, identified compounds were annotated using the KEGG database. Differential metabolites were screened based on $|\log2FC| > 1.0$, VIP > 2.0, and $P < 0.01$. Associations among gut microbiota, metabolites, and phenotypic traits were evaluated by two-sided Spearman's correlation analysis with BH-adjusted p-values. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. In Vitro Function Screening of FB 3-14's Postbiotics or Combination with Dietary Herbs

To compare the functional differences among FB 3-14 derived postbiotics, we performed in vitro screening of their cholesterol-binding and antioxidant capacities. Postcell showed the strongest cholesterol micellar binding ability among all postbiotic forms ($P < 0.05$), while its DPPH radical-scavenging activity was the lowest (Figure 1A, B). Based on these results, Postcell was chosen as the postbiotic form for combination with DH in subsequent in vivo experiments. Meanwhile, the hypocholesterolemic effects of the different postbiotic forms and LiveBL were also evaluated in vivo to further confirm the lipid-lowering potential of Postcell.

3.2. Effect of FB 3-14's Postbiotics and Dietary Herbs on Fat Accumulation

Body weight changes over the 12-week experimental period are shown in Figure 1C. Compared with the Control group, mice in the HFHC group exhibited a significant increase in body weight after 7 weeks ($P < 0.01$). Moreover, the HFHC group showed the highest food efficiency, serum leptin level, epididymal fat mass, and adipocyte area ($P < 0.05$; Figure 1D-H), confirming the successful establishment of the diet-induced obesity.

No significant difference in body weight gain was observed between the LiveBL and PostBL groups, although the LiveBL group showed lower food efficiency ($P < 0.01$; Figure S1A, B). Among all FB 3-14 derived postbiotics, the Postcell group maintained the lowest body weight throughout the intervention period (Figure S1A, B). Notably, the Postcell_DH led to a pronounced suppression of weight gain after 1 week, compared with the Control, HFHC, and all postbiotic groups (Figure 1C). Consistently, the Postcell_DH group also showed the lowest food efficiency and reduction of serum leptin, epididymal fat mass, and adipocyte hypertrophy ($P < 0.01$; Figure 1D-H), highlighting a potentiated anti-obesity effect conferred by DH supplementation.

Collectively, among the different FB 3-14 derived postbiotic forms, Postcell exhibited potential effects on body weight control and food efficiency. Moreover, its combination with DH further

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enhanced these beneficial effects, suggesting a synergistic role in attenuating weight gain and improving energy utilization.

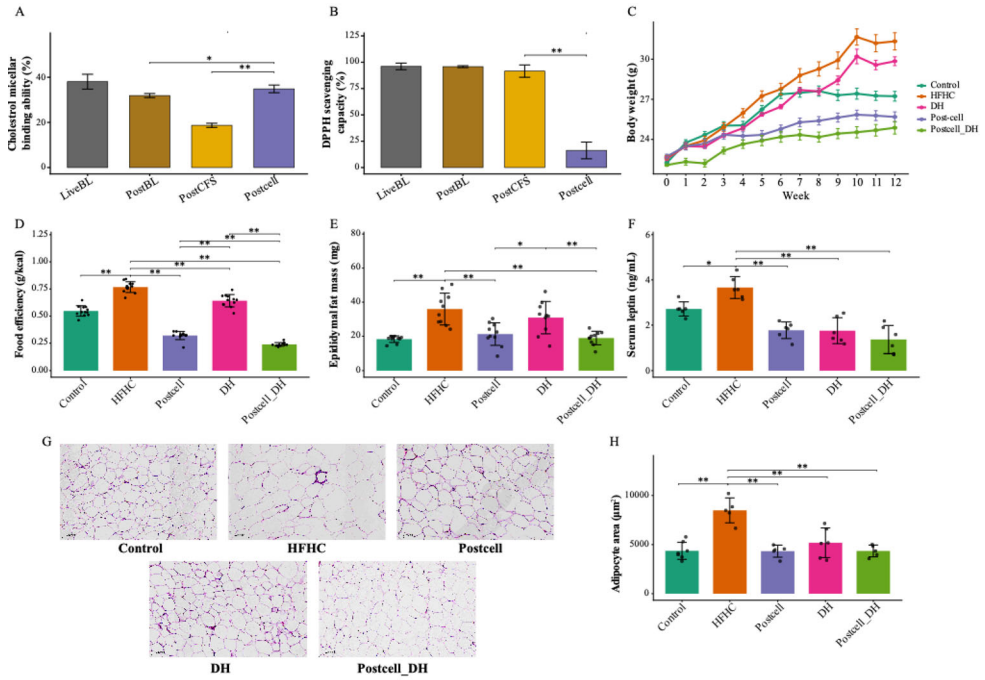

Figure 1. In vitro function screening of FB 3-14 derived postbiotics, alone or in combination with dietary herbs, and their anti-obesity effect in hyperlipidemia mice. (**A**) Cholesterol micellar binding ability. (**B**) DPPH radical scavenging capacity (%). (**C**) Body weight over the entire experimental period. (**D**) Food efficiency. (**E**) Epididymal fat mass. (**F**) Serum leptin levels. (**G**) Representative hematoxylin and eosin (H&E)-stained images of epididymis adipose tissue. (**H**) Quantification of adipocyte area. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

3.3. Effect of FB 3-14's Postbiotics and Dietary Herbs on Lipid Metabolism

To evaluate the effects of different interventions on lipid metabolism, serum lipid profiles and intestinal lipid accumulation were assessed. The HFHC diet significantly elevated serum TC, TG, LDL, and jejunum TG compared with the Control group (all $P < 0.01$; Figure 2A-D), confirming the successful establishment of the hyperlipidemia model. Among all postbiotics, Postcell showed a strong lipid-lowering effect, particularly in reducing jejunal TG accumulation, indicating an advantage in improving intestinal lipid deposition ($P < 0.01$; Figure S2H). Additionally, the Postcell_DH group demonstrated the most pronounced improvement with either treatment alone (Figure 2A, B, D). Specifically, jejunum TG content was most effectively reduced in the Postcell_DH group, suggesting that DH supplementation enhanced the lipid-lowering efficacy of Postcell, especially in limiting intestinal lipid accumulation and improving systemic lipid homeostasis.

Histopathological evaluation of liver sections by H&E staining further supported these biochemical findings. In the Control group, hepatic lobular architecture was intact, and hepatocytes were regularly arranged without obvious pathological alterations. In contrast, the HFHC group showed evident hepatic injury, characterized by disorganized hepatocyte arrangement, increased vacuolar degeneration, and inflammatory cell infiltration. Both Postcell and DH treatment alleviated these pathological changes, whereas the Postcell_DH group exhibited the most marked improvement, with a more preserved hepatic architecture, fewer vacuolated hepatocytes, and reduced inflammatory infiltration (Figure 2E). Consistently, liver oil Red O staining further showed that hepatic lipid droplet accumulation induced by the HFHC diet was visibly ameliorated, with the most pronounced improvement observed in the Postcell_DH group (Figure 2F).

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Taken together, these results indicate that Postcell had a remarkable effect on intestinal and systemic lipid metabolism, and that its combination with DH further strengthened these benefits, producing a more comprehensive improvement in lipid metabolic disorders and liver pathological damage.

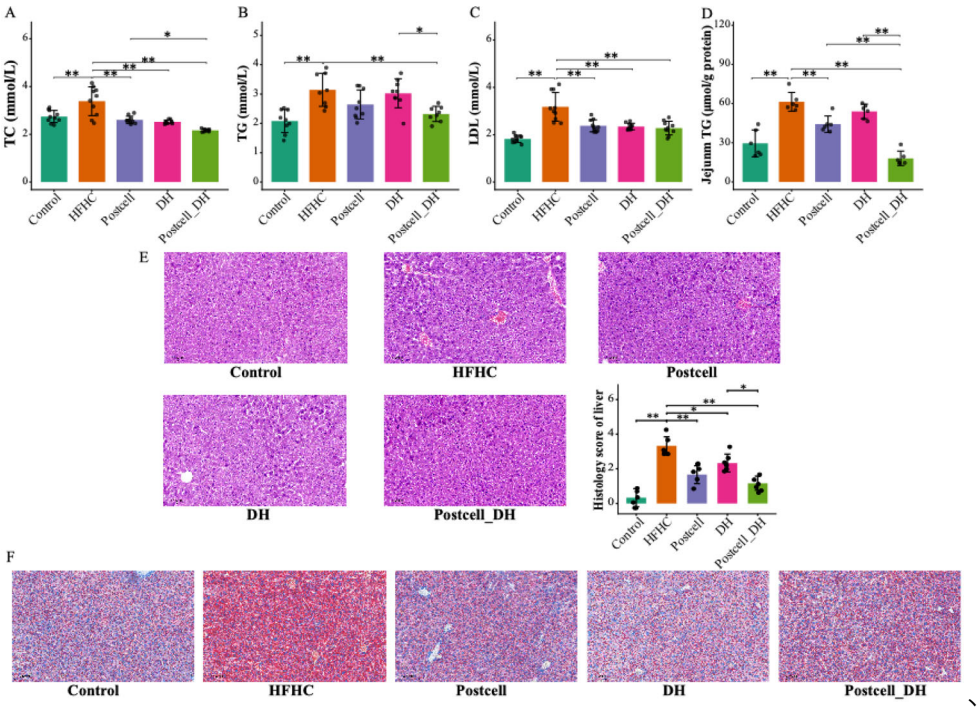

Figure 2. Effect of FB 3-14 derived postbiotics and dietary herbs on lipid metabolism. (**A**) Serum TC. (**B**) Serum TG. (**C**) Serum LDL. (**D**) Representative histologic images of hematoxylin and eosin (H&E) staining of liver sections. (**E**) Histology score of the liver. (**F**) Representative Oil Red O-stained images of liver sections. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

3.4. Effect of FB 3-14's Postbiotics and Dietary Herbs on Liver Function and Bile Acid Metabolism

Compared with the Control group, mice fed the HFHC diet showed significantly elevated hepatic ALT and AST levels, indicating liver injury under HFHC feeding ($P < 0.05$; Figure 3A, B). The HFHC diet also markedly increased total bile acid concentrations in the liver, ileum, and serum, and was accompanied by a significant elevation in hepatic CYP7A1 activity, suggesting expansion of the bile acid pool together with enhanced cholesterol-to-bile acid conversion ($P < 0.01$; Figure 3C-F).

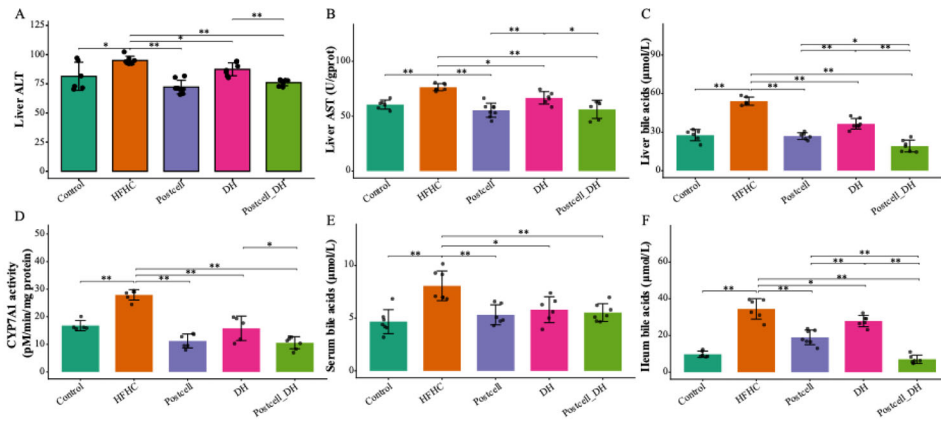

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Figure 3. Effect of FB 3-14 derived postbiotics and dietary herbs on liver function and bile acid metabolism. (**A**) Liver ALT. (**B**) Liver AST. (**C**) Liver bile acids. (**D**) CYP7A1 activity. (E) Serum bile acids. (**F**) Ileum bile acids. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

Among all FB 3-14's postbiotics, Postcell exerted the strongest hepatoprotective and bile acid–modulating effects, reducing hepatic ALT levels, bile acid accumulation, and CYP7A1 activity ($P < 0.05$; Figure S1G-L). Notably, the combination of Postcell with DH further lowered hepatic and ileal bile acid levels compared with either treatment alone ($P < 0.05$; Figure 3C, F), indicating enhanced regulation of bile acid homeostasis.

These findings suggest that the efficacy of Postcell, especially when combined with DH, is closely linked to improved regulation of cholesterol catabolism and enterohepatic bile acid circulation, thereby contributing to protection against HFHC-induced hepatic metabolic injury.

3.5. Effect of FB 3-14's Postbiotics and Dietary Herbs on Brain Function

After 12 weeks of HFHC feeding, mice exhibited severe impairment in brain-related behavioral, histological, and inflammatory levels. In the OF test, HFHC-fed mice showed significantly reduced exploration of the center area, as reflected by lower center time and center distance ($P < 0.05$; Figure 4A, B). In the EMP test, the HFHC group displayed reduced open-arm exploration ($P < 0.05$; Figure 4C, D). Histological examination revealed hippocampal neuronal injury, including lighter staining, fewer neurons, and disrupted cellular organization (Figure 4E, F). In parallel, brain inflammatory cytokines, particularly IL-1β and TNF-α, were elevated in the HFHC group, indicating that the HFHC diet induced substantial neuroinflammation ($P < 0.01$; Figure 4G, H).

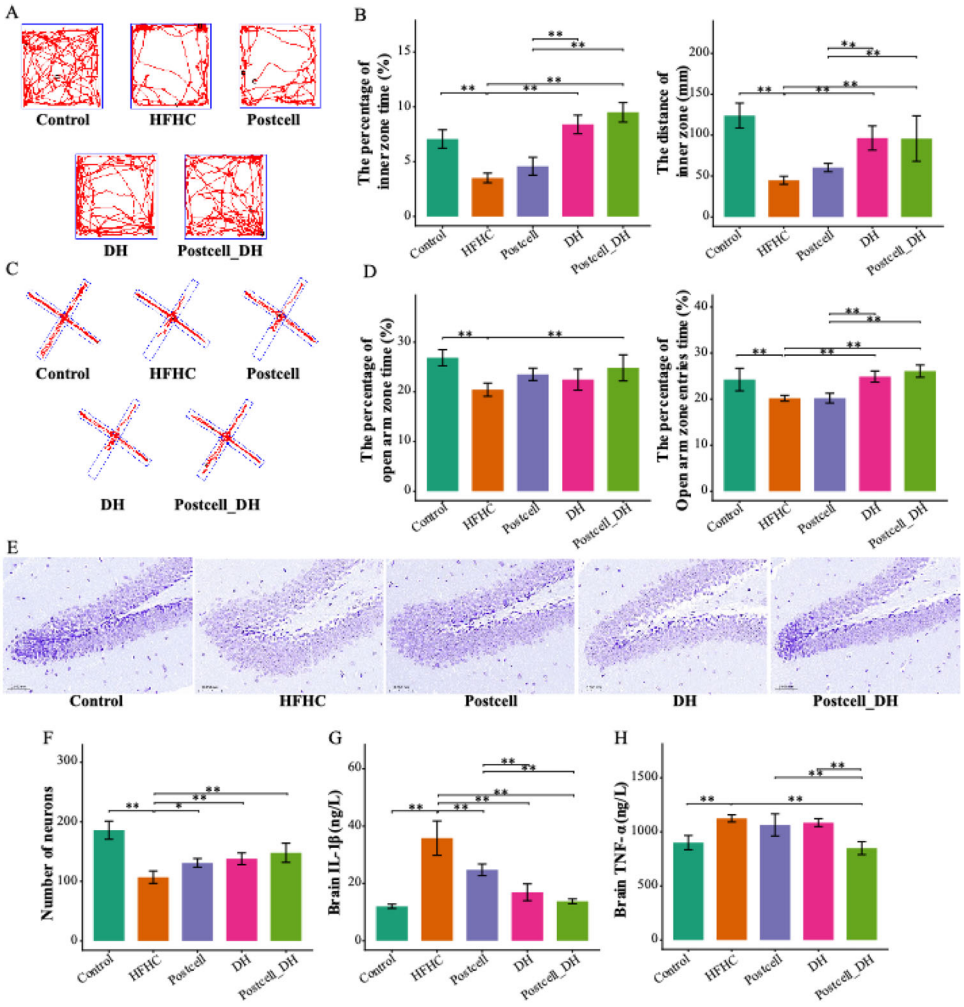

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Figure 4. Effect of FB 3-14 derived postbiotics and dietary herbs on brain function and neuroinflammation. (**A**) Representative trajectories in the open-field (OF) test. (**B**) Time spent in the center area and total distance traveled in the OF test. (**C**) Representative trajectories in the elevated plus maze (EPM). (**D**) Time spent in open arms and the number of entries into open arms (EPM). (**E**) Nissl staining of the hippocampal region. (**F**) Number of neurons. (**G**) Brain IL-10. (**H**) Brain TNF-α. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

Among the FB3-14-derived postbiotics, all postbiotic treatments reduced brain IL-1beta relative to HFHC ($P < 0.05$; Figure S1O), but none clearly improved TNF-α ($P > 0.05$; Figure S1P), indicating that FB3-14-derived postbiotics alone afforded only partial protection. DH alone significantly improved behavioral performance in both the OF and EMP tests, as well as brain IL-1β levels ($P < 0.01$; Figure 4).

Notably, the combination of Postcell with DH produced more pronounced improvements in brain-related outcomes than either treatment alone. In the Postcell_DH group, hippocampal histological abnormalities were markedly alleviated, with clearer cellular morphology, more orderly arrangement, and better preservation of neuronal structure (Figure 4E, F). Moreover, although Postcell or DH alone did not significantly reduce brain TNF-α levels, the combined intervention significantly lowered TNF-α, indicating a stronger suppressive effect on neuroinflammation ($P < 0.01$; Figure 4H).

These findings demonstrate that FB3-14 derived postbiotics alone provided only partial protection, and DH produced clearer improvements in brain-related outcomes. More importantly, the combination of Postcell and DH conferred the most comprehensive neuroprotective effect, suggesting that DH complemented the limited efficacy of Postcell on the gut-brain axis and enhanced its anti-neuroinflammatory activity.

3.6. Effect of FB 3-14's Postbiotics and Dietary Herbs on Gut Microbiota

To determine whether the metabolic benefits of the interventions were associated with gut microbiota remodeling, 16S rRNA sequencing was performed. Compared with the Control group, the HFHC diet markedly reduced both the Shannon index and Chao1 richness, indicating a substantial loss of microbial diversity and richness. None of the interventions fully restored these alpha-diversity indices to Control levels (Figure 5A, B). Beta-diversity analysis showed clear separation between the Control and HFHC groups, confirming that HFHC feeding strongly altered community structure (Figure 5C). Among all intervention groups, only the DH group showed a significant difference from the HFHC group (PERMANOVA, $Padj = 0.02$), suggesting that DH exerted the most evident effect on microbiota structure.

At the genus level, the HFHC diet increased several taxa associated with a metabolic dysfunction, including *Akkermansia*, *Faecalibaculum*, and *Coriobacteriaceae_UCG-002* (Figures 5D and F). These genera were positively associated with bile acid circulation–related indices, brain inflammatory markers, and LDL levels (Figure 5E).

Compared with the HFHC group, DH treatment increased the relative abundance of *Clostridioides* and reduced *Lachnoclostridium* ($P < 0.01$; Figure 5F). Correlation analysis showed that *Clostridioides* was negatively associated with serum leptin and TC levels, whereas *Lachnoclostridium* was positively correlated with brain inflammatory markers, LDL, and serum bile acids ($P < 0.05$; Figure 5E). Moreover, Postcell treatment significantly increased the relative abundance of *Clostridium_sensu_stricto_1* and *Paludicola*, and this enrichment was specific to the Postcell intervention among the tested postbiotic forms ($P < 0.05$; Figure S2A). Correlation analysis further showed that both genera were negatively associated with several adverse phenotypes ($P < 0.05$; Figure 5E). The Postcell_DH group showed a similar tendency in these genura, although these changes did not reach statistical significance. Notably, the relative abundance of *Holdemania* was higher in the Postcell_DH group than in both the HFHC and Control groups ($P < 0.05$; Figure 5F). Correlation analysis indicated that *Holdemania* was significantly negatively associated with serum leptin and TC levels ($P < 0.05$; Figure 5E).

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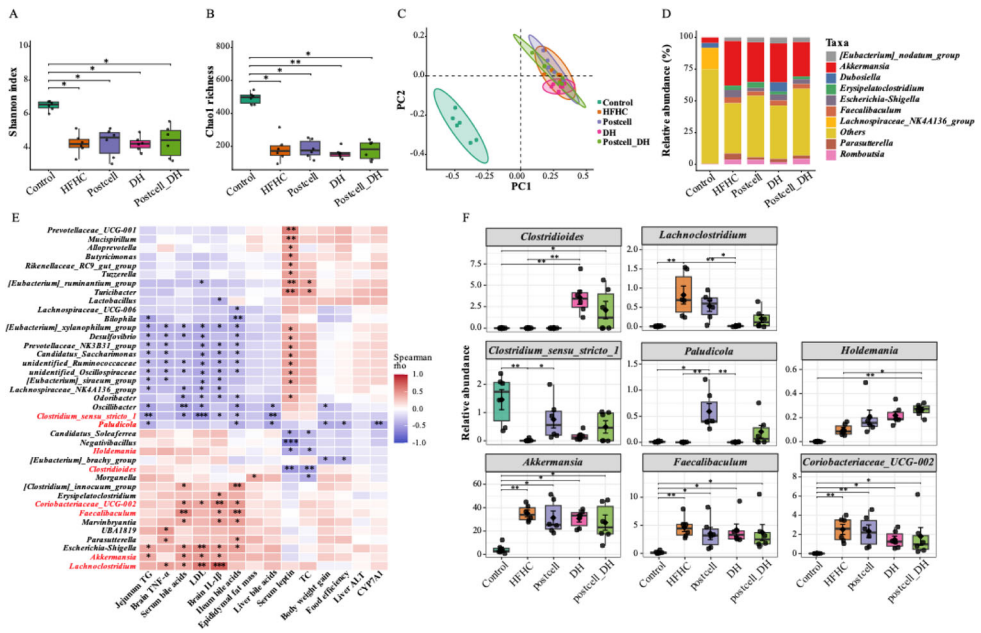

Figure 5. Effects of FB 3-14 derived postbiotics and dietary herbs on gut microbiota composition. **(A)** Shannon index. **(B)** Chao1 richness. **(C)** Beta diversity analysis based on Bray–Curtis distance (PCoA). **(D)** Relative abundance of dominant taxa at the genus level. **(E)** Correlation analysis between microbial taxa and metabolic parameters. **(F)** Differentially abundant microbial taxa among groups. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

Collectively, these findings indicate that both Postcell and DH contributed to the partial restoration of microbial homeostasis, while the combined Postcell_DH intervention showed a broader corrective effect on HFHC-associated dysbiosis, shifting the gut microbiota toward a profile associated with improved lipid metabolism, bile acid homeostasis, and reduced neuroinflammation. These results suggest that remodeling of the gut microbiota may represent an important mechanism underlying the synergistic metabolic benefits of Postcell and DH.

3.7. Effect of FB 3-14's Postbiotics and Dietary Herbs on Cecal Metabolites

To further elucidate the potential anti-obesity effects of Postcell, we first compared the HFHC and Postcell groups. A total of 277 cecal metabolites were significantly altered, including 148 downregulated and 129 upregulated metabolites in the Postcell group relative to the HFHC group (Figure S2B). Differential metabolites enriched in the Postcell group were associated with nucleotide metabolism and pyrimidine metabolism, represented by 4'-Thiothymidine, and also included Quercetol B and the lipid-related metabolites N-Lauroyl Methionine and N-Oleoyl Arginine (Figure S2C, D).

To further investigate the metabolic effects of the combined intervention, untargeted metabolomics was performed in the Control, HFHC, Postcell, DH, and Postcell_DH groups. Principal coordinates analysis based on Bray–Curtis distance demonstrated that the overall metabolomic profiles differed significantly among groups in both negative- and positive-ion modes (negative mode: $R^2 = 0.368$, $P = 0.001$; positive mode: $R^2 = 0.309$, $P = 0.001$). Pairwise PERMANOVA further showed that only the Postcell_DH group differed significantly from the HFHC group ($R^2 = 0.186$, *Padj* = 0.045), indicating that the combined treatment induced the most pronounced shift in the metabolic profile (Figure 6A). Compared with the HFHC group, the Postcell_DH group showed 466 differential metabolites, including 313 downregulated and 153 upregulated features (Figure 6B). KEGG enrichment analysis highlighted pyruvate metabolism and steroid hormone biosynthesis among the significantly enriched pathways (Figure 6C).

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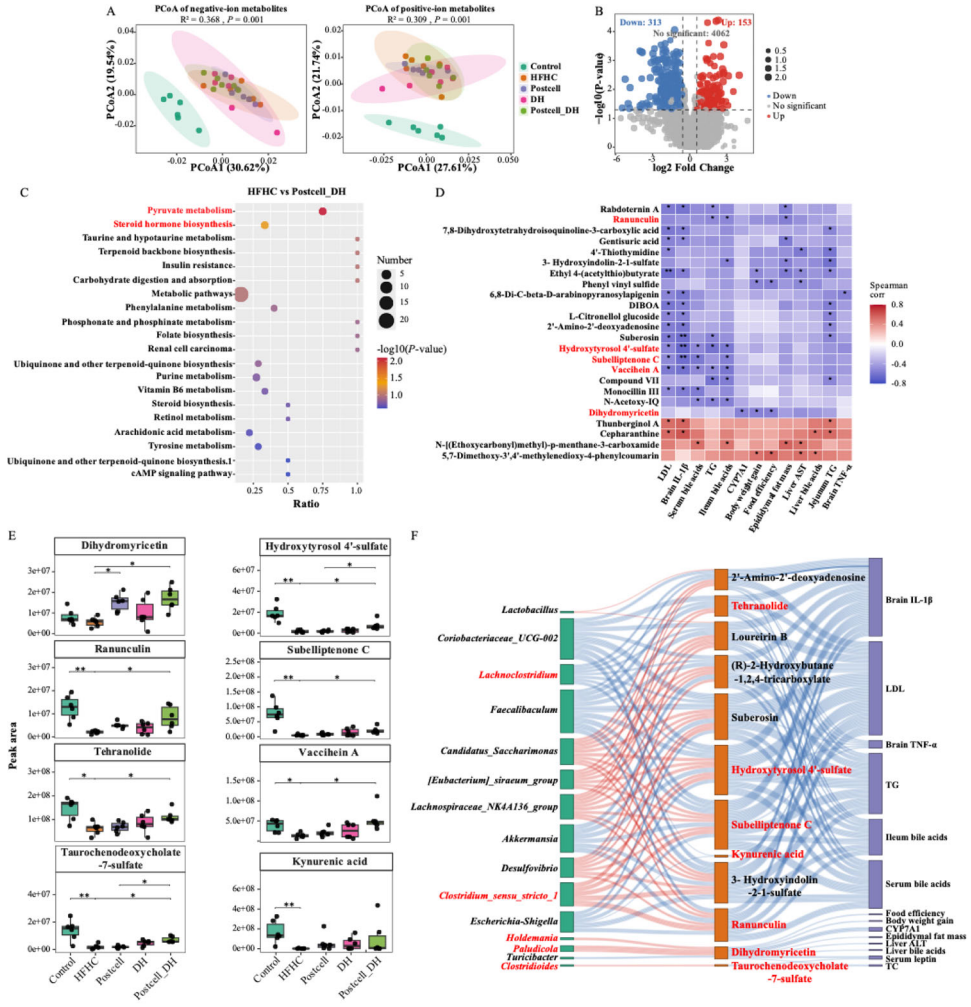

Figure 6. Effects of FB 3-14 derived postbiotics and dietary herbs on metabolomic profiles and multi-omics integration. **(A)** Principal coordinate analysis (PCoA) of metabolomic profiles. **(B)** Volcano plot of differential metabolites. **(C)** KEGG pathway enrichment analysis. **(D)** Heatmap of key differential metabolites. **(E)** The peak area of key differential metabolites. **(E)** Correlation network of microbiota, metabolites, and metabolic phenotypes. Data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$.

Correlation analysis further identified metabolites associated with at least three hyperlipidemia-related phenotypes. Compared with the HFHC group, several metabolites were enriched in the Postcell_DH group, including hydroxytyrosol 4’-sulfate, ranunculin, subelliptenone C, tehranolide, vaccihein A, taurochenodeoxycholate-7-sulfate, and kynurenic acid. These metabolites also showed a relationship with adiposity, dyslipidemia, bile acid-related indices, and neuroinflammatory markers ($P < 0.05$; Figure 6D, E). Additionally, dihydromyricetin was increased in both the Postcell and Postcell_DH groups compared with the Control and HFHC groups ($P < 0.05$; Figure 6E).

3.8. Construction of “Gut Microbiota-Metabolites-Index” Axis

Spearman correlation analysis was next performed to summarize the key correlations among microbial taxa, metabolites, and metabolic index (Figure 6F). *Clostridioides* and taurochenodeoxycholate-7-sulfate, both enriched in the Postcell_DH group, were positively correlated with each other and negatively associated with serum TC and leptin levels. Kynurenic acid was negatively correlated with *Escherichia-Shigella* and *Coriobacteriaceae_UCG-002*, as well as with LDL-related traits. In addition, 3-Hydroxyindolin-2-1-sulfate was positively associated with *Clostridium_sensu_stricto_1*, a taxon enriched in the Postcell group that was negatively correlated with brain inflammatory indices, lipid parameters, and bile acid-related traits. *Holdemania*, enriched in the

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Postcell_DH group, was positively associated with dihydromyricetin as well as CYP7A1, serum leptin, and total cholesterol. Together, these findings suggest that the beneficial metabolic effects of Postcell_DH may be mediated through coordinated remodeling of the gut microbiota–metabolite–phenotype interactions.

4. Discussion

Compared with live probiotics, postbiotics offer several practical advantages, including improved safety, greater stability, longer shelf-life, and better batch-to-batch consistency [7]. In our previous study, *Bifidobacterium longum* subsp. *infantis* FB 3-14 showed anti-obesity and lipid-lowering effects in high-fat-fed mice [24]. However, whether its postbiotic derivatives exert comparable benefits, and which postbiotic form is most suitable for intervening in hyperlipidemia-associated obesity, especially under conditions involving bile acid dysregulation and cognitive impairment, remained unclear. In the present study, LiveBL and PostBL did not differ significantly in their effects on body weight gain, lipid metabolism, or brain inflammatory markers, suggesting that heat inactivation did not substantially impair the major metabolic benefits of the parental strain. Notably, however, PostBL produced a greater reduction in hepatic bile acid levels than LiveBL. This finding indicates that postbiotic preparations may retain, and in some aspects even enhance, specific metabolic functions of the live strain, particularly in regulating bile acid homeostasis.

Different postbiotic fractions are not necessarily functionally equivalent. Among the various postbiotic forms reported to date, SCFAs, cell fragments, and heat-killed probiotics have been considered major contributors to anti-obesity activity [5]. In line with this concept, our study demonstrated that FB 3-14-derived postbiotics possess distinct functional properties. Interestingly, inactivated bacterial cells (Postcell) represent the most effective form for ameliorating hyperlipidemia-associated obesity. Postcell exhibited the strongest cholesterol micellar binding activity in vitro and showed the clearest improvement in lipid-related phenotypes in vivo, including attenuation of body weight gain, reduction of jejunal triglyceride accumulation, improvement of serum lipid profiles, and alleviation of hepatic injury and bile acid disturbance.

Previous studies have shown that some lactic acid bacteria (LAB), even in a non-viable state, can remove cholesterol through surface binding, membrane incorporation, and bile-related interactions [25,26]. In addition, EPS produced by LAB have also been reported to exert cholesterol-lowering effects in vitro [22,27]. Based on these findings and our current results, it is reasonable to speculate that the superiority of Postcell may be attributed to the retention of structural bacterial components after inactivation. These components may physically interact with cholesterol micelles and possibly bile salts within the intestinal lumen, thereby limiting intestinal lipid absorption, particularly in the jejunum, and subsequently reducing downstream metabolic burden.

Moreover, Postcell treatment markedly reshaped the gut microbial composition, characterized by a significant enrichment of *Clostridium_sensu_stricto_1* and *Paludicola*. Previous studies have indicated that *Clostridium_sensu_stricto_1* is involved in bile acid metabolism, particularly by promoting bile acid deconjugation in the intestine, thereby influencing enterohepatic circulation and lipid homeostasis [28].In addition, *Paludicola* has been reported to be negatively associated with body weight, HOMA-IR, and fasting plasma glucose, while positively correlated with energy expenditure and insulin sensitivity, suggesting a beneficial role in metabolic regulation [21]. Consistently, our correlation analysis demonstrated that both genera were negatively associated with jejunal triglyceride levels as well as intestinal and hepatic bile acid concentrations, supporting their potential contribution to improved lipid metabolism and bile acid homeostasis. At the metabolomic level, Postcell intervention induced distinct metabolic reprogramming, with differential metabolites primarily enriched in nucleotide and pyrimidine metabolism pathways, exemplified by 4′-thiothymidine. In addition, several bioactive compounds, including Quercetol B and lipid-related metabolites such as N-lauroyl methionine and N-oleoyl arginine, were significantly enriched in the Postcell group. These metabolites may contribute to improved lipid handling and energy metabolism, further supporting the anti-obesity effects of Postcell. These findings suggest that FB 3-

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14-derived Postcell represents a promising alternative to live probiotics, combining practical advantages in stability, safety, and formulation flexibility with marked efficacy in improving body weight control, intestinal triglyceride absorption, and hepatic function. Importantly, these beneficial effects seem to be mediated not only by direct interactions with dietary lipids, but also by coordinated remodeling of the gut microbiota and metabolome.

However, the effect of Postcell alone on the gut–brain axis appeared relatively limited. This may be because, compared with cell-free supernatant, inactivated bacterial cells contain fewer metabolites that can directly regulate neuroinflammation and gut–brain signaling [30]. In contrast, DH alone showed pronounced neuroprotective and anti-inflammatory effects in the present study, which is consistent with its reported antioxidant, anti-inflammatory, and neuroregulatory activities [14,15]. Specifically, DH significantly improved performance in the OF and EPM test, suggesting enhanced exploratory behavior and reduced anxiety-like behavior in rodents. In addition, DH treatment was associated with better hippocampal neuronal preservation and reduced brain IL-1β levels, further supporting its protective effects against HFHC-induced neuroinflammation and neuronal injury. Taken together, these findings indicate that DH may compensate for the relatively limited gut–brain regulatory activity of Postcell, thereby broadening the functional spectrum of the combined intervention.

Recently, increasing attention has been given to postbiotics generated through probiotic fermentation of dietary herbs, which have shown promising health benefits [17,18]. In contrast, studies investigating the combined use of heat-inactivated bacterial cells and herbal components remain relatively limited. Notably, this combination strategy offers several practical advantages, including improved quality control, precise dose standardization, greater formulation flexibility, and preservation of the native bioactive components from both sources [19]. In the present study, our findings are therefore of particular interest, as the Postcell_DH combination exhibited more pronounced protective effects than either component alone, especially in terms of neuroinflammation, bile acid homeostasis, and metabolic regulation. This enhanced efficacy may be attributed to functional complementation between the two components. Specifically, Postcell primarily acts at the intestinal level by modulating cholesterol handling and bile acid balance, whereas DH exerts broader systemic effects, including antioxidant activity, neuroinflammatory regulation, and gut–brain axis protection. Our findings highlight a feasible and effective strategy for integrating postbiotics and herbal interventions to achieve broader metabolic and neuroprotective benefits, particularly in the context of hyperlipidemia-associated liver–gut–brain dysfunction.

The small intestine is a key site for dietary cholesterol absorption and bile acid reabsorption, thereby linking intestinal lipid handling with hepatic metabolism through the enterohepatic cycle [30,31]. As cholesterol-derived metabolites, bile acids play a central role in maintaining lipid homeostasis. However, chronic HFHC feeding disrupts this balance by enhancing CYP7A1 activity, elevating bile acid synthesis and accumulation, which contributes to dyslipidemia and liver injury [2,3]. Consistently, in the present study, 12 weeks of HFHC feeding markedly elevated total bile acid levels in the serum, liver, and ileum, accompanied by increased CYP7A1 activity, indicating exaggerated cholesterol-to-bile-acid conversion and disrupted enterohepatic homeostasis.

Notably, the Postcell_DH intervention effectively reversed these alterations and showed a stronger effect than either Postcell or DH alone. Given that CYP7A1 is the rate-limiting enzyme in bile acid synthesis, its downregulation, particularly in the Postcell_DH group, suggests that the combined treatment alleviated upstream cholesterol burden and suppressed excessive bile acid production. In addition, both *Clostridioides* and taurochenodeoxycholate-7-sulfate were significantly enriched in the Postcell_DH group and showed a positive correlation. Previous studies have demonstrated that *Clostridioides* can express bile salt hydrolases and is closely involved in bile acid metabolism, with its growth and activity being strongly influenced by the composition of the bile acid pool in a species- and metabolite-specific manner [32]. Taurochenodeoxycholate-7-sulfate, a sulfated bile acid derivative, has been implicated in bile acid detoxification and homeostasis, and its elevation may indicate enhanced bile acid turnover and reduced metabolic burden. Consistent with

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our findings, Zhang et al. [33] reported that bile acid biosynthesis is a key pathway disrupted by the HFHC diet. In particular, several primary bile acids, including taurocholic acid, taurochenodeoxycholic acid, glycocholic acid, and taurochenodeoxycholic acid, are markedly elevated. In this study, the co-enrichment of *Clostridioides* with taurochenodeoxycholate-7-sulfate, together with their negative associations with serum TC and leptin, suggests that the Postcell_DH intervention may promote a coordinated remodeling of bile acid metabolism. Specifically, *Clostridioides* may contribute to bile acid transformation, while the accumulation of sulfated bile acids supports detoxification and stabilization of the bile acid pool, ultimately contributing to improved metabolic homeostasis.

Increasing evidence suggests that chronic HFHC feeding not only induces metabolic disorders, but also impairs brain function, resulting in cognitive decline and anxiety-like behaviors [4,34]. In the present study, the Postcell_DH intervention significantly improved spatial working memory and anxiety-like behavior, accompanied by increased neuronal preservation. Notably, Postcell_DH markedly reduced brain TNF-α levels, whereas Postcell or DH alone showed no significant effect, indicating a stronger neuroprotective efficacy of the combined treatment. This pattern suggests that the two components may act in a functionally complementary manner to alleviate HFHC-induced neuroinflammation and neuronal injury. Multi-omics analysis further supported the involvement of tryptophan-related microbial metabolites in this process. Kynurenic acid (KYNA), an important metabolite of the tryptophan pathway, has attracted attention because of its immunomodulatory and neuroprotective properties. KYNA can interact with GPR35 expressed on macrophages and other immune cells, and is linked to the regulation of cytokine production and inflammatory responses [35]. In our study, KYNA was negatively correlated with *Escherichia-Shigella* and *Coriobacteriaceae_UCG-002*, as well as with LDL-related traits, suggesting that enrichment of this metabolite may be associated with reduced neuroinflammation and improved metabolic status. Consistent with our results, *Coriobacteriaceae_UCG-002* has been proven to be associated with cognitive performance [36] and to participate in bile acid biotransformation, including 6β-epimerization and 7α-dehydroxylation [37]. In addition, 3-hydroxyindolin-2-one sulfate, another tryptophan-related metabolite, was positively associated with *Clostridium_sensu_stricto_1*, which itself was negatively correlated with brain inflammatory indices, lipid parameters, and bile acid-related traits. This finding suggests a potential microbiota–tryptophan metabolite axis contributing to both metabolic and neurological improvement.

Our study highlights that different FB 3-14 derived postbiotic fractions possess distinct functional properties and that the inactivated cell fraction represents a preferred candidate for lipid-targeted intervention. Moreover, it provides evidence that a physically combined postbiotic–herbal strategy achieves broader efficacy than either component alone, encompassing improvements in obesity, dyslipidemia, hepatic dysfunction, bile acid imbalance, gut microbial dysbiosis, and cognitive impairment in hyperlipidemic mice. However, some limitations need to be addressed in future research. First, the associations identified among gut microbiota, metabolites, and host phenotypes are primarily based on correlation analyses. The underlying causal relationships and synergistic mechanisms of Postcell_DH treatment require further study. Second, only a single ratio of the Postcell_DH combination was evaluated in this study to facilitate initial mechanistic exploration. Future studies incorporating multiple formulation ratios are needed to better define dose–response relationships.

5. Conclusions

In this study, the cholesterol-lowering capacity of FB 3-14 derived postbiotics was first screened in vitro and in vivo. Postcell exhibited the strongest cholesterol-binding capacity and efficacy in attenuating body weight gain, jejunal TG accumulation, and hepatic dysfunction compared with other postbiotic forms. Moreover, Postcell_DH exerted broader metabolic benefits, including reductions in weight gain, food efficiency, bile acid dysregulation, and neuroinflammation, along with improvements in brain function. Multi-omics analyses suggested that the observed benefits are

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likely linked to coordinated alterations in the gut microbiota and metabolome, particularly pathways related to bile acids and tryptophan metabolism. Notably, *Clostridioides* and taurochenodeoxycholate-7-sulfate were negatively associated with TC and leptin, whereas *Clostridium_sensu_stricto_1* and 3-Hydroxyindolin-2-1-sulfate were negatively correlated with brain inflammatory level, lipid, and bile acid-related index. These findings support a practical development strategy that combines lipid-oriented postbiotic fractionation with herbal functional complementation to ameliorate hyperlipidemia-associated obesity and cognitive impairment.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Effect of different FB 3-14 derived postbiotics in mice fed a high-fat and high-cholesterol diet; Figure S2: Effect of different FB 3-14 derived postbiotics on microbiome and metabolomic profiles.

Author Contributions: Conceptualization, S.W. and Y.S.; methodology, Y.S. and Y.Z.; software, Z.Y.; validation, Y.S., Y.Z., and Z.Y.; formal analysis, H.Y.; investigation, Y.Z. and H.C.; resources, S.W. and Y.S.; data curation, Y.S., H.L., and H.X.; writing—original draft preparation, Y.S.; writing—review and editing, Y.S., J.W., and D.F.; visualization, Y.S., B.Z. and H.L.; supervision, project administration and funding acquisition, S.W. and Y.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program (No. 2024YFD2100701) and Tianjin Natural Science Foundation Project (24JCQNJC02020).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Committee of Nankai University (protocol code 2025-SYDWLL-000454 and date of approval 8th March 2025).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DH	Dietary herbs
HFHC	High-fat, high-cholesterol
TC	Total cholesterol
TG	Total triglycerides
LDL	Low-density lipoprotein cholesterol
EPS	Extracellular polysaccharides
SCFAs	Short-chain fatty acids
OF	Open-field
EPM	Elevated maze
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
LAB	Lactic acid bacteria
KYNA	Kynurenic acid

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