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Combined Administration of *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827 Attenuates Obesity in High-Fat Diet-Induced Obese Mice

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ABSTRACT: Obesity, a major global health issue, is increasingly associated with gut microbiota dysbiosis and chronic inflammation. Probiotics and their derivatives have emerged as promising modulators of metabolic health. This study aimed to evaluate the anti-obesity effects of *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827, isolated from healthy human fecal samples, administered in either live or heat-inactivated form in high-fat diet-induced obese mice. The live combination (BA+LP) significantly reduced body weight gain, adiposity, and serum lipid levels, outperforming single strains. The heat-inactivated formulation (h-kBA+LP) yielded moderate phenotypic benefits but more prominently altered gut microbiota composition, notably increasing *Akkermansia*. Both formulations downregulated pro-inflammatory and lipogenic gene expression. Metabolomic analysis revealed reduced deoxycholic acid and increased beneficial lipid-related metabolites, contributing to improved metabolic profiles. These findings support the complementary roles of v iable and non-viable probiotics in obesity management and highlight the potential of postbiotics as functional metabolic modulators.

Key words: Probiotics; *Bifidobacterium adolescentis*; *Lactobacillus plantarum*; postbiotics; Obesity

1. Introduction

Obesity and the related metabolic syndrome are considered as major global public health challenges. According to the World Health Organization (2024), more than 1 billion people worldwide are obese, including 650 million adults, 340 million adolescents, and 39 million children. The global prevalence of obesity has nearly tripled since 1975, making it a major public health concern[1, 2]. Metabolic syndrome, characterized by central obesity, hypertension, dyslipidemia, and insulin resistance, is a cluster of metabolic abnormalities with an estimated adult prevalence of about 12.5%–31.4% globally[3]. Obesity and metabolic

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syndrome can significantly increase the risk of various chronic diseases. For example, an estimated 44% of type 2 diabetes cases and 23% of ischemic heart disease cases are attributable to obesity[4] . These figures indicate that the obesity epidemic not only affects quality of life but also imposes a substantial burden in terms of disease and healthcare costs. Therefore, the prevention and control of obesity is of great public health importance.

Although dietary intervention remains the first-line for obesity management, its long-term effectiveness is often compromised by poor adherence due to behavioral and environmental influences. In recent years, increasing attention has been directed toward probiotics as a complementary approach. Probiotics, defined by the World Health Organization as "live microorganisms that confer a health benefit on the host when administered in adequate amounts," play an important role in modulating host metabolism and gut microbiota[5]. In view of the close link between obesity and gut microbiota dysbiosis as well as chronic inflammation, modulating the gut microbiota with probiotics has been proposed as an anti-obesity strategy in recent years. For example, according to Ramirez-Olea et al. (2025), supplementation with a *Bacillus licheniformis*-based probiotic in a high-fat diet-induced obese mouse model significantly reduced body weight gain and visceral adiposity, while restoring gut microbiota diversity and modulating SCFA-producing bacteria, ultimately improving metabolic inflammation[6].Recent findings by Wang et al. (2025) demonstrated that supplementation with *Ligilactobacillus animalis* LA-1 in a high-fat diet-induced obese mouse model markedly reduced body weight gain and hepatic lipid accumulation, while reshaping gut microbiota composition—particularly normalizing the Firmicutes/Bacteroidetes ratio and enhancing SCFA-producing taxa—thereby exerting notable anti-obesity effects[7].One of the most widely studied mechanisms involves the enhanced production of short-chain fatty acids (SCFAs), which promote lipid metabolism and fatty acid oxidation[8]. In addition, probiotics have been shown to modulate appetite-related hormones and inhibit the intestinal absorption of dietary fats, thereby contributing to reduced energy intake[9]. Furthermore, probiotics help strengthen the intestinal barrier and reduce endotoxin translocation, which is closely linked to chronic low-grade inflammation in obesity[10]. Collectively, these mechanisms underscore the translational potential of probiotics as an effective adjunctive therapy for obesity and metabolic syndrome.

Among the multiple probiotic species, *Bifidobacterium adolescentis* (*B. adolescentis*) and *Lactobacillus plantarum* (*L. plantarum*) have attracted considerable attention due to their notable functional properties. Previous studies have shown that supplementation with certain *B. adolescentis* strains reduces weight gain in high-fat diet-induced obese mice through promoting the expression of genes related to fat oxidation and alleviating the obesity-related inflammation[11, 12]. Similarly, *L. plantarum* has exhibited significant anti-obesity effects in animal models. The intake of *L. plantarum* effectively suppressed weight gain and fat accumulation in obese mice, while improving insulin sensitivity and glucose tolerance[13-16]. The above evidence suggests that these two probiotic strains hold promise in ameliorating obesity-related phenotypes.

Current research on probiotic-mediated obesity management has primarily focused on single-strain interventions, with limited exploration of multi-strain synergies or combined probiotic-functional ingredient

approaches. While some studies hypothesize that co-administration of probiotics with bioactive compounds may enhance metabolic regulation compared to monotherapy [17], experimental and clinical evidence supporting such combinatorial strategies remains scarce, necessitating further validation.

In addition, the emerging concept of "postbiotics" has garnered attention in recent years. Postbiotics refer to non-viable microbial cells or their bioactive metabolites. Postbiotics offer advantages such as reduced risks of microbial translocation and improved standardization for manufacturing[18]. Unlike live probiotics, postbiotics do not require viable bacteria to exert their beneficial effects, making them more stable, easier to store, and less dependent on environmental conditions during transport. Moreover, postbiotics have a lower risk of bacterial translocation or infection, which can be a concern with live probiotics, particularly in immunocompromised individuals. Postbiotics can still modulate gut microbiota, improve metabolic outcomes, and exhibit anti-inflammatory effects similar to live probiotics. Importantly, postbiotics offer enhanced safety and stability, providing an alternative strategy for managing obesity and metabolic syndrome. For instance, heat-inactivated *Lactobacillus* strains have demonstrated efficacy in ameliorating obesity-associated metabolic dysfunction and adipose tissue inflammation[19, 20]. Proposed mechanisms include reinforcement of intestinal barrier integrity, modulation of gut microbiota composition and metabolite profiles (e.g., increased short-chain fatty acid [SCFA] production), and regulation of energy metabolism via gut hormone signaling[19]. However, research on postbiotics for obesity intervention is still nascent, with their therapeutic potential incompletely characterized.

Based on the above background, an intervention study was conducted in a high-fat diet (HFD)-induced obese mouse model, to systematically evaluate the anti-obesity effects of two probiotic strains: *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827, both isolated from fecal samples of healthy donors in the Shenzhen region. The experimental design incorporated four distinct intervention groups: (1) monotreatment with live *B. adolescentis*, (2) monotreatment with live *L. plantarum*, (3) combination therapy with both live strains, and (4) combination therapy with heat-inactivated strains (postbiotic formulation). The study specifically evaluated the impacts of the two live strains individually and these two combined interventions on body weight gain, lipid metabolism, and inflammatory cytokine levels in mice, while simultaneously analyzing changes in gut microbiota composition and gut metabolic profiles. Through these analyses, the study aimed to clarify the differences in anti-obesity efficacy and potential mechanisms between interventions using live probiotics versus inactivated probiotics. The findings were expected to provide scientific evidence for the application of multi-strain synergistic probiotic interventions and postbiotics in the prevention and treatment of obesity.

Although postbiotics (e.g., heat-inactivated probiotics) have shown remarkable effects in modulating fat accumulation in high-fat diet-induced obese mice, the therapeutic potential of live probiotics remains superior due to their metabolic activity and sustained colonization capacity. However, maintaining probiotic viability during industrial processing, storage, and gastrointestinal transit presents significant challenges, as most probiotic strains exhibit sensitivity to environmental stressors including thermal processing, gastric acidity,

bile salts, and oxidative conditions. To address these limitations, we developed an encapsulation system specifically designed to protect viable probiotics throughout the product lifecycle. The system utilizes a composite matrix of gelatin, glycerol, and low-ester pectin as wall materials, selected for their synergistic protective properties and industrial scalability. *L. plantarum* was chosen as the model probiotic for encapsulation due to its facultative anaerobic nature and documented health benefits, while *B. adolescentis* was excluded from this phase of research due to its strict anaerobic requirements that exceed current technical capabilities. According to previous publication, the encapsulation system is not only scalable for industrial production but also produces microspheres with a particle size suitable for human dietary consumption and effective gastrointestinal delivery, ensuring that a higher number of viable bacteria reach the gut to exert their health benefits[21].

In this study, we employed a high-fat diet (HFD)-induced obese mouse model to systematically evaluate the anti-obesity effects of *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827 administered either as live cells or heat-inactivated postbiotics. Mice received single-strain (BA or LP), combined live (BA+LP), or combined postbiotic (h-kBA+LP) interventions for 11 weeks. Primary outcomes included body-weight gain, adiposity, serum lipids, and adipose tissue histology, expression of key genes related to lipid metabolism and inflammation, gut microbiota composition, and fecal metabolomic profiles. By comparing live and inactivated formulations, we aimed to clarify their distinct mechanistic contributions and provide experimental evidence for the development of multi-strain probiotic and postbiotic strategies against obesity and associated metabolic disorders.

2. Materials and Methods

2.1 Bacterial isolation, inactivation, and powder preparation

Fecal samples from healthy adult donors in Shenzhen, China, were cryopreserved at −80 ℃ until use. Under strict anaerobic conditions, equal volumes of individual fecal samples were pooled to prepare a homogenized composite sample. A 0.5 mL aliquot of the mixture was inoculated into an anaerobic blood culture bottle and incubated statically at 37 ℃ for 72 h. Following enrichment, 0.1 mL of the culture was serially diluted (10^{-1} to 10^{-7}) in phosphate-buffered saline (PBS, Solarbio, P1020) supplemented with 1 g/L L-cysteine hydrochloride. A 100 μL aliquot of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions was spread onto pre-reduced YCFA agar plates (triplicates per dilution) and incubated anaerobically at 37 ℃ for 72 h. Individual colonies were selected and cultured in pre-reduced brain heart infusion (BHI) broth under anaerobic conditions for an additional 72 h.

Each isolate was assigned a unique code and subjected to Gram staining, physiological and biochemical characterization, and 16S rRNA gene sequencing for taxonomic identification. Strains exhibiting typical morphological and functional characteristics were selected for further study. Among them, *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827 were confirmed as representative strains based on taxonomic and functional profiling. These strains were provided by Shenzhen Unknown BioTech Co., Ltd.

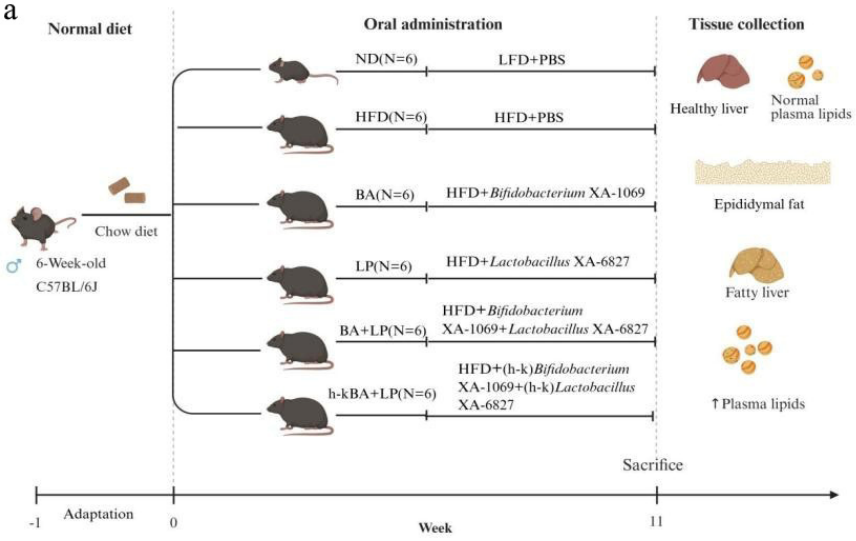
(Shenzhen, China), and have been deposited at the China General Microbiological Culture Collection Center (CGMCC) under accession numbers CGMCC No. 31381 and CGMCC No. 31383, respectively.

The selected strains were subcultured twice and inoculated into suitable fermentation media (MRS or mGAM broth) at defined inoculum ratios. Cultures were incubated anaerobically at 37 ℃ for 15–18 h. The viable cell counts were determined via colony-forming unit (CFU) enumeration on MRS or mGAM agar plates (incubated anaerobically at 37 ℃ for 48 h). Cultures were subsequently heat-inactivated by autoclaving at 121 ℃ for 20 min. Bacterial pellets were collected via centrifugation (Lynx 6000, Beckman Coulter; 8000 × g, 10 min), resuspended in a cryoprotectant solution, and subjected to freeze-drying to yield bacterial powders. The CFU equivalents per gram of powder for XA-1069 and XA-6827 were calculated based on the pre-inactivation viable counts and the final dry weight of each powder. Complete inactivation was confirmed by the absence of colony growth after anaerobic incubation on MRS/mGAM agar at 37 ℃ for 48 h.

Equal amounts (w/w) of the heat-inactivated powders of XA-1069 and XA-6827 were mixed to formulate the postbiotic combination referred to as h-kBA+LP, which was used in subsequent animal experiments.

2.2 Animal experiment design

All animal experiments were approved by the Health Bureau of Macau (AL008/DICV/DIS/2024). Thirty-six male C57BL/6J mice (6-week-old, (18 ± 2) g) obtained from Zhuhai Best Biotechnology Co., Ltd. (Guangdong, China) were housed under controlled conditions ((25 ± 2)℃, (55 ± 5)% humidity, 12 h light/dark cycle). All mice were allowed to take food freely. After one week adaptation, mice were randomly divided into six groups (n = 6): (1) ND group: standard diet (10% kcal fat, D12450J; Research Diets Inc., USA) + 200 μLPBS gavage; (2) HFD group: high-fat diet (60% kcal fat, D12492; same manufacturer) + 200 μLPBS; (3) BA group: HFD + *Bifidobacterium adolescentis* XA-1069 (2×10^9 CFU/day in 200 μL PBS); (4) LP group: HFD + *Lactobacillus plantarum* XA-6827 (2×10^9 CFU/day in 200 μL PBS); (5) BA+LP group: HFD + 1:1 combination of viable strains (total 2×10^9 CFU/day in 200 μL PBS); (6) h-kBA+LP group: HFD + 1:1 mixture of heat-inactivated strains (autoclaved at 121 ℃ for 20 min, equivalent CFU in 200 μL PBS). Daily oral gavage was performed for 11 consecutive weeks with body weight monitoring every week (Figure 1a).



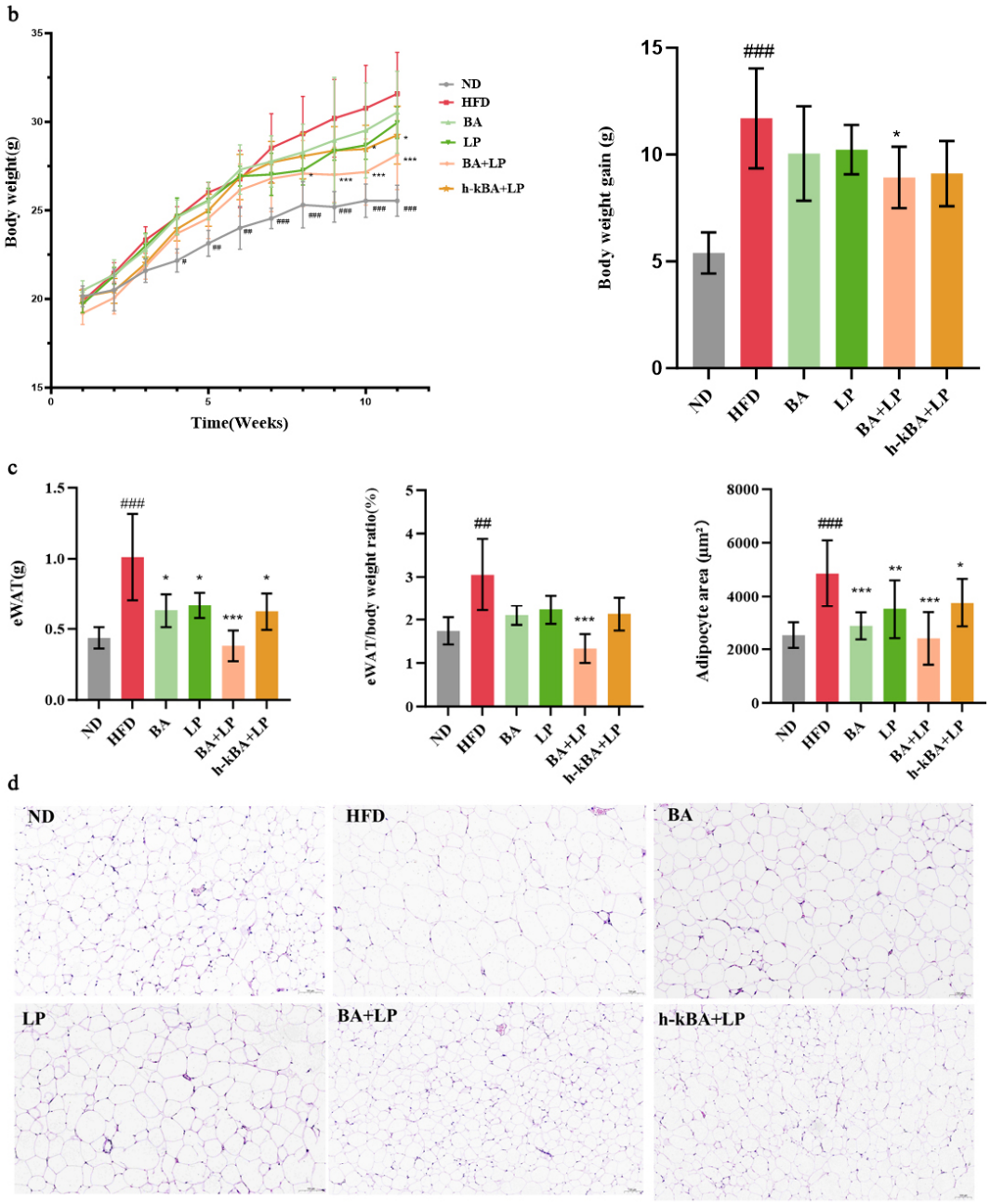


Figure 1. Experimental design and anti-obesity effects of probiotic intervention in high-fat diet-induced obese mice. (a) Schematic diagram of the animal experiment. Mice were administered either *Bifidobacterium adolescentis* XA-1069 (BA), *Lactobacillus plantarum* XA-6827 (LP), a combination of both (BA+LP), or their heat-inactivated form (h-kBA+LP) for 11 weeks; (b) Body weight progression over the experimental period (left), and body weight gain at the 11th week (right); (c) Epididymal white adipose tissue (eWAT) weight, eWAT-to-body weight ratio, eWAT index, and adipocyte area; (d) Representative histological sections of adipocytes (scale: 100 μm). Hash symbol (#) indicates significant differences between ND and HFD groups: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; Asterisk (*) indicates significant differences between HFD and probiotics treatment (BA, LP, BA+LP and h-kBA+LP) groups: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. ND, low fat diet control with sterile water control; HFD, high fat diet control with sterile water control; BA, high fat diet with 2×10^9 CFU/day *Bifidobacterium adolescentis* XA-1069 in 200 μL PBS treatment; LP, high fat diet with 2×10^9 CFU/day *Lactobacillus plantarum* XA-6827 in 200 μL PBS treatment; BA+LP, high fat diet with 1:1 combination of viable strains (total 2×10^9 CFU/day) in 200 μL PBS treatment; h-kBA+LP, high fat diet with1:1 mixture of heat-inactivated strains (autoclaved at 121 ℃ for 20 min, equivalent CFU) in 200 μL PBS treatment.

2.3 Sample collection

At the end of the 11th week, fecal samples of mice were collected and stored at -80 ℃ for further research. Following a 12-hour fasting period with free access to water, mice were sacrificed via CO_2 asphyxiation. Blood samples were spontaneously collected by cardiac puncture. Then liver tissues and epididymal white adipose tissues (eWAT) were harvested and weighed for further analysis.

2.4 Serum lipid measurements

Blood samples were collected after a 4-hour fast and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifugation at 3,000 × g for 15 minutes at 4 ℃, aliquoted, and stored at −80 ℃ until analysis. Then, levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined. TG was detected by the glycerol phosphate oxidase-phenol aminophenazone peroxidase (GPO-PAP) method. TC was detected by the cholesterol oxidase-phenol aminophenazone peroxidase (COD-PAP) method. HDL-C and LDL-C were determined by direct selective enzymatic colorimetric methods. The colorimetric assay kits for TG (Product No.: E-BC-K261-M), TC (Product No.: E-BC-K109-M), HDL-C (Product No.: E-BC-K221-M), and LDL-C (Product No.: E-BC-K205-S) were from Elabscience Biotechnology Co., Ltd. (Wuhan, Hubei province, China). Absorbance was measured using a SpectraMax M5 microplate reader (Molecular Devices, San Jose, CA, USA) at the appropriate wavelengths specified in each assay protocol. All measurements were performed in triplicate to ensure accuracy.

2.5 Histological examination

Mouse liver and epididymal adipose tissue were analyzed using hematoxylin-eosin (H&E) staining. A portion of the mouse liver and epididymal fat was fixed in a 4% paraformaldehyde solution, embedded in paraffin, and stained with H&E. Then, tissues were observed under a light microscope (BX53, Olympus Corporation, Tokyo, Japan) with an imaging system.

2.6 Quantitative real-time PCR analysis

Liver tissues were stored at -80℃ for further analysis. RNA was extracted using a commercial RNA extraction kit (Solarbio Science & Technology Co., Ltd., Beijing, China; Catalog No. R1200-50t) through the following steps including initial homogenization in provided lysis buffer, chloroform-mediated phase separation, and purification of the aqueous RNA-containing phase via silica-based spin columns. RNA quality was assessed immediately after extraction using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) with DEPC-treated water as the blank. All samples exhibited A260/A280 ratios between 1.87 and 1.98 (mean ± SD: 1.93 ± 0.03) and A260/A230 ratios between 1.95 and 2.10 (2.02 ± 0.04), indicating high purity and minimal protein or organic contaminant carryover. These spectrophotometric values met the generally accepted criteria for downstream quantitative real-time PCR analysis.

Complementary DNA (cDNA) synthesis was performed with a reverse transcription kit (Promega Corporation, Madison, WI, USA; Catalog No. A2801). Gene-specific primers (Table 1) for target transcripts

(PPAR-γ, FAS, TNF-α, IL-1β, etc.) were synthesized by ATCG Inc. (Hongkong, China), and qPCR reactions were conducted using qPCR Master Mix (Promega, Madison, WI, USA) on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method with normalization to GAPDH as the endogenous control.

Table 1 Primer sequences and amplicon size of the differentially expressed genes coding MCP, ACOX, FAS, PPAR-γ, and TNF-α used in the qPCR reaction.

Gene symbol	Forward primer	Reverse primer
MCP	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
ACOX	CCTGATTCAGCAAGGTAGGG	TCGCAGACCCTGAAGAAATC
FAS	GCTGAGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
PPAR-γ	GCTGTTATGGGTGAAACTCT	TGGCATCTCTGTGTCAACCA
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
GAPDH (Reference gene)	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

2.7 Intestinal microbial sequencing and analysis

Total genomic DNA was extracted from fecal samples using a magnetic bead-based extraction kit (Guangzhou Magen Biotechnology Co., Ltd., Guangzhou, China) following the manufacturer's instructions. DNA concentration and purity were measured prior to amplification. PCR amplification targeting the V3-V4 hypervariable region of bacterial 16S rDNA was conducted using 30 ng template DNA and fusion primers (338F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTWTCTAAT) in a 50 μL reaction volume. Thermal cycling parameters were optimized as: 95℃ initial denaturation for 3 min; 30 cycles of 95℃ (15 s), 56℃ (15 s), and 72℃ (45 s); followed by final extension at 72℃ for 5 min.

2.8 Metabolomic methodology

Fecal samples (25 mg) were thawed at 4℃, followed by the homogenization with two beads at 50 Hz for 5 min in 800 μL of extraction solvent (methanol: acetonitrile: water = 2:2:1, v:v:v, pre-cooled at -20℃). The homogenate was sonicated for 10 min at 4 ℃ using a VCX130 ultrasonic processor (Sonic & Materials, USA) with a 3 mm microtip set to 30% amplitude (≈ 40 W nominal) under an ice-water bath, following commonly employed conditions for fecal metabolite extraction. After centrifugation at 25,000 × g for 15 min (4℃), 600 μL of supernatant was transferred to a refrigerated vacuum concentrator. Then 600 μL of the reconstituted solution (methanol: water = 1:9, v:v) was added, vortexed and shaken for 1 min, and sonicated at 4℃ for 10 min. The mixture was centrifuged at 4℃ for 15 minutes at 25,000 × g, and the supernatant was transferred to a sample bottle. For system stability evaluation, 50 μL aliquots from each sample were pooled to generate quality control samples, which were analyzed alongside experimental samples.

LC-MS analysis was conducted in both positive and negative ionization modes. Metabolites meeting the following criteria were defined as differentially abundant metabolites (DAMs): statistical significance ($P < 0.05$), fold change (FC) > 2 or < 0.5, and variable importance in projection (VIP) ≥ 1.

2.9 Statistical analysis

All data are expressed as mean ± standard deviations (SD). Figures showing the results of animal experiments were plotted using GraphPad Prism (version 10.1.2), and data were analyzed using one-way ANOVA followed by Duncan's multiple range test to determine significant differences between groups. A p-value of < 0.05 was considered statistically significant, and *P*-values < 0.001 were reported where applicable. Metabolomics analyses and 16S rRNA sequencing analyses were performed using R 4.3.0.

3. Results

3.1 Oral administration of probiotics reduces the body weight of HFD-induced obese mice

In this study, the fat control benefits of *Bifidobacterium adolescentis* XA-1069 (BA), *Lactiplantibacillus plantarum* XA-6827 (LP), the two viable probiotics combination (BA+LP), and heat-inactivated combination (h-kBA+LP, as postbiotics) in HFD-fed mice were determined. The experimental procedure is depicted in Figure 1a. After 7 weeks of intervention, HFD-fed mice exhibited significantly higher weight gain compared to the low-fat diet (ND) group ($P < 0.001$, Figure 1b). At the 11-week endpoint, BA+LP-treated mice showed a markedly lower body weight versus the HFD group ($P < 0.001$), while the h-kBA+LP group demonstrated a moderate but statistically significant decrease of body weight gain ($P < 0.05$). Although BA and LP monotherapy groups exhibited numerically lower body weights than the HFD group, but not statistically significant. Notably, BA+LP intervention reduced cumulative weight gain by 23.68% in the final week compared to HFD group ($P < 0.05$), highlighting the synergistic efficacy of dual-strain probiotic therapy.

The BA+LP treatment exerted pronounced inhibitory effects on eWAT expansion (Figure 1c). Compared to the ND group, HFD feeding significantly increased eWAT mass of mice ($P < 0.001$). Single-strain intervention BA or LP as well as the heat-killed dual-strain formulation (h-kBA+LP) moderately attenuated eWAT accumulation, reducing tissue weight by 35.63%, 34.61%, and 37.64% ($P < 0.05$), respectively. In contrast, the viable dual-strain combination (BA+LP) demonstrated superior efficacy, achieving a 65.37% reduction in eWAT mass compared to HFD ($P < 0.001$) and significantly lowering the eWAT/body weight ratio ($P < 0.001$).

Histological analysis via H&E staining corroborated these findings. Adipocyte hypertrophy in the HFD group was significantly higher than that in ND group ($P < 0.001$). Probiotic interventions reduced adipocyte area by 40% (BA, $P < 0.05$), 27% (LP, $P < 0.01$), 50% (BA+LP, $P < 0.001$), and 22% (h-kBA+LP, $P < 0.05$) compared to HFD, respectively (Figure 1c). Consistently, HE staining (Figure 1d) also revealed a marked reduction in adipocyte hypertrophy following probiotic intervention, further confirming the morphological improvements induced by the treatments. Subsequent lipid profiling revealed that 11 weeks of HFD feeding markedly elevated serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels ($P < 0.05$) while numerically reducing high-density lipoprotein cholesterol (HDL-C) (Figure 2); In contrast, the combined probiotic intervention ameliorated this lipid metabolic dysregulation, demonstrating statistically significant reductions in TG and LDL-C compared to HFD group ($P < 0.05$, Figure 2).

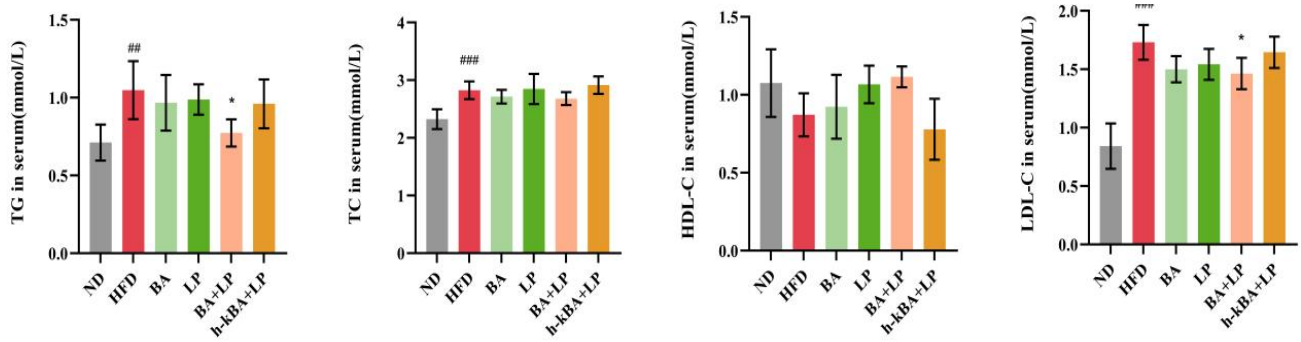


Figure 2. Effects of probiotic and postbiotic intervention on serum lipid profiles in HFD-fed mice. Serum concentrations of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) after 11 weeks of dietary intervention. Hash symbol(#) indicates significant differences between ND and HFD groups: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$,$^{\#\#\#}P < 0.001$; Asterisk (*) indicates significant differences between HFD and probiotics treatment (BA, LP, BA+LP and h-kBA+LP) groups*: * P < 0.05, ** P < 0.01 and *** P < 0.001. ND*, low fat diet control with sterile water control; HFD, high fat diet control with sterile water control; BA, high fat diet with 2×10^9 CFU/day *Bifidobacterium adolescentis* XA-1069 in 200 μL PBS treatment; LP, high fat diet with 2×10^9 CFU/day *Lactobacillus plantarum* XA-6827 in 200 μL PBS treatment; BA+LP, high fat diet with 1:1 combination of viable strains (total 2×10^9 CFU/day) in 200 μL PBS treatment; h-kBA+LP, high fat diet with1:1 mixture of heat-inactivated strains (autoclaved at 121 ℃ for 20 min, equivalent CFU) in 200 μL PBS treatment.

3.2 Supplementation with probiotics affected the levels of some transcription factors and inflammatory factors in mouse liver

As shown in Figure 3, compared with the ND group, the HFD treatment significantly upregulated the transcriptional expression of adipocyte differentiation- and inflammation-related factors—PPAR-γ ($P < 0.01$), FAS ($P < 0.001$), TNF-α ($P < 0.001$), and MCP ($P < 0.001$). BA intervention group reduced the expression of PPAR-γ ($P < 0.001$), FAS ($P < 0.001$), and TNF-α ($P < 0.001$), respectively; the BA+LP intervention decreased PPAR-γ ($P < 0.01$) , FAS ($P < 0.001$), TNF-α ($P < 0.001$), and MCP ($P < 0.01$) expression; and the h-kBA+LP group showed reductions in PPAR-γ ($P < 0.001$), FAS ($P < 0.001$), TNF-α ($P < 0.001$), and MCP ($P < 0.001$) *expression*. Specifically, comparing with the ND group, the HFD group significantly down-regulated the fatty acid oxidation-related enzyme ACOX ($P < 0.01$), while LP treatment and BA+LP treatment restored its expression, with $P < 0.001$ and $P < 0.05$, respectively.

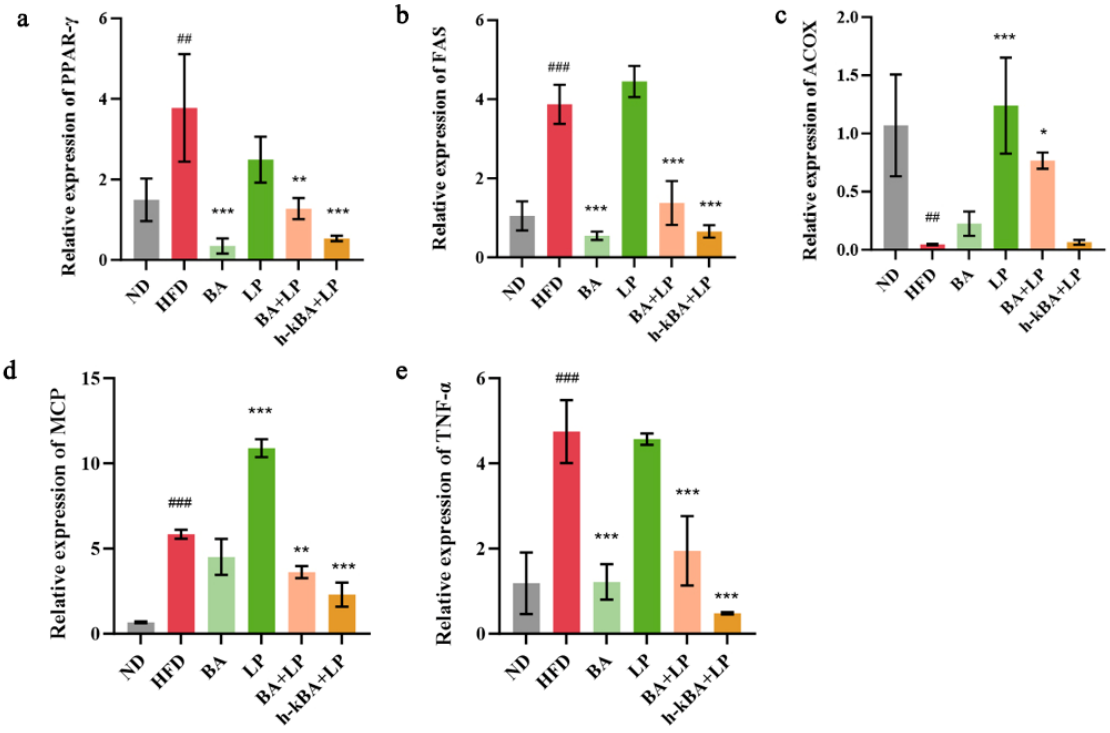
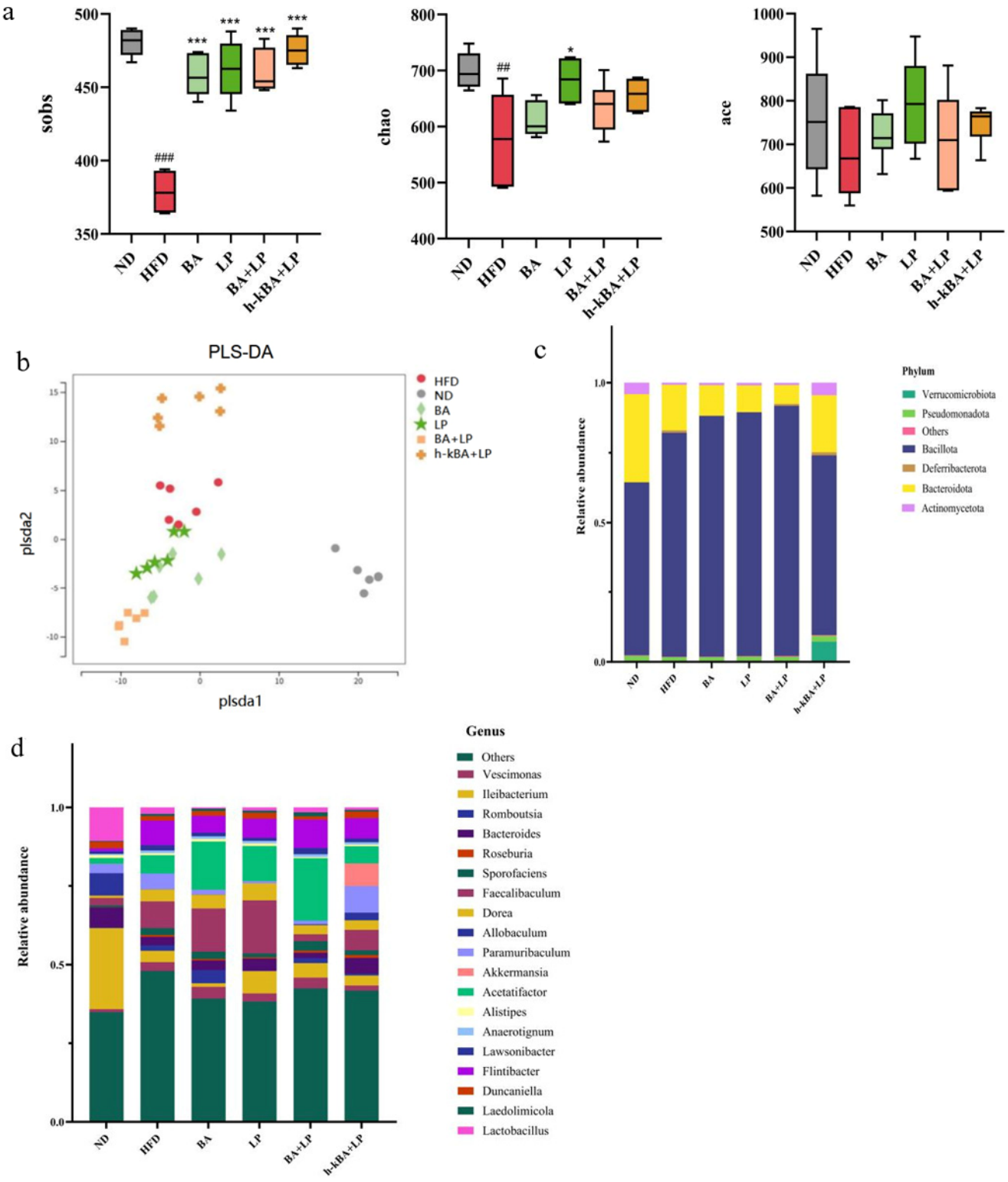


Figure 3. Effects of probiotic and postbiotic treatment on the expression of genes related to lipid metabolism and inflammation in liver tissue. Relative mRNA expression levels of (a) PPAR-γ (peroxisome proliferator-activated receptor gamma), (b) FAS (fatty acid synthase), (c) ACOX (acyl-CoA oxidase), (d) MCP (monocyte chemoattractant protein-1), and (e) TNF-α (tumor necrosis factor-alpha). Hash symbol(#) indicates significant differences between ND and HFD groups: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; Asterisk (*) indicates significant differences between HFD and probiotics treatment (BA, LP, BA+LP and h-kBA+LP) groups*: * P < 0.05, ** P < 0.01 and *** P < 0.001. ND*, low fat diet control with sterile water control; HFD, high fat diet control with sterile water control; BA, high fat diet with 2×10^9 CFU/day *Bifidobacterium adolescentis* XA-1069 in 200 μL PBS treatment; LP, high fat diet with 2×10^9 CFU/day *Lactobacillus plantarum* XA-6827 in 200 μL PBS treatment; BA+LP, high fat diet with 1:1 combination of viable strains (total 2×10^9 CFU/day) in 200 μL PBS treatment; h-kBA+LP, high fat diet with1:1 mixture of heat-inactivated strains (autoclaved at 121 °C for 20 min, equivalent CFU) in 200 μL PBS treatment.

3.3 Supplementation with probiotics improved gut microbiota dysbiosis

The composition of mice gut microbiota in the six treatments was determined via 16S rRNA sequencing. As shown in Figure 4a, the sobs index was significantly elevated in all probiotic-treated groups compared to the HFD group, indicating improved observed species richness. In contrast, both the chao and ace indices exhibited numerical increases; however, these changes did not reach statistical significance. Partial least squares discriminant analysis (PLS-DA) further confirmed distinct compositional clusters of gut microbiota among the ND, HFD, BA, LP, BA+LP, and h-kBA+LP groups (Figure 4b). Notably, the PLS-DA revealed statistically significant differences between the HFD group and each treatment group, with adjusted p-values as follows: HFD vs ND: 9.77×10^{-8}; HFD vs BA:8.53×10^{-8}; HFD vs LP: 2.81×10^{-5}; HFD vs BA+LP: 6.43×10^{-13}; and HFD vs h-kBA+LP: 2.17×10^{-10}. These findings further support that each intervention group induced a distinct shift in gut microbial structure compared to the HFD group.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Relative abundance
Phylum
Verrucomicrobiota
Pseudomonadota
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Bacillota
Deferribacterota
Bacteroidota
Actinomycetota
d
Relative abundance
Genus
Others
Vescimonas
Ileibacterium
Romboutsia
Bacteroides
Roseburia
Sporofaciens
Faecalibaculum
Dorea
Allobaculum
Paramuribaculum
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Lawsonibacter
Flintibacter
Duncaniella
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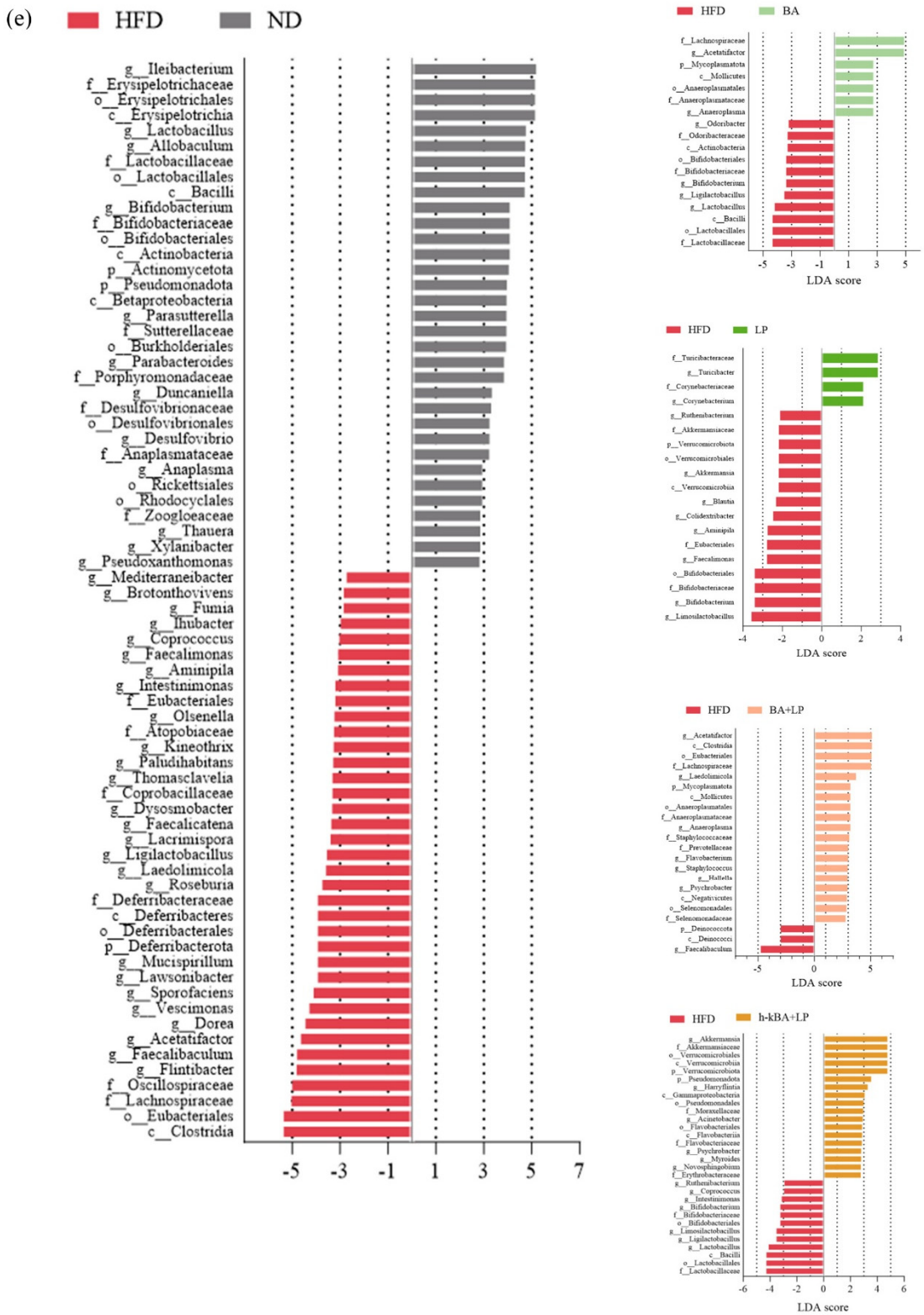

Figure 4. Effects of probiotic and postbiotic treatment on gut microbiota diversity and composition in HFD-fed mice. (a) Index of alpha diversity (Sobs, Chao, and Ace); (b) Partial least squares discriminant analysis (PLS-DA) score plot showing distinct clustering of microbial community structures between groups based on β-diversity; (c) Relative abundance of dominant bacterial phyla across groups; (d) Relative abundance of dominant bacterial genera across groups. (e) Linear discriminant analysis effect size (LEfSe) analysis; Hash symbol(#) indicates significant differences between ND and HFD groups: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; Asterisk (*) indicates significant differences between HFD and probiotics treatment (BA, LP, BA+LP and h-kBA+LP) groups: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. *ND*, low fat diet control with sterile water control; HFD, high fat diet control with sterile water control; BA, high fat diet with 2×10^9 CFU/day *Bifidobacterium adolescentis* XA-1069 in 200 μL PBS treatment; LP, high fat diet with 2×10^9 CFU/day *Lactobacillus plantarum* XA-6827 in 200 μL PBS treatment; BA+LP, high fat diet with 1:1 combination of viable strains (total 2×10^9 CFU/day) in 200 μL PBS treatment; h-kBA+LP, high fat diet with1:1 mixture of heat-inactivated strains (autoclaved at 121 °C for 20 min, equivalent CFU) in 200 μL PBS treatment.

Subsequently, a composition analysis was conducted to determine the relative abundance of gut microbiota at phylum level in each group. As depicted in Figure 4c, Bacillota and Bacteroidota were the dominant phyla. Compared with the ND group, the abundance of Actinomycetota was reduced in the HFD group, but was restored by the treatment of h-kBA+LP. Additionally, the abundance of Verrucomicrobiota was significantly increased in the h-kBA+LP group. At the genus level (Figure 4d), compared with the HFD group, the combined treatment BA+LP upregulated the proportions of *Flintibacter* and *Acetatifactor*. In contrast, the heat-killed bacterial mixture (h-kBA+LP group) increased the relative abundance of *Paramuribaculum*. Notably, the relative abundance of *Akkermansia* (which belongs to Verrucomicrobiota) was significantly elevated in the h-kBA+LP group.

Linear discriminant analysis effect size (LEfSe) results revealed distinct microbial composition alterations among different groups (Figure 4e). Compared with the ND group, HFD feeding significantly enriched *Dorea*, *Faecalibaculum*, *Lawsonibacter*, and *Vescimonas*, while reducing *Allobaculum* abundance. Both BA+LP and h-kBA+LP treatments effectively attenuated HFD-induced enrichment of *Dorea* and *Faecalibaculum*. Notably, the h-kBA+LP group demonstrated superior regulatory effects, showing significant suppression of *Lawsonibacter* and *Vescimonas* proliferation compared to other intervention groups. Furthermore, h-kBA+LP administration uniquely promoted *Akkermansia* and *Allobaculum* colonization, exhibiting a 2.3-fold increase relative to HFD controls.

3.4 Supplementation with probiotics altered fecal metabolites in HFD Mice

Given the significant alterations in certain gut microbial taxa observed by 16S rRNA sequencing following probiotic supplementation, the differences in fecal metabolites using non-targeted liquid chromatography-mass spectrometry (LC-MS) were further analyzed. Differential metabolites were identified using established thresholds: variable importance in projection (VIP) ≥1, fold change ≥1.2 or ≤0.83, and $P < 0.05$. Comparative analysis revealed marked metabolic profile alterations between groups. The HFD group exhibited 500 upregulated and 525 downregulated metabolites versus ND controls (total 1,025 differential features). Intervention groups showed distinct regulatory patterns: BA+LP administration modulated 429 metabolites (225 upregulated, 204 downregulated) compared to HFD, while h-kBA+LP treatment adjusted 374 metabolites (172 upregulated, 202 downregulated).

Targeted analysis identified significant nutrient-metabolic shifts. HFD feeding significantly decreased circulating levels of D-(+)-mannose, 5-hydroxyindole-3-acetic acid, calcitriol, and cholecalciferol compared to ND controls, while accumulating deoxycholic acid and palmitic acid (Figure 5a). BA+LP intervention partially reversed these alterations, restoring cholecalciferol ND levels and reducing deoxycholic acid. This group also showed unique upregulation of palmitoleic acid and docosapentaenoic acid versus HFD (Figure 5b). Notably, h-kBA+LP treatment demonstrated complementary effects, normalizing calcitriol and 5-hydroxyindole-3-acetic acid while similarly attenuating deoxycholic acid accumulation (Figure 5c).

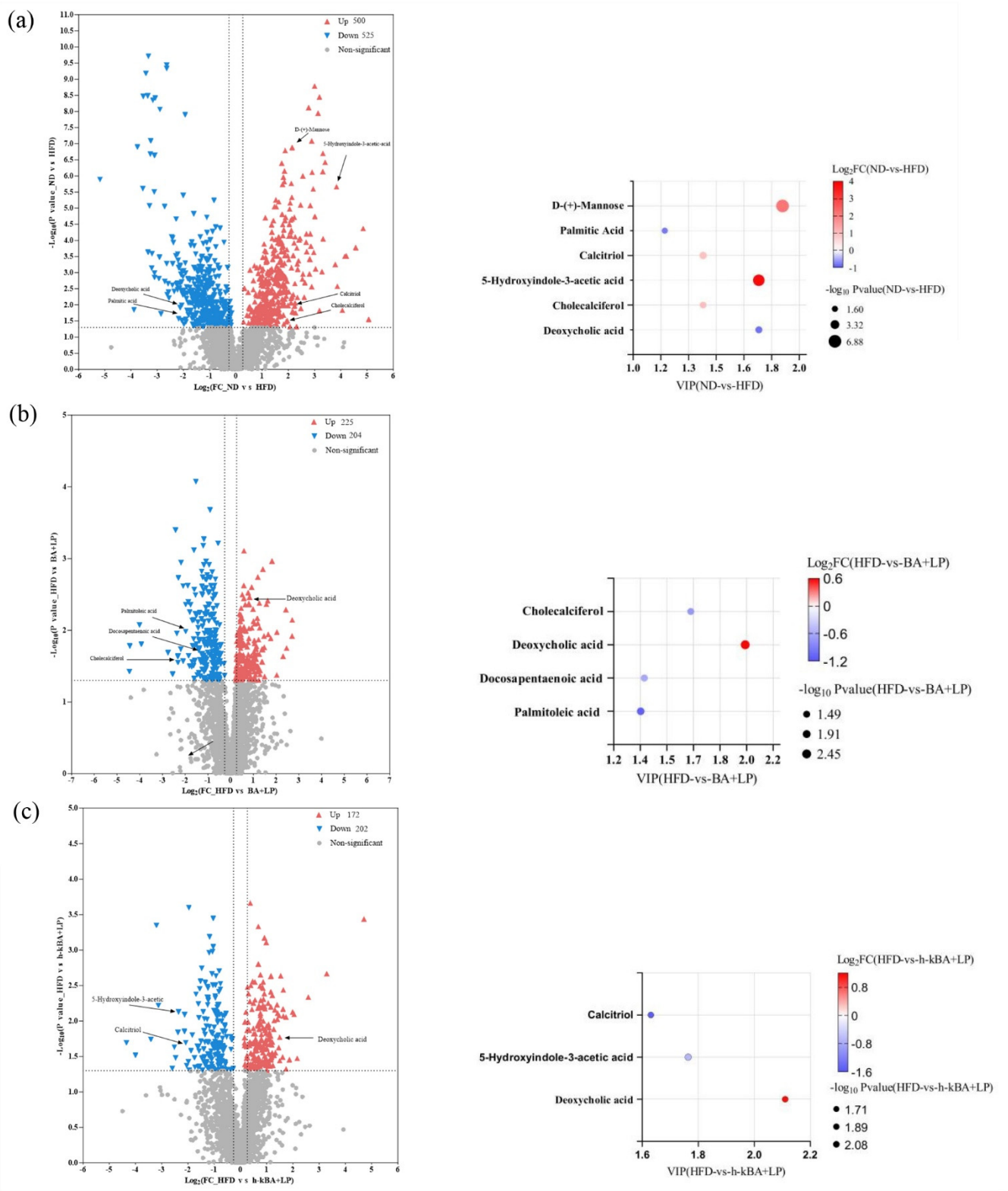

Figure 5. Differential fecal metabolites associated with probiotic and postbiotic intervention in HFD-fed mice. (a) Volcano plot and variable importance in projection (VIP) bubble plot showing differential metabolites between the ND and HFD groups; (b) Volcano plot and VIP plot showing differential metabolites between the HFD and BA+LP groups; (c) Volcano plot and VIP plot showing differential metabolites between the HFD and h-kBA+LP groups. Red and blue dots in the volcano plots represent significantly upregulated and downregulated metabolites, respectively ($P < 0.05$, fold change >1.5). Larger bubble size in the VIP plots indicates higher -log$_{10}$(p) values, and bubble color represents log$_2$(fold change).

3.5 Correlation analysis between gut microbiota, fecal differential metabolites, and host phenotypes

Pearson correlation analysis was conducted to explore the relationships between gut microbiota, metabolites, and host phenotypes. As shown in Figure 6(a), at the genus level, *Dorea*, *Faecalibaculum*,

Lawsonibacter, and *Vescimonas* were positively correlated with body weight, eWAT, TG, TC, LDL-C, and pro-inflammatory mediators (MCP and TNF-α), but negatively correlated with HDL-C and PPAR-γ. Although the underlying mechanisms remain unclear, similar associations have been observed under high-fat diet conditions, suggesting a possible link that warrants further investigation. In contrast, the abundance of *Akkermansia* and *Allobaculum* was negatively correlated with body weight, eWAT, TG, TC, LDL-C, and inflammatory markers (TNF-α), as well as obesity-related genes (PPAR-γ and FAS), while positively correlated with HDL-C and gene expression.

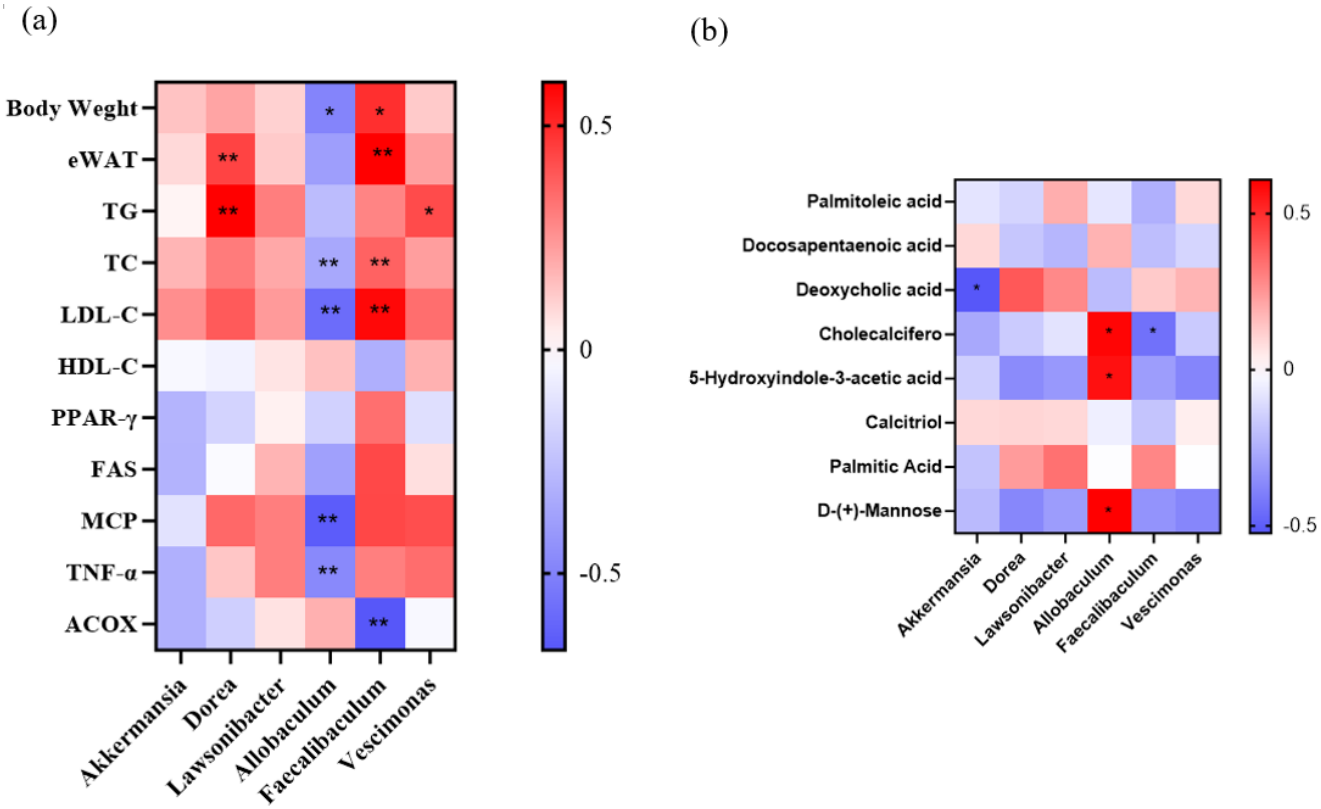

Figure 6. Correlation analysis between gut microbiota, metabolic indicators, and key metabolites. Pearson correlation heatmap showing associations between dominant bacterial genera and physiological or biochemical parameters, including body weight, epididymal white adipose tissue (eWAT), serum lipid levels (TG, TC, LDL-C, HDL-C), and gene expression markers *(*PPAR-γ, FAS, MCP TNF-α, and ACOX). (b) Pearson correlation heatmap showing relationships between key bacterial genera and representative fecal metabolites modulated by probiotic or postbiotic intervention. Color intensity reflects the strength and direction of the correlation (red = positive, blue = negative). Hash symbol(#) indicates significant differences between ND and HFD groups: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$; Asterisk (*) indicates significant differences between HFD and probiotics treatment (BA, LP, BA+LP and h-kBA+LP) groups*: * P < 0.05, ** P < 0.01 and *** P < 0.001. ND*, low fat diet control with sterile water control; HFD, high fat diet control with sterile water control; BA, high fat diet with 2×10^9 CFU/day *Bifidobacterium adolescentis* XA-1069 in 200 μL PBS treatment; LP, high fat diet with 2×10^9 CFU/day *Lactobacillus plantarum* XA-6827 in 200 μL PBS treatment; BA+LP, high fat diet with 1:1 combination of viable strains (total 2×10^9 CFU/day) in 200 μL PBS treatment; h-kBA+LP, high fat diet with1:1 mixture of heat-inactivated strains (autoclaved at 121 ℃ for 20 min, equivalent CFU) in 200 μL PBS treatment.

Furthermore, diet-induced metabolic alterations and probiotic interventions exhibited microbiota-dependent modulation of fecal metabolites. According to Figure 6(b), deoxycholic acid concentrations negatively correlated with *Akkermansia* and *Allobaculum* abundances. *Allobaculum* further displayed positive associations with cholecalciferol, 5-hydroxyindole-3-acetic acid, docosapentaenoic acid, and D-(+)-mannose. Contrastingly, *Dorea*, *Faecalibaculum*, *Lawsonibacter*, and *Vescimonas* showed inverse metabolic signatures: negative correlations with beneficial metabolites (docosapentaenoic acid,

D-(+)-mannose, cholecalciferol, 5-hydroxyindole-3-acetic acid) and positive associations with deoxycholic acid and palmitic acid. These dichotomous association profiles suggest divergent metabolic roles: *Dorea* and *Faecalibaculum* may potentiate metabolic dysregulation through pro-inflammatory and lipogenic pathways, whereas *Akkermansia* and *Allobaculum* likely confer metabolic protection via lipid-remodeling mechanisms and anti-inflammation.

4. Discussion

The present study investigated the anti-obesity effects of *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827, administered both as live strains and heat-inactivated strains (postbiotics), in high-fat diet (HFD)-induced obese mice. The results demonstrated that the combined administration of live strains (BA+LP) significantly reduced body weight gain, epididymal white adipose tissue (eWAT) mass, and serum lipid levels compared to the HFD group. Interestingly, although the BA+LP intervention group exhibited a plateau in body weight from week 6 to week 10, a slight rebound in weight gain was observed after week 11. This phenomenon may be attributed to adaptive metabolic responses that diminish the efficacy of probiotics over time. Long-term dietary interventions, even when beneficial, can lead to homeostatic compensation mechanisms such as increased energy harvest efficiency or microbial resilience[22]. Additionally, it is possible that the probiotic strains used in this study reached a colonization threshold, beyond which no further beneficial modulation occurred. Environmental factors, age-related metabolic slowdown, or variability in host–microbiota interactions over time could also contribute to this observation. Similar patterns have been reported in previous long-term probiotic intervention studies, where initial benefits plateau or decline after extended durations. Additionally, the heat-inactivated combination (h-kBA+LP) also showed moderate but statistically significant effects on body weight and eWAT reduction. Furthermore, both interventions modulated gut microbiota composition and fecal metabolites, with distinct regulatory patterns observed between live and heat-inactivated strains. Although *Lactobacillus plantarum* XA-6827 alone did not elicit statistically significant improvements in key metabolic and phenotype compared to the HFD group, its inclusion in this study was based on several considerations. First, *L. plantarum* is widely recognized as a probiotic species exhibiting acid and bile tolerance, intestinal adhesion ability, and anti-inflammatory properties across various strains[23, 24]. Second, previous studies have reported strain-specific benefits of *L. plantarum* in metabolic disorders[25], suggesting its possible efficacy in combination formulations. Indeed, in our results, XA-6827 demonstrated a synergistic effect when administered with *Bifidobacterium adolescentis* XA-1069, leading to superior anti-obesity outcomes. These findings underscore the importance of strain compatibility and combinatorial application in probiotic interventions.

Bifidobacterium adolescentis has garnered considerable attention for its reproducible anti-obesity activity. Multiple groups have shown that separate strains of *B. adolescentis* markedly attenuate HFD-induced weight gain, visceral fat expansion, and nuclear factor κB (NFκB) activation while improving insulin sensitivity in rodents[11, 26, 27]. Parallel work with *Lactobacillus plantarum* (e.g., strains K10, ATCC 14917 and KC28) likewise demonstrated significant reductions in adiposity, serum lipids, and glucose intolerance in

obese mice[13, 28]. Building on these single-strain observations, Landmann et al.[29] reported that a three-strain blend containing *Bifidobacterium lactis Lafti* B94, *Lactobacillus plantarum* HA-119 and *Lactobacillus helveticus Lafti* L10, when administered in combination, lowered body-weight gain, improved insulin resistance, and prevented hepatic steatosis and oxidative stress after 7 weeks of HFD feeding, highlighting the multi-target advantages of combination therapy.

Many of those studies reported significant benefits on weight and metabolic parameters, even though the bacteria were no longer alive[30]. For example, heat-killed *Lactobacillus spp.* in general have demonstrated anti-adiposity activity, ameliorating HFD-induced obesity by modulating gut microbiota and host signaling pathways involving key enzymes such as AMPK, NF-κB, and SIRT1, which are involved in pathways regulating energy metabolism, inflammation, and cellular stress responses[31, 32]. The transcriptional levels of the factors such as PPAR-γ, FAS, TNF-α, and MCP were analyzed, as they are closely linked to lipid metabolism and inflammatory responses. Although AMPK, NF-κB, and SIRT1 were not directly tested, these factors are known to play roles in relative metabolic and inflammatory pathways. Specifically, PPAR-γ is a critical transcription factor that regulates adipogenesis and fat metabolism, and its activity is modulated by AMPK and SIRT1. AMPK has been found to indirectly suppress PPAR-γ activity by enhancing SIRT1's deacetylase function, thus affecting fat storage and energy metabolism[33, 34]. Moreover, FAS, a key enzyme involved in apoptosis and inflammatory responses, has been shown to regulate lipid metabolism by modulating adipogenesis and fat accumulation in adipocytes[35]. TNF-α, a pro-inflammatory cytokine, activates NF-κB by promoting IκBα phosphorylation and degradation, allowing NF-κB to translocate into the nucleus and upregulate downstream targets[35]. This process enhances the expression of lipogenic and inflammatory mediators such as FAS and MCP, which are involved in adipogenesis and immune cell recruitment, respectively[36]. These genes are likely linked to the activation of AMPK, NF-κB, and SIRT1, providing a clearer understanding of the broader metabolic and inflammatory regulatory networks involved.

The consistent findings across studies highlight the robustness of *B. adolescentis* and *L. plantarum* as complementary anti-obesity agents. These results also reinforce the concept that multi-strain probiotic interventions—and, notably, their postbiotic forms—may exert broad metabolic benefits through shared or converging mechanisms, such as modulation of inflammation, lipid metabolism, and gut microbiota composition.

The BA+LP treatment appears to reverse several obesogenic microbiome and metabolite alterations. Notably, the combined probiotic group showed marked suppression of the genera *Dorea* and *Faecalibaculum*. These taxa are well-documented as obesity-associated microbes: for example, *Dorea* species (e.g. *D. longicatena*, *D. formicigenerans*) are enriched in overweight/obese and NASH patients and correlate positively with body weight and BMI[37, 38]. Similarly, *Faecalibaculum* is expanded by high-fat diet feeding and has been linked to diet-induced gut inflammation and metabolic disorders[39, 40]. Overgrowth of these genera has been associated with intestinal barrier dysfunction and pro-inflammatory signaling, so their downregulation by BA+LP is consistent with the improved metabolic and inflammatory phenotypes observed.

In parallel, fecal deoxycholic acid (DCA)- a cytotoxic secondary bile acid that rises on high-fat diets was significantly reduced in BA+LP mice. Elevated DCA is known to damage the intestinal mucosa, consequently induce inflammation[41, 42]. Mechanistically, excess DCA disrupts epithelial tight junctions through reducing ZO-1 and occludin, activates the NLRP3 inflammasome and pro-IL-1β signaling, and promotes pathogenic Th17 differentiation[41]. Thus, the BA+LP treatment declined DCA may potentially alleviate bile-acid-mediated intestinal injury and metabolic inflammation.

Conversely, several beneficial metabolites were enriched in BA+LP group mice. Cholecalciferol (vitamin D_3) levels rose; vitamin D is known to modulate lipid homeostasis and immune function, with the active form calcitriol suppressing TNF-α, IL-1β, IL-6 and other proinflammatory cytokines[43, 44]. Docosapentaenoic acid (DPA, 22:5n-3) - an ω-3 fatty acid-also increased; DPA has potent anti-inflammatory effects, strongly inhibiting pro-inflammatory eicosanoids (PGE_2, LTB_4) and reducing TNF-α and IL-1β while elevating IL-10 in colitis models[45]. Palmitoleic acid (C16:1n7), an omega-7 MUFA, was similarly upregulated; this lipid has been reported to improve adipose tissue function and attenuate obesity-induced inflammation (e.g. reducing adipocyte hypertrophy and stromal cell cytokine release)[46]. Collectively, the increases in cholecalciferol, DPA, and palmitoleic acid align with the observed enhancements in lipid metabolism and reduced inflammatory markers in BA+LP mice. These results illustrate a multi-targeted action of the BA+LP combination on the gut–microbiome–metabolome axis. This synergism likely underlies the superior improvements in weight control, lipid profiles and inflammatory status seen in the BA+LP group, as multiple pathways (microbiota composition, bile acid balance, host metabolites) are simultaneously normalized.

In our study, an intriguing finding was the selective enrichment of *Akkermansia* in the heat-inactivated probiotic group. *Akkermansia* is a mucin-degrading genus often exhibiting negative association with obesity and metabolic dysfunction[47]. The notable increase in *Akkermansia* abundance observed in the heat-inactivated (postbiotic) treatment group suggests that a distinct, non-colonization-based mechanism may be involved. One plausible explanation is that structural components of the inactivated *B. adolescentis* and *L. plantarum* stimulated host pattern recognition receptors (PRRs), thereby enhancing mucosal immune responses without the need for bacterial colonization. This immunological activation could promote mucin secretion and strengthen the intestinal barrier, ultimately creating a favorable ecological niche for mucin-degrading bacteria such as *Akkermansia*[47, 48]. The rise of *Akkermansia* in our heat-killed group coincided with distinct shifts in host metabolites, hinting at a linked interplay. Specifically, the heat-killed treatment led to metabolic changes including upregulation of calcitriol (the active form of vitamin D) and 5-hydroxyindole-3-acetic acid (5-HIAA), alongside downregulation of DCA. The enrichment of *Akkermansia* may both reflect and contribute to these metabolic changes. Improved calcitriol, the hormonally active form of vitamin D derived from cholecalciferol, plays an essential role not only in calcium homeostasis but also in direct immunomodulation. It suppresses pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, thereby reducing systemic inflammation and enhancing insulin sensitivity[49, 50]. Unlike cholecalciferol,

calcitriol exerts more immediate regulatory effects on immune cells and gut mucosal responses, which may contribute to shaping the gut microbiota and promoting the proliferation of beneficial microbes such as *Akkermansia*.

Meanwhile, the elevated levels of 5-hydroxyindole-3-acetic acid (5-HIAA) in h-kBA+LP group, a major metabolite of tryptophan catabolism, suggest activation of the aryl hydrocarbon receptor (AhR) pathway, known to promote regulatory T-cell differentiation and IL-10 expression at the transcription level, thus maintaining immune tolerance in the gut[51]. This mechanism may contribute to the anti-inflammatory milieu fostered by heat-inactivated probiotic treatment. As described above, DCA level enhances with HFD treatment, which induces gut inflammation. In our study, the heat-inactivated probiotic (h-kBA+LP) group exhibited a significant reduction in fecal deoxycholic acid (DCA) levels, accompanied by a notable increase in *Akkermansia* abundance. Although our findings are observational and do not establish causality, Wu et al. reported that supplementation with *A. muciniphila* in HFD-fed mice significantly reduced levels of secondary bile acids, including DCA and lithocholic acid (LCA), in both the caecum and liver[52]. These consistent patterns raise the possibility of an inverse association between *Akkermansia* and DCA, potentially through modulation of bile acid metabolism.

Previous studies demonstrated that *Akkermansia muciniphila* modulates bile acid metabolism by suppressing the intestinal Farnesoid X Receptor-Fibroblast Growth Factor 15 (FXR-FGF15) signaling axis, thereby reshaping the bile acid pool and reducing levels of harmful secondary bile acids such as deoxycholic acid DCA and LCA[52]. The FXR-FGF15 axis represents an essential regulatory pathway in the orchestration of host metabolism, emerging as a promising therapeutic target in the management of obesity and metabolic-associated fatty liver disease[53]. Activation of the FXR-FGF15 axis suppresses hepatic bile acid production, thereby reducing toxic bile acid accumulation including DCA. Additionally, modulation of bile salt hydrolase activity by gut commensals may enhance bile acid deconjugation and detoxification, further lowering DCA levels and alleviating its suppressive effects on beneficial microbes[54]. Thus, the h-kBA+LP intervention likely reshapes the gut metabolic environment by promoting *Akkermansia* expansion, which in turn regulates bile acid homeostasis via the FXR-FGF15 pathway and bile salt hydrolase-mediated detoxification. This integrated modulation may underlie the observed improvements in intestinal barrier integrity, reduced inflammation, and metabolic outcomes in treated mice.

The findings of this study align well with the current conceptual framework of the gut microbiota-host metabolic axis, which posits that the gut microbiome modulates host energy metabolism and immune homeostasis through complex metabolite signaling and barrier integrity regulation[55, 56]. Specifically, the enrichment of *A. Muciniphila*, the concomitant changes in vitamin D metabolites, and bile acid profiles observed in heat-inactivated probiotic group fit within the paradigm that microbiota-derived metabolites and host receptors such as the FXR act synergistically to regulate metabolic inflammation and lipid homeostasis[57, 58]. These findings were further supported by fecal metabolomic analysis. Notably, the reduction of deoxycholic acid (DCA), a cytotoxic secondary bile acid, may alleviate lipid accumulation and metabolic

inflammation by preserving intestinal epithelial integrity—mechanisms commonly impaired in obesity. Meanwhile, elevated levels of tryptophan-derived metabolites such as 5-hydroxyindole-3-acetic acid (5-HIAA) have been associated with anti-inflammatory signaling and gut–brain axis modulation, potentially contributing to appetite regulation and metabolic homeostasis. Increases in omega-3 polyunsaturated fatty acids, including docosapentaenoic acid (DPA), further support enhanced lipid oxidation and reduced adipose tissue inflammation. These results highlight the importance of microbial-derived metabolites as downstream effectors in mediating the anti-obesity benefits of both viable and heat-inactivated probiotic formulations.

Taken together, these results underscore the complexity of host-microbe interactions and highlight that probiotic efficacy cannot be evaluated solely based on viability or colonization potential. Although both live and heat-inactivated formulations of *Bifidobacterium adolescentis* XA-1069 and *Lactobacillus plantarum* XA-6827 attenuated HFD-induced metabolic dysfunction, their effector profiles were notably divergent. Live cells (BA+LP) achieved superior body weight and lipid-lowering effects. These changes coincided with a sustained increase in *Flintibacter* and *Acetatifactor*, along with elevated fecal levels of palmitoleic acid and docosapentaenoic acid compared to the HFD group. In contrast, the h-kBA+LP group did not show statistically significant improvements in phenotypic indicators such as body weight gain or serum lipid profiles, it elicited substantial regulatory effects at the molecular and microbial levels. These included pronounced suppression of lipogenic and inflammatory gene expression (PPAR-γ, FAS, MCP, TNF-α) and notable modulation of gut microbiota composition and metabolite signatures. This divergence may stem from the indirect and delayed mode of action of postbiotics, which, unlike live probiotics, do not dynamically colonize the gut or interact continuously with host tissues. Instead, their bioactive components, such as cell wall fragments or secreted metabolites, act in a paracrine or systemic manner to modulate immune and metabolic responses. Similar phenomena have been observed in other studies, where heat-inactivated strains or purified postbiotics improve inflammatory markers and microbial profiles without inducing overt phenotypic changes[59]. Moreover, it is possible that longer intervention durations or higher dosages of postbiotics are required to achieve measurable systemic outcomes such as weight loss or serum lipid reduction. Future research may focus on optimizing dosing strategies and identifying the most bioactive components within the postbiotic formulation. Collectively, these findings support a dual-pathway model: live probiotics primarily operate through colonisation-driven metabolite modulation, whereas postbiotics rely on cell-surface pathogen-associated molecular patterns (e.g., lipoteichoic acid, peptidoglycan) to reinforce barrier integrity and shape community composition via host signalling. Consequently, alternating or combining viable and inactivated formulations may represent a rational next-generation strategy to harness both metabolic and immune arms of the gut microbiota–host axis against obesity.

Despite the promising findings, this study has several limitations that warrant acknowledgment. First, although the modulation of gut microbiota and metabolite profiles was clearly demonstrated, direct mechanistic evidence linking specific microbial metabolites to host metabolic signaling pathways—such as FXR activation or immune cell modulation—remains to be established. Second, while we observed significant

downregulation of adipogenic markers such as PPAR-γ, key upstream regulators like AMPK and SIRT1 were not examined. This limits our ability to fully delineate the signaling cascades involved. Future studies should aim to validate the AMPK–SIRT1–PPAR-γ axis in the context of probiotic-mediated metabolic regulation. Third, although short-chain fatty acids (SCFAs)—such as acetate, propionate, and butyrate—are well recognized for their roles in energy homeostasis and immunomodulation, they were not quantified in the present study due to limitations of the untargeted metabolomics platform. Subsequent research using targeted SCFA detection methods (e.g., GC–MS) will be essential to clarify their contribution. Additionally, the sample size used in this study was relatively small, which may limit the statistical power and generalizability of the findings. Some functional assays, such as measurements of intestinal barrier integrity proteins and host bile acid receptor expression, were also not performed. These assessments could provide deeper insight into host–microbe interactions. Furthermore, although the survival of probiotics under simulated gastric conditions was tested, we did not evaluate bacterial viability under simulated intestinal digestion conditions. This remains an important aspect for comprehensively assessing microsphere functionality during gastrointestinal transit and should be addressed in future work. Lastly, the long-term effects and safety profile of heat-inactivated probiotics remain unclear and require further investigation prior to clinical application. Taken together, future studies integrating multi-omics approaches, expanded functional assays, and mechanistic validation experiments will be vital to advancing the understanding of probiotic and postbiotic actions in metabolic disease models.

In this study, microencapsulation of *Lactobacillus plantarum* effectively protected the probiotic cells from harsh gastric and intestinal environments, markedly improving their viability compared to free bacterial cells. This enhanced survival during gastrointestinal transit likely contributed to the superior metabolic improvements observed in the live probiotic groups, as higher numbers of viable bacteria can better colonize the gut and interact with the host. Therefore, microencapsulation represents a promising delivery strategy to overcome viability challenges in oral probiotic administration and may underlie the more pronounced anti-obesity effects seen with the viable dual-strain intervention.

5. Conclusion

This study demonstrated that combined administration of *B. adolescentis* XA-1069 and *L. plantarum* XA-6827 effectively ameliorates high-fat diet-induced obesity and metabolic disturbances in mice. Both live and heat-inactivated (postbiotic) formulations improved body weight control, adipose tissue accumulation, serum lipid profiles, and inflammatory markers, though through distinct mechanisms. The live probiotic combination exhibited stronger anti-obesity effects, likely due to the presence of viable bacteria capable of colonizing the gut and modulating microbial communities and host metabolism. Heat-inactivated strains also conferred metabolic benefits, potentially via immune modulation and alteration of key gut metabolites such as calcitriol, 5-hydroxyindole-3-acetic acid, and bile acids, accompanied by increased abundance of *Akkermansia muciniphila*. These findings underscore the therapeutic potential of both multi-strain probiotic and postbiotic interventions targeting the gut microbiota-metabolite-host axis in obesity management. Future

research should further explore the molecular mechanisms and long-term effects to support clinical translation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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