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Hawthorn leaf and its extract alleviate high-fat diet-induced obesity and modulate gut microbiome in mice

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ABSTRACT

Obesity has emerged as a global health issue with its prevalence continuously increasing and being associated with multiple comorbidities. Although existing medications are effective, they often come with significant side effects, making dietary therapy an advantageous alternative. Hawthorn leaves and their active component, vitexin, have shown potential in regulating lipid metabolism and improving gut microbiota imbalance. This study utilized a high-fat diet-induced obese mouse model, administering different doses of hawthorn leaves and vitexin for 13 weeks, and employed 16S rRNA sequencing and metabolomics to analyze the composition of gut microbiota and metabolites. The results demonstrated that hawthorn leaves and vitexin significantly slowed body weight gain, improved glucose tolerance, regulated blood lipid levels, and downregulated the expression of obesity-related gene in mice (*ppar-α, ppar-γ, fas*). Additionally, the treatment groups showed a significant improvement in gut microbiota diversity. Both vitexin and hawthorn leaves increased the abundance of *Kineothrix*, *Paramuribaculum*, *Lawsonibacter* (which belong to the Bacillota phylum) and *Olsenella* (Actinobacteria phylum), while reducing the abundance of *Anaerotignum* (Bacillota phylum). Moreover, the hawthorn leaves and vitexin treatments may alleviate obesity-related symptoms by increasing the fecal content of testosterone propionate, formoterol, and isoleucyl-prolyl-proline, and decreasing the content of Trolox. These findings highlight the potential of hawthorn leaves and vitexin as functional foods for obesity management by modulating gut microbiota pathways, offering a promising dietary therapy approach.

1. Introduction

In recent years, obesity has become a global health issue, with its prevalence on the rise worldwide (Haslam and James, 2005). Obesity is associated with multiple comorbidities, such as type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease. Its direct cause mainly stems from excessive fat accumulation, which is linked to gut microbiota imbalance and metabolic disorders (Zhao, 2013). Over the years, numerous drugs—including orlistat, dapagliflozin, sibutramine, and liraglutide—have been employed to manage obesity. However, these drugs generally come with notable side effects (Heck et al., 2000; Müller et al., 2022; Tak and Lee, 2021). In contrast, dietary therapy offers significant advantages as an alternative for alleviating obesity and related metabolic diseases.

Hawthorn (*Crataegus oxyacantha* L.), a thorny shrub of the Rosaceae family, holds potential in the management of obesity. Traditionally, it has been used for symptoms such as diarrhea, asthma, insomnia, angina pectoris, and hypertension. Research indicated that extracts from the fruits, leaves, and flowers of *C. pinnatifida* can help prevent heart failure and hypertension (Dehghani et al., 2019). Hawthorn leaves, for instance, contain large amounts of flavonoids and polysaccharides that play important roles in lipid regulation (Kuo et al., 2009). Studies suggest that the flavonoids in hawthorn leaves can reduce fat accumulation in the liver tissue of diabetic patients and lower blood lipid levels in

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individuals with hyperlipidemia (Kawser Hossain et al., 2016). In recent years, several animal experiments have confirmed that total flavonoids at doses of 200–450 mg/kg body weight (bw) can significantly reduce fasting blood glucose levels and improve insulin secretion in type 2 diabetic mice (Lü et al., 2009; Shu et al., 2009). Vitexin, a major bioactive flavonoid compound derived from hawthorn (about 6–8 mg/g of leaf), has been reported to possess diverse biological activities, including anti-cancer, anti-inflammatory, antioxidant, and antihypertensive effects (Dong et al., 2011). Vitexin extracted from bamboo leaves using methanol (MeOH) and the ethyl acetate (EtOAc) fraction, as well as from *Lemna minor*, inhibits adipogenesis and reduces the protein expression of the key adipogenic genes *c/ebpα* and *ppar-γ* (Yang et al., 2017). In addition, studies have shown that the intake of vitexin-rhamnoside can reduce dyslipidemia and fat accumulation in mice fed a high-fat diet mice (Kim et al., 2010).

Gut microbiota is a critical environmental factor for maintaining the body's metabolic balance (Brial et al., 2018). A healthy microbial ecosystem helps stabilize lipid metabolism by regulating hepatic cholesterol metabolism, promoting lipid oxidation in muscle, supporting energy storage in adipose tissue, and preserving the integrity of the intestinal barrier (He and You, 2020; Jia et al., 2021). Once a dysbiosis occurs, it can lead to the proliferation of potential pathogens and disrupt immune homeostasis, triggering the abnormal production of inflammatory cytokines and adipokines, which is closely associated with the development of chronic conditions such as hyperlipidemia, obesity, diabetes, and atherosclerosis (Ren et al., 2021; Stephens et al., 2018). Moreover, damage to the intestinal mucosal barrier caused by a high-fat diet exacerbates this imbalance. Studies show that long-term high-fat consumption not only diminishes the relative abundance of beneficial genera like *Lactobacillus* and *Oscillospira* but also increases the *Firmicutes/Bacteroidetes* ratio, interfering with the secretion of gut peptides related to satiety and resulting in increased food intake (Rautmann and De La Serre, 2021). Meanwhile, various plant polysaccharides can improve the gut microbial environment by enriching bacteria that produce short-chain fatty acids (SCFAs), thus alleviating liver damage and treating hyperlipidemia (Chen et al., 2024). However, the specific regulatory effects of hawthorn leaf flavonoids on the gut microbiota in diet-induced obese mouse models have yet to be fully elucidated.

Although existing research has demonstrated the potential benefits of hawthorn leaves and their extracts in lowering blood lipids and improving glucose-lipid metabolism, a comprehensive phenotypic evaluation and detailed mechanism of action in high-fat diet-induced obesity models remain unclear. In this study, we established a high-fat diet-induced mouse model of obesity and utilized high-throughput metabolomics and 16S rRNA sequencing to systematically elucidate the potential of hawthorn leaves and vitexin in maintaining gut health and improving obesity. This research provides important evidence supporting the development of hawthorn leaves and their extract, vitexin, as functional foods and functional factors to mitigate obesity.

2. Materials and methods

2.1. Experimental design and treatments

All animal experiments were approved by the animal ethics committee (IAC23W346). Six-week-old male C57BL/6J mice with an average weight of 20 ± 2 g were purchased from Zhuhai BestTest Bio-Tech Co, Ltd. (Zhuhai, Guangdong Province, China), and were housed at a constant temperature (25 ± 2 °C) and humidity (55 ± 5%) room with the 12h light-dark cycle.

After adaptive feeding for 7 days, all 60 mice were randomly divided into six groups (n = 10 in each group): the Control group, High fat diet (HFD) group, 100 mg/kg bw/day Hawthorn leaves treatment (HL100) group, 500 mg/kg bw/day Hawthorn leaves treatment (HL500) group, 6 mg/kg bw/day Vitexin treatment (Vitexin6) group and 30 mg/kg bw/day Vitexin treatment (Vitexin30) group. Hawthorn leaves (powders, purity≥98%) and vitexin (purity≥98%) were bought from Chengdu Pufei De Reference Substances Technology Co., Ltd. (Chengdu, Sichuan Province, China). The mice in Control group and HFD group were provided with low fat diet or high fat diet respectively, and others were provided with high fat diet mixed with Hawthorn or Vitexin for 13 weeks. All mice were allowed to take food freely, and body weights of them were recorded weekly (Fig. 1(a)).

2.2. Sample collection

At the 12th week, fecal samples of mice were collected and stored at -80 °C for further research. At week 13, the glucose tolerance test (GTT) was conducted 3 days before the sacrifice of mice. After fasting for 12h, mice were sacrificed by CO_2 asphyxiation, and blood samples, liver tissues, and epididymal white adipose tissues (eWAT) were collected for further analysis.

2.3. Glucose tolerance test

The mice were subjected to GTT according to Xiao et al. with modifications (Xiao et al., 2017). All mice were fasted for 6h, and then 2 mg/kg bw glucose was injected into the intraperitoneal area of the mice. Blood glucose levels were obtained from a tail vein in the mice, and determined at 0 min, 15 min, 60 min and 120 min. The area under the curve (AUC) was calculated and used to assess glucose tolerance in mice.

2.4. Plasma lipid measurements

The blood samples were centrifugated to acquire plasma and then stored at -80 °C. The triglyceride (TG) (Product No.: E-BC-K261-M), total cholesterol (TC) (Product No.: E-BC-K109-M), high-density lipoprotein cholesterol (HDL-C) (Product No.: E-BC-K221-M), and low-density lipoprotein cholesterol (LDL-C) (Product No.: E-BC-K205-S) colorimetric assay kits were from Elabscience Biotechnology Co., Ltd. (Wuhan, Hubei province, China). To determine the levels of TG, TC, HDL-C and LDL-C, the following methods were utilized: the glycerol phosphate oxidase-phenol aminophenazone peroxidase (GPO-PAP) method for TG, the cholesterol oxidase-phenol aminophenazone peroxidase (COD-PAP) method for TC, and direct selective enzymatic colorimetric methods for HDL-C and LDL-C.

2.5. Histological assessment

Fresh eWAT was fixed with a 4% paraformaldehyde solution and then embedded in paraffin (Zhou et al., 2023). Tissue sections (5 μm) were cut, stained with hematoxylin and eosin (H&E) stain, and observed under a light microscope (200-fold) and with an imaging system.

2.6. Quantitative Real-Time PCR analysis

Fresh liver tissues were stored at -80 °C until used. RNA extraction and quantification were performed using commercial assay kits (product No.: R1200-50t) from Solarbio Science & Technology Co., Ltd (Beijing, China). The procedure involved initial lysis of the samples with the provided lysis buffer, followed by separation of nucleic acids and proteins using chloroform. The RNA-containing aqueous phase was then purified through a spin column to remove impurities. After multiple washing steps, the purified RNA was eluted with RNase-free water. This method effectively yielded high-quality RNA suitable for downstream applications.

Then the isolated RNA was converted into cDNA using a cDNA synthesis kit (product No: A2801) from Promega Biotech Co., Ltd (Madison, USA). The primers of the obesity-related genes (*ppar-α*, *ppar-γ* and *fas*) (Table 1) were synthesized by Bio Basic Canada Inc. (Markham, Ontario, Canada), and the qPCR Master Mix was provided by Promega Biotech Co., Ltd (Madison, USA). Quantitative Real-Time PCR was

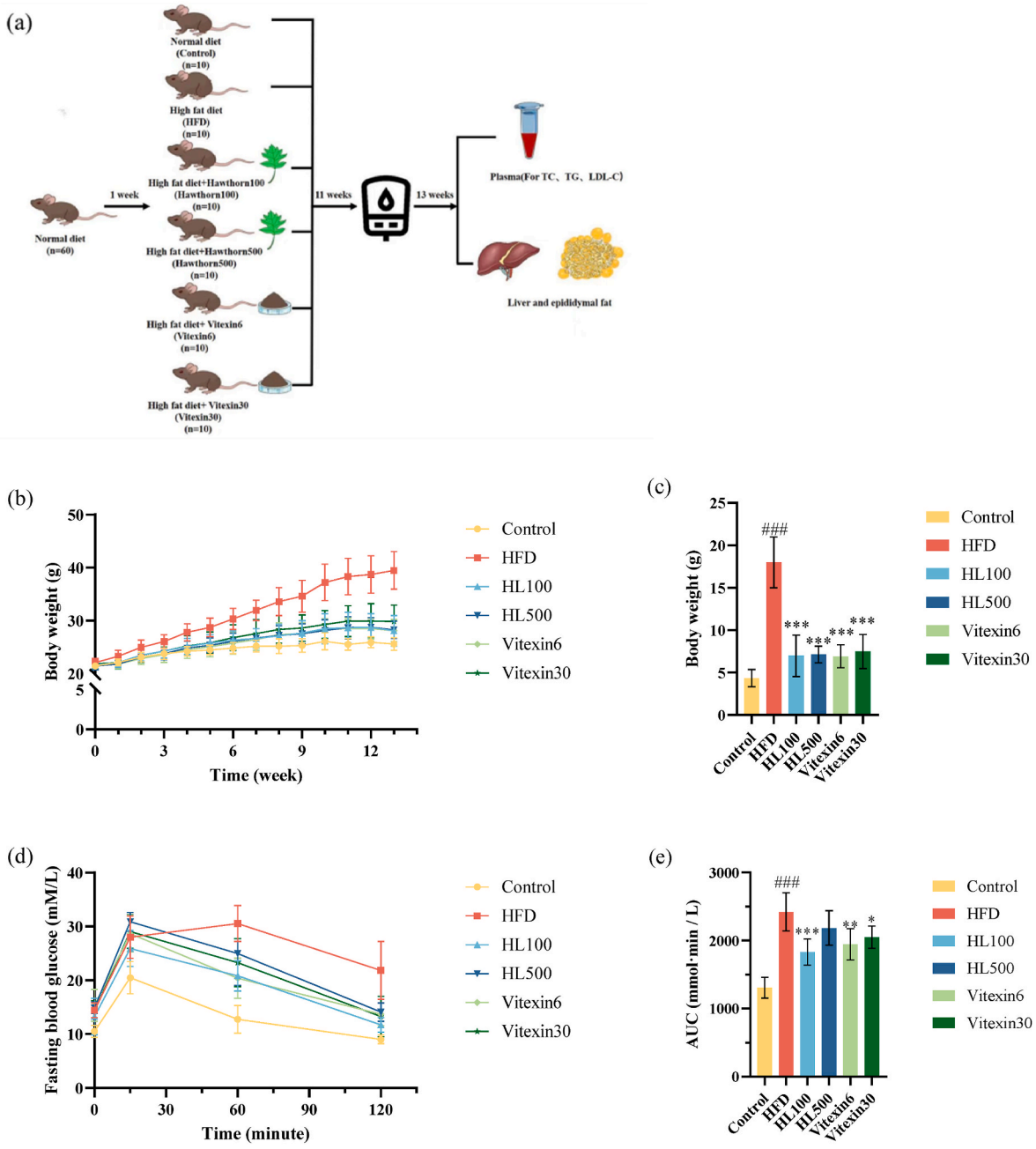


Fig. 1. Hawthorn leaves and vitexin treatments decreased body weights and glucose tolerance. (**a**) Schematic diagram of the study design: Mice were given Hawthorn leaves or vitexin mixed with high fat diet; (**b**) Changes in body weight during Hawthorn leaves and vitexin treatment over 13 weeks and (**c**) body weight gain at the 13th week; (**d**) Glucose tolerance test (GTT) and (**d**) area under the curve (AUC). Hash symbol ($^{\#}$) indicates significant differences between Control and HFD groups: $^{\#\#\#}p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

Table 1
Primer sequences and amplicon size of the differentially expressed genes *ppar-α*, *ppar-γ* and *fas* used in the qPCR reaction.

Gene symbol	Forward primer	Reverse primer
ppar-α	CCTCAGGGTACCACTACGGAGT	GCCGAATAGTTCGCCGAA
ppar-γ	GCTGTTATGGGTGAAACTCT	TGGCATCTCTGTGTCAACCA
fas	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
gapdh (Reference gene)	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

performed using StepOnePlus Real-Time PCR System from Thermo Fisher Scientific Inc. (Waltham, USA). The relative expressions of genes were studied and estimated on the relative $2^{-\Delta\Delta C_T}$ method (Raza et al., 2022).

2.7. Intestinal microbial sequencing and analysis

DNA was extracted from fecal samples using magnetic bead fecal and soil genome extraction kit from Magen Biotechnology Co. (Guangzhou, Guangdong province, China) according to manual instruction. The DNA concentration and purity were measured. PCR enrichment was performed in a 50 μL reaction containing 30 ng template and fusion PCR primers. The V3V4 variable region of 16S rDNA of bacteria was amplified by forward and reverse PCR degenerate primers F and R (338F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTWTCTAAT). PCR cycling conditions were 95 °C for 3 min; 30 cycles of 95 °C for 15 s, 56 °C for 15 s, 72 °C for 45 s and final extension at 72 °C for 5 min. PCR products were purified by DNA magnetic beads from Beijing Genomics Institute (BGI) (Shenzhen, Guangdong province, China). The validated libraries were used for sequencing on Illumina MiSeq/Hiseq platform (BGI, Shenzhen, China) following the standard pipelines of Illumina, and generating 2 × 250/300 bp paired-end reads. Raw data were filtered to generate high quality clean reads according to He et al. (He et al., 2013), and operational taxonomic units (OTUs) were clustered according to 97% sequence similarity (Magoč and Salzberg, 2011). Then OTU representative sequences were aligned against the database for taxonomic annotation by RDP classifier (v2.2) software (sequence identity is set to be 0.6). Alpha diversity and Beta diversity analysis were done by software mothur (v.1.31.2) and QIIME (v1.80) respectively.

2.8. Metabolomic methodology

Sample (25 mg) was thawed slowly at 4 °C, 800 μL of extraction solution (methanol: acetonitrile: water = 2:2:1, v:v:v, pre-cooled at -20 °C), 10 μL of the internal standard, and two small steel beads were placed in a tissue grinder and ground at 50 Hz for 5 min. The mixture was then sonicated for 10 min at 4 °C, and allowed to stand in the refrigerator for 1 h at -20 °C. After centrifugation at 4 °C and 25000 g for 15 min, 600 μL of the supernatant was extracted and 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 °C for 10 min. The mixture was centrifuged at 4 °C for 15 min at 25000 g, and the supernatant was transferred to a sample bottle (Lin et al., 2024). To ensure the reliability of the liquid chromatography-mass spectrometry (LC-MS) analysis, 50 μL of supernatant from each sample was meticulously combined with synthetic quality control (QC) samples to evaluate both repeatability and stability. Mass spectrometric analysis was conducted in both positive and negative ion modes. Metabolites with $P < 0.05$, fold change (FC) > 1.2 or < 0.83, and VIP ≥1 were considered as differentially abundant metabolite (DAMs).

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 9.5.1), and data for multiple variable comparisons were assessed via one-way analysis of variance (ANOVA). $P < 0.05$ was deemed statistically significant. 16S rRNA and metabolomics analyses were performed using R 4.3.0.

3. Results

3.1. Hawthorn leaves and vitexin treatments ameliorated obesity in adult male mice

As shown in Fig. 1(b) and (c), all hawthorn and vitexin treatments had reduced body weight gain compared to HFD-fed mice. The body weights of mice in the HL100, HL500, Vitexin6 and Vitexin30 groups were significantly lower than those in the HFD group by 28.87%, 28.46%, 28.81% and 24.44% respectively at the last week. The results of the GTT indicated the effects of hawthorn leaves and vitexin on glucose tolerance in obese mice (Fig. 1(d)). During the test, peak blood glucose levels were observed at 15 min in the Control, HL100, HL500, Vitexin6, and Vitexin30 groups, whereas the HFD group exhibited a delayed peak at 45 min (Table 2). Additionally, the AUC values in the HL100, Vitexin6, and Vitexin30 groups were significantly lower compared to the HFD group (Fig. 1(e)).

Additionally, the eWAT weight, organ index and cell area in the HL100, HL500, Vitexin6 and Vitexin30 groups were significantly lower than those in the HFD group (Fig. 2). Both levels of HDL-C and LDL-C in the HL100, HL500, Vitexin6 and Vitexin30 groups were reduced compared to the HFD group, though the difference was not statistically significant. The intervention groups showed reduced levels of TC and TG compared to HFD group, while no significant differences were observed between the Control and HFD group (Table 3).

Fig. 3 shows that HFD treatment significantly increased the expression of obesity-related genes *ppar-α*, *ppar-γ*, and *fas* compared with the Control group ($P < 0.05$). Notably, vitexin treatment (in the Vitexin6 and Vitexin30 groups) significantly reduced the expression levels of these three genes ($P < 0.05$). Specifically, the expression level of *ppar-α*, *ppar-γ*, and *fas* in Vitexin6 group were reduced by 97.91%, 87.90% and 93.50% than those in HFD group ($P < 0.05$) respectively; compared to the HFD group, in the Vitexin30 group, these reductions were 97.93%, 78.45% and 66.02%, respectively.

3.2. Hawthorn leaves and vitexin treatments modified the diversity and richness of the gut microbiota in adult male mice

Both hawthorn leaves and vitexin treatments modified gut microbiota composition (Fig. 4(a)). Diversity analysis of gut microbiota revealed that following vitexin treatment (especially at the dosage of 6 mg/kg bw vitexin), there was a notable increase in the Sobs, Chao and Ace index (Fig. 4 (b)). Partial Least Squares Discriminant Analysis (PLS-DA) further delineated distinct compositional clusters within the gut microbiota among Control, HFD, HL100, HL500, Vitexin6 and Vitexin30 groups (Fig. 4(c)). These findings underscore the importance of conducting additional analyses to elucidate changes in the intestinal flora. At the phylum level, following vitexin treatment, there was a marked increase in the proportion of Bacillota, accompanied by a significant decrease in the proportion of Bacteroidota, Cyanobacteriota and Fusobacteriota; following hawthorn leaves treatment, the proportions of Mycoplasmatota and Candidatus Saccharibacteria increased significantly, while those of Actinomycetota and Cyanobacteriota decreased notably (Fig. 4(d)). At the genus level, a notable increase in the proportion of *Kineothrix*, *Paramuribaculum*, *Olsenella* and *Lawsonibacter* within both vitexin and hawthorn leaves treatments was observed (Fig. 4(e)). Furthermore, an increase proportion of *Laedolimicola* and *Sporofaciens* was observed in the Vitexin6 and Vitexin30 groups, and an increase in *Anaerotignum* was noticed in HL100 and HL500 groups. Interestingly, the genus *Olsenella* is classified within the phylum Actinobacteria, while other mentioned genera belong to the phylum Bacillota. Linear discriminant analysis effect size (LEfSe) analysis demonstrated that *Acetatifactor* and *Eubacteriales* (which belong to the phylum Bacillota) were enriched in the Vitexin6 and Viteixn30 groups. Conversely, *Paramuribaculum* and *Salmonella* were enriched in the HL100 and HL500 groups (Fig. 4 (f)). Through Kyoto Encyclopedia of

Table 2
Effects of Hawthorn leaves and vitexin treatments on fasting blood glucose (FBG) ($N = 8$).

	0 min FBG (mmol/L)	15 min FBG (mmol/L)	60 min FBG (mmol/L)	120 min FBG (mmol/L)
Control	10.51 ± 1.01	20.50 ± 2.79	12.76 ± 2.40	9.00 ± 0.75
HFD	14.40 ± 1.28 ##	28.01 ± 3.66 ###	30.54 ± 3.10 ###	21.86 ± 4.98 ###
HL100	13.29 ± 3.12	25.90 ± 3.10	20.83 ± 2.61 ***	11.70 ± 1.26 ***
HL500	14.89 ± 0.98	30.89 ± 1.61	24.97 ± 5.52	14.07 ± 1.59 ***
Vitexin6	15.34 ± 2.74	28.67 ± 3.12	20.39 ± 3.45 ***	13.67 ± 1.96 ***
Vitexin30	14.33 ± 1.79	29.06 ± 2.89	23.29 ± 4.13 **	13.27 ± 3.48 ***

Note: Hash symbol (#) indicates significant differences between Control and HFD groups: $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: $^{**}p < 0.01$ and $^{***}p < 0.001$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

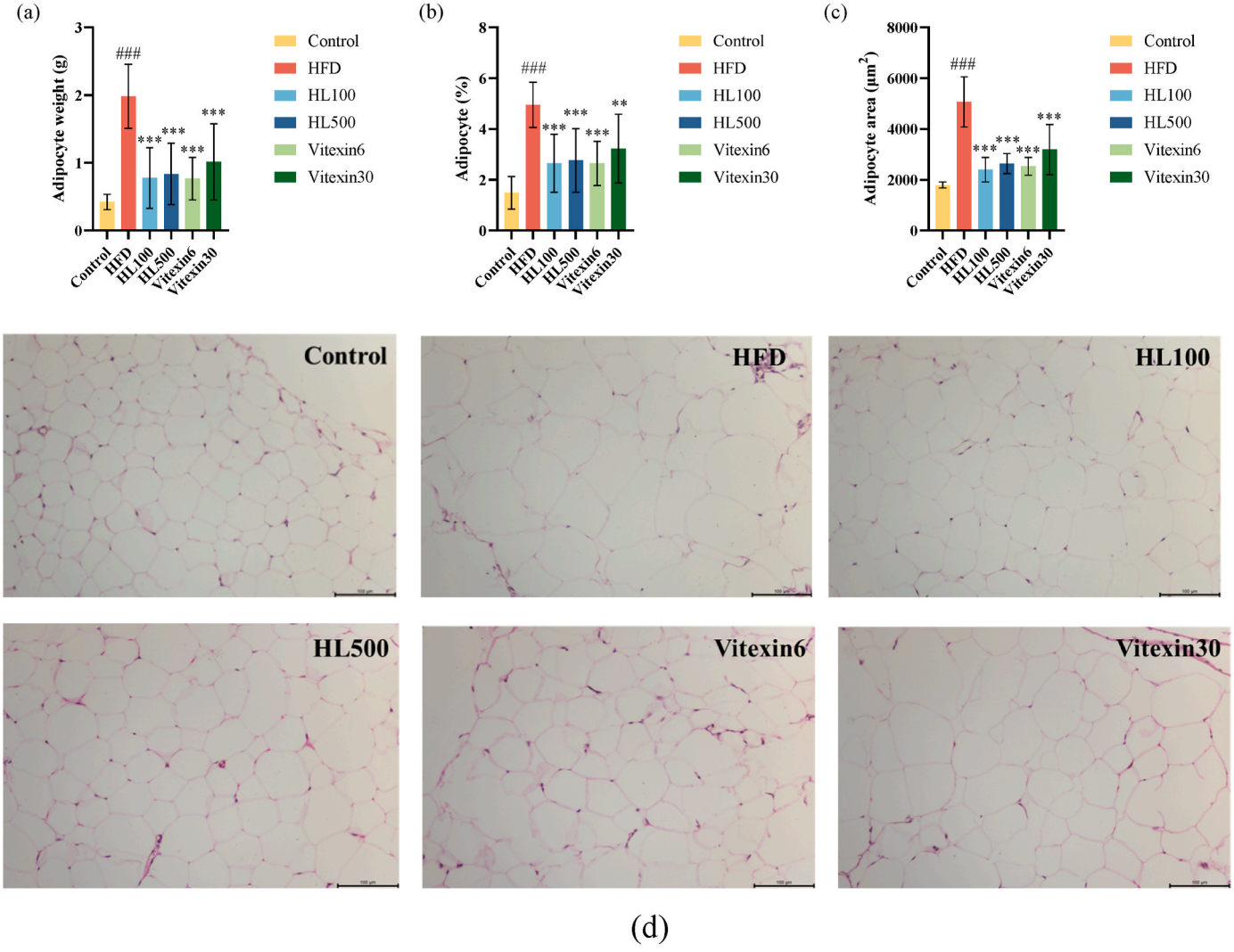


Fig. 2. Hawthorn leaves and vitexin treatments decreased adipocyte. (**a**) Adipocyte weight; (**b**) Adipocyte index; (**c**) Adipocyte area and (**d**) Representative histological sections of adipocyte (scale: 100 μm). Hash symbol (#) indicates significant differences between Control and HFD groups: $^{\#\#\#}p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: $^{**}p < 0.01$ and $^{***}p < 0.001$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

Genes and Genomes (KEGG) pathway enrichment analysis of 16S rRNA data, a total of 31 pathways with $P < 0.05$ were identified in the comparison between Control and HFD groups. Among these, the microbial communities in the HFD vs HL100 and HFD vs HL500 comparisons were significantly enriched in the Chlorocyclohexane and chlorobenzene degradation pathway. Additionally, the microbial communities in the HFD vs Vitexin6 and HFD vs Vitexin30 groups were significantly enriched in both the Chlorocyclohexane and chlorobenzene degradation and Bacterial secretion system pathways (Fig. 4(g)).

The Spearman correlation network analysis elucidated the complex

Table 3
Effects of Hawthorn leaves and vitexin treatments on blood lipids ($N = 8$).

	LDL-C (mmol/L)	HDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)
Control	0.74 ± 0.33	0.79 ± 0.38	4.23 ± 0.27	0.85 ± 0.18
HFD	2.05 ± 0.32 ###	1.86 ± 0.45 ###	4.21 ± 0.37	1.02 ± 0.33
HL100	1.43 ± 0.23	1.49 ± 0.20	4.00 ± 0.41	0.56 ± 0.25 **
HL500	1.77 ± 0.44	1.45 ± 0.37	3.46 ± 0.16 ***	0.96 ± 0.17
Vitexin6	1.85 ± 0.41	1.78 ± 0.34	3.15 ± 0.23 ***	0.94 ± 0.41
Vitexin30	2.02 ± 0.57	1.86 ± 0.40	3.28 ± 0.35 ***	0.71 ± 0.16

Note: Hash symbol (#) indicates significant differences between Control and HFD groups: ###$p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: **$p < 0.01$ and ***$p < 0.001$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

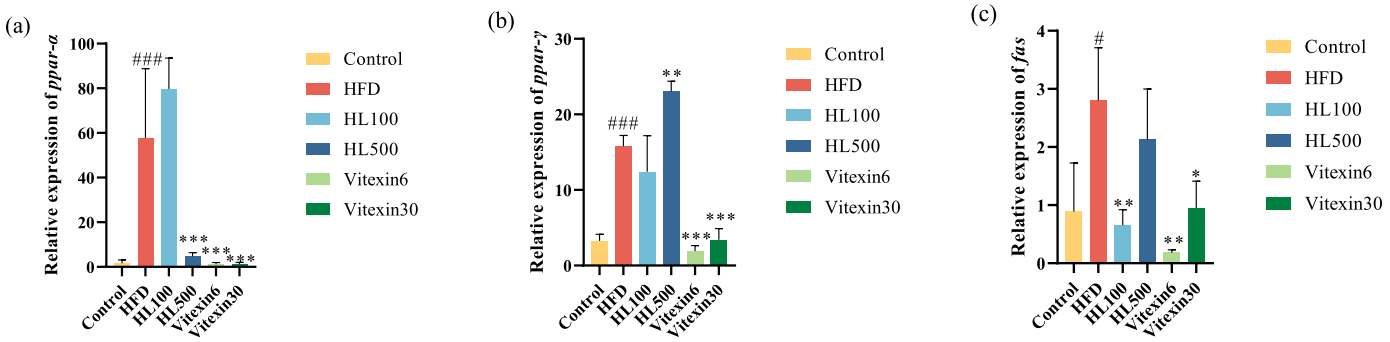


Fig. 3. Vitexin treatments decreased expression level of obesity-related genes (*ppar-α*, *ppar-γ* and *fas*). Expression levels of (**a**) *ppar-α*, (**b**) *ppar-γ* and (**c**) *fas*. Hash symbol (#) indicates significant differences between Control and HFD groups: #$p < 0.05$ and ###$p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

interplay between the gut microbiota and host physiological responses in the permethrin intervention. The genus *Anaerotignum* was significantly positive correlated with obesity biomarkers, including body weight, *ppar-α* and *ppar-γ*; In contrast, *Flintibacter* showed a strong negative correlation with TG, *ppar-α* and *ppar-γ* (Fig. 4(h)).

3.3. Hawthorn leaves and vitexin treatments modified the content and composition of fecal metabolites

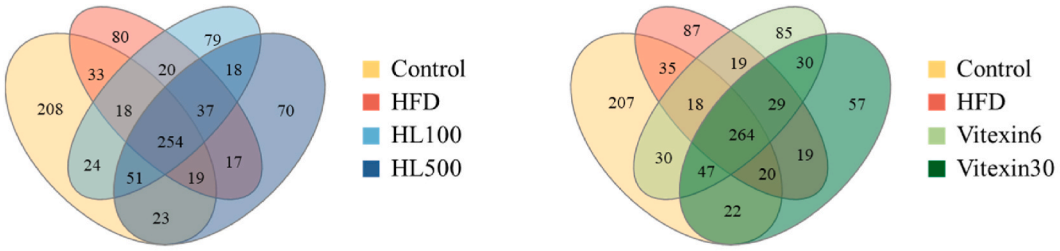
The composition of fecal metabolites following hawthorn leaves and vitexin treatments were analyzed using GC-MS to explore the changes in metabolites contents (Fig. 5(a)).We performed cluster analysis on DAMs with VIP≥2, and found that the contents of testosterone propionate, formoterol, and isoleucyl-prolyl-proline (IPP) in feces were significantly increased in the HL100, HL500, Vitexin6 and Vitexin30 groups, while the content of Trolox was markedly decreased (Fig. 5(b) and (c)) compared to the HFD group.

Notably, in the HL100 and HL500 groups, DAMs were enriched in Sphingolipid metabolism, Arginine and proline metabolism, and Pyrimidine metabolism pathways. Meanwhile, in the Vitexin6 and Vitexin30 groups, DAMs were enriched in Primary bile acid biosynthesis and Arginine and proline metabolism pathways (Fig. 5(d) and (e)).

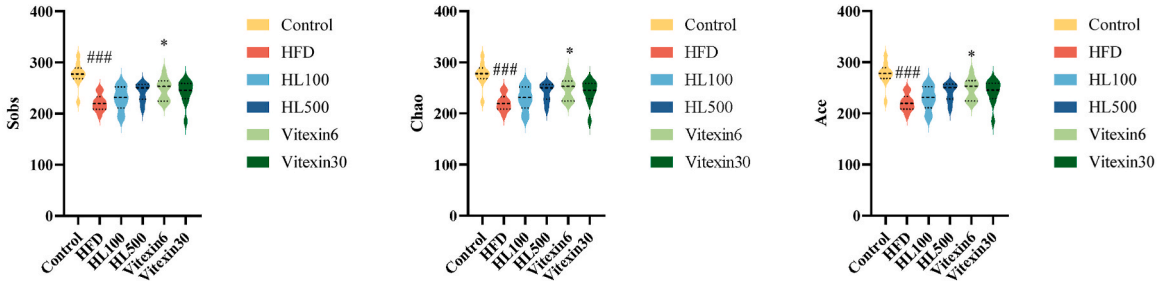
4. Discussion

This paper provides novel insights into the beneficial effects of a plant-based diet, specifically hawthorn leaves and their extract vitexin, in alleviating obesity. We administered hawthorn leaves and vitexin, which significantly reduced body weight gain and fat accumulation in an obese mouse model fed a high-fat diet. Furthermore, the treatments improved abnormalities in sugar metabolism and impaired glucose clearance, which are common in obese individuals. The observed changes in gut microbiota composition, such as the increased abundance of *Kineothrix*, *Paramuribaculum*, and *Lawsonibacter*, and the decreased abundance of *Olsenella*, suggested that these treatments may modulate the gut microbiota to support metabolic health. Additionally, the hawthorn leaves and vitexin treatments also increased the fecal content of testosterone propionate, formoterol, and IPP, and decreased content of Trolox. In conclusion, this study highlights the potential of hawthorn leaves and vitexin in supporting gut health and mitigating obesity.

Dietary components, such as flavonoids, not only directly impact the host's metabolism but also influence it indirectly by regulating microbial functions and the production of secondary metabolites, offering a range of health benefits including the prevention of obesity, diabetes, and cardiovascular diseases. In a double-blind trial, overweight adults treated with CEM (a combination of extracts from *C. pinnatifida* leaves and *Citrus unshiu* peels) achieved significantly greater decreases in their body fat percentages, body weights, and TG levels compared with those treated with the placebo (Song et al., 2024). Other previous research has shown that *C. pinnatifida* leaf and *Ci. unshia* peel extracts were able to reduce TC and TG levels in serum, and reduce the expression of *fas* gene (Lee et al., 2015). Wang, et al. reported that the flavonoids fraction of *C. pinnatifida* leaves showed inhibitory effects on TG and glucose absorption (Wang et al., 2011). These results were in line with our research, which suggested that hawthorn leaves decreased body weight by regulating glucose tolerance and reducing blood lipids and fatty acid synthase gene (*fas*) expression. Orlistat, dapagliflozin, sibutramine and liraglutide are weight loss drugs that have been used on the market. Orlistat can reduce the weight of mice by 10%, mice treated with dapagliflozin by 12%, and mice treated with liraglutide lose an average of 15% in 2 weeks (Deng et al., 2022). In our experiment, the body weights of the HL100, HL500, Vitexin 6, and vitexin 30 groups were 15.5%, 16.1%, 16.5%, and 13.7% lower, respectively, compared with the HFD group.



(a)



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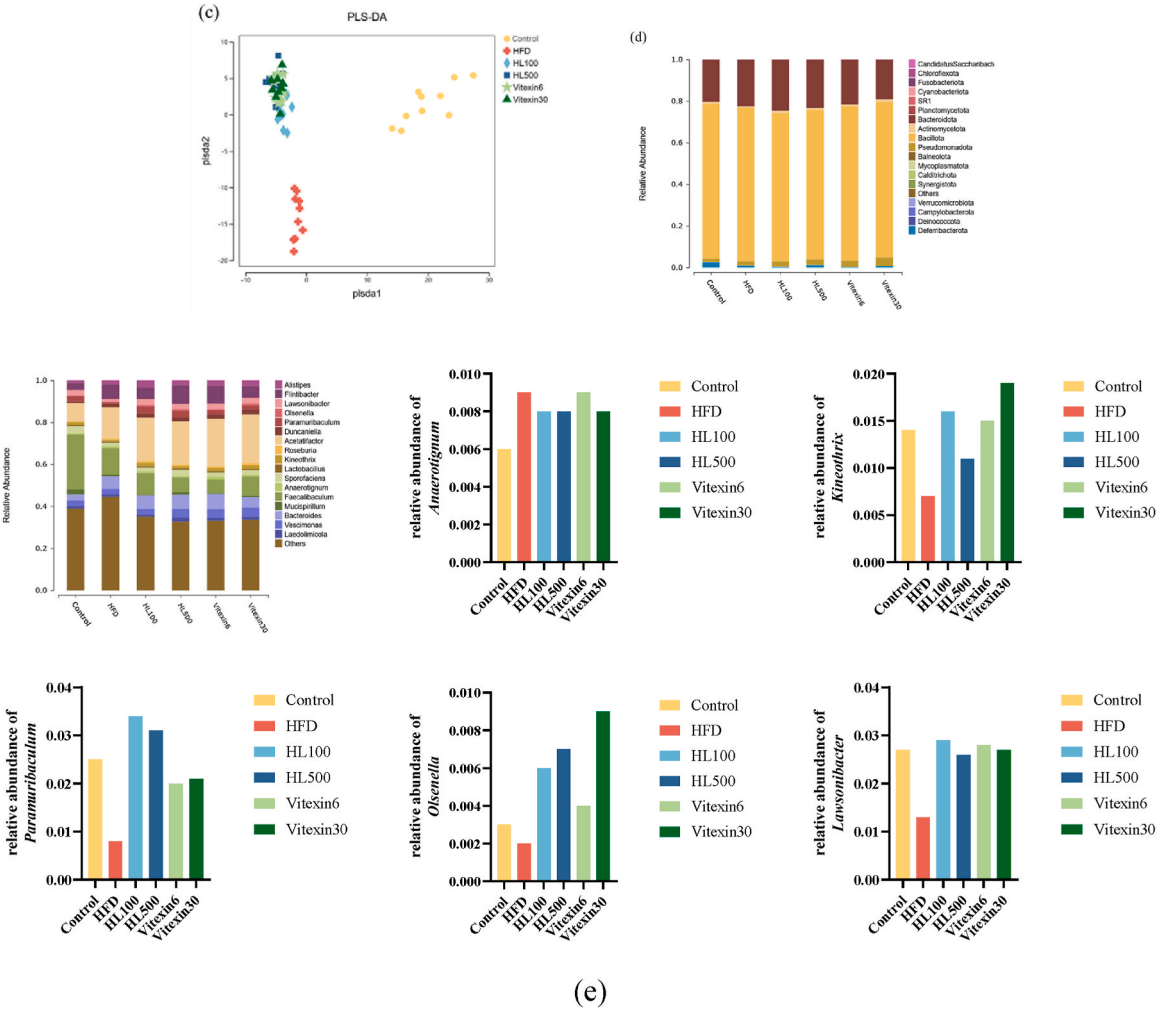
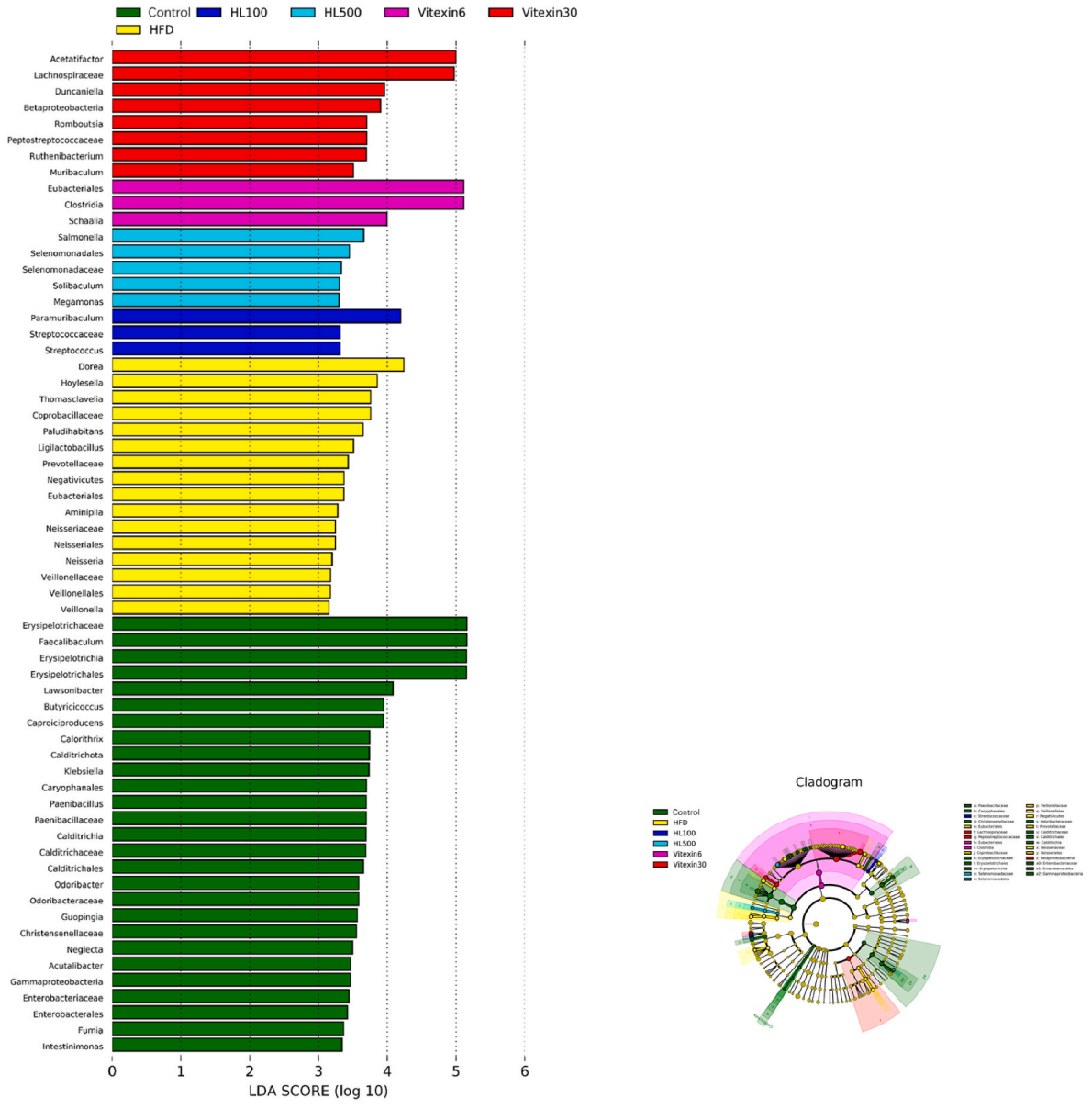


(e)

Fig. 4. Hawthorn leaves and vitexin treatments modified gut microbiota composition. (**a**) Venn plots of Control, HFD, HL100 and HL500/Vitexin6 and Vitexin30 groups; (**b**) Index of alpha diversity (Sobs, Chao, Ace, Shannon and Simpson); (**c**) Partial Least Squares Discriminant Analysis (PLS-DA); Differences in species

composition at the phylum level (**d**) and genus level (**e**); (**f**) Linear discriminant analysis effect size (LEfSe) analysis; (**g**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis; (**h**) Correlation analysis among the significant difference phylum, genus, and physiological parameters. Hash symbol (#) indicates significant differences between Control and HFD groups: $^{###}p < 0.001$; Asterisk (*) indicates significant differences between HFD and Hawthorn leaves/vitexin treatment (HL100, HL500, Vitexin6 and Vitexin30) groups: $^{*}p < 0.05$. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

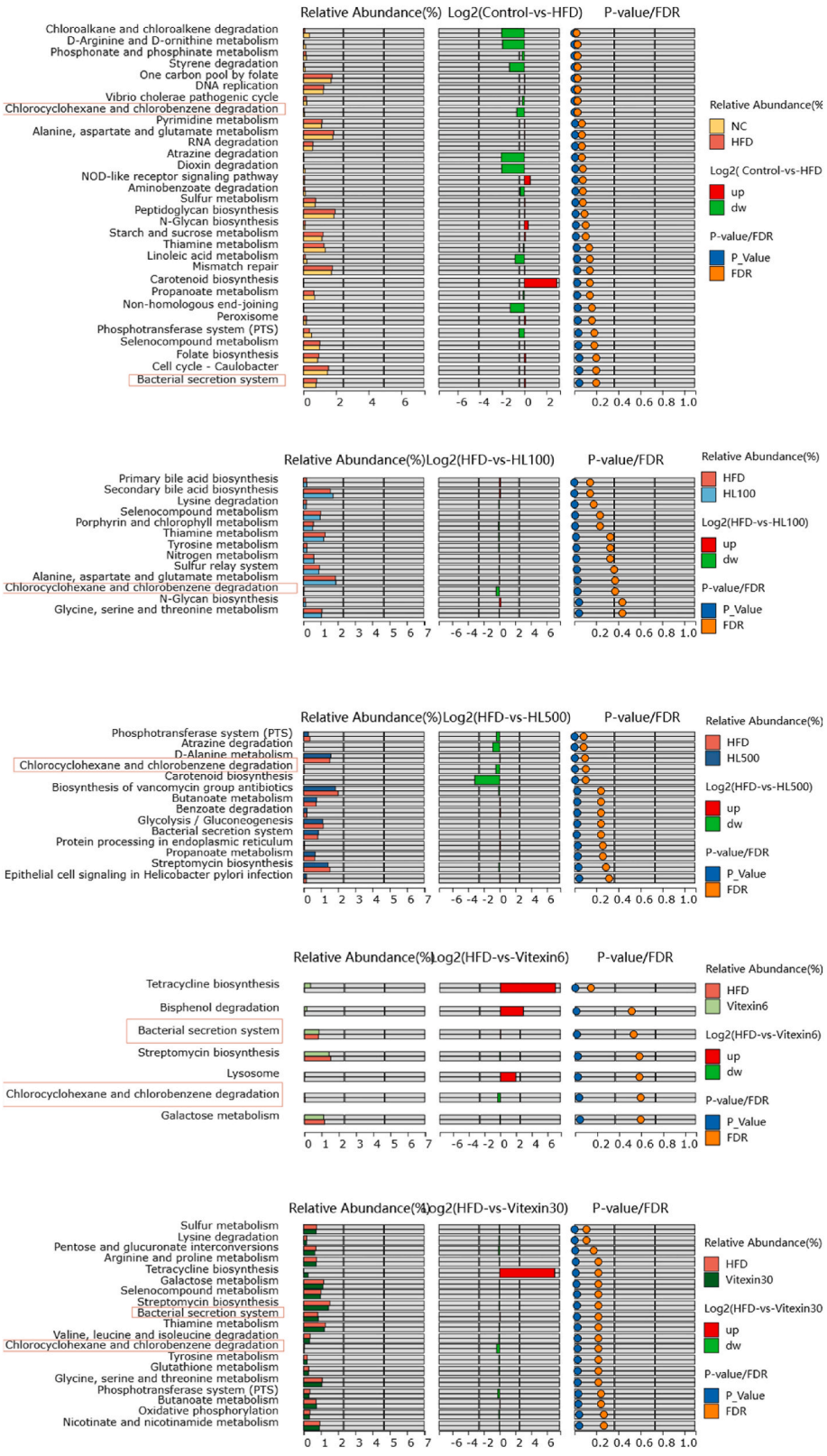


(f)

Fig. 4. (*continued*).

Additionally, vitexin, a naturally occurring flavonoid, was also observed with an anti-obesity activity and suppressed de novo lipogenesis by downregulating expression of *ppar-γ*, *fas*, *Acetyl-CoA Carboxylase* (*acc*), etc. (Inamdar et al., 2019). Vitexin increased the translocation of *GLUT4* from the cytoplasm to the membrane, indicating that vitexin promoted glucose uptake (Gire et al., 2021). Our previous study found that *ampk-α* is the key molecular target for vitexin-mediated lipid reduction; In detail, it was vitexin that activated *ampk-α*, which controls fat accumulation, and inhibited two promoters of fat production and adipocyte differentiation, *C/EBPα* and *fas* (Peng et al., 2019). In our research, vitexin could alleviate obesity by regulating fatty acid synthase proteins, especially *ppar-γ* and *fas*. Oral administration of vitexin was reported to significantly decrease postprandial blood glucose content (Choo et al., 2012), which was in line with the present research.

The gut microbiota and its metabolites may play a role in the development of various diet-related diseases, including obesity and its comorbidities, by affecting the body's ability to acquire nutrients and regulate energy use (Liu et al., 2021; Boulangé et al., 2016). In this study, HFD induced gut inflammation and damage, which was also observed in other studies. In our HFD obese mouse model, the abundance of *Negativicutes, Eubacteriales, Coprobacillaceae, Ligilactobacillus, Prevotellaceae*, *Neisseriaceae*, *Veillonellaceae*, and *Dorea* was higher, consistent with the results of most studies (Byrne et al., 2016; Eckburg et al., 2005; Precup and Vodnar, 2019; Silberbauer et al., 2000). Among



(g)

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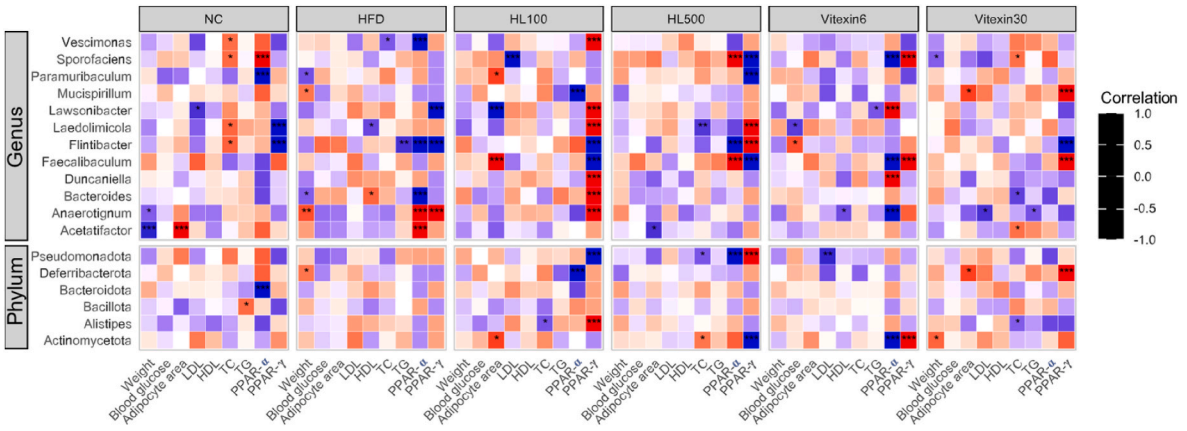

(h)

Fig. 4. (*continued*).

them, the abundance of *Dorea* was the highest at the genus level. Previous studies have confirmed its positive correlation with BMI, waist circumference, and diastolic blood pressure. Individuals with a high abundance of *Dorea* in the gut face more difficulties in weight loss (Precup and Vodnar, 2019; Guo et al., 2020). However, the impact of flavonoids, especially vitexin, on the gut microbiota has not been reported. In addition, although there are many beneficial bacteria that produce healthy fatty acids in the obese model group of this study, such as *Megamonas*, current studies have shown that excessive production of fatty acids by gut commensal microbiota can exacerbate obesity (Li et al., 2024; Takeuchi et al., 2023). In the low-dose hawthorn leaf treatment group, the abundance of *Selenomonadales*, *Selenomonadaceae*, and *Paramuribaculum* was higher, which may improve metabolic health by promoting the production of SCFAs (such as butyrate and propionate) (Silberbauer et al., 2000; Canfora et al., 2015; Louis and Flint, 2017). In the high-concentration hawthorn leaf group, the components of hawthorn leaves may lead to the excessive proliferation of *Streptococcaceae*, *Streptococcus*, and *Megamonas*, which is related to the deterioration of metabolic diseases (for the D.E.S.I.R. Study Group et al., 2011; Zhou et al., 2022). Currently, there are numerous studies on flavonoids that can alleviate obesity, including quercetin, kaempferol, curcumin, and naringin (Azuma et al., 2013; Bian et al., 2018a, 2018b; Fu et al., 2014).

Moreover, the increase in *Acetatifactor* abundance is associated with the alleviation of gut microbiota dysbiosis (Wang et al., 2024). A study from the Netherlands showed that in C57BL/6J mice treated with high sugar and high fat, the abundance of *Acetatifactor* and *Duncaniella* was significantly reduced, and they may improve the risk of metabolic disorders in the process of obesity improvement by reducing the expression of liver fat and pro-inflammatory markers, which is consistent with the results of this study (Riezu-Boj et al., 2022). *Lachnospiraceae*, *Betaproteobacteria*, and *Eubacteriales* were found to have higher abundance in non-obese mice compared to obese mice in current studies, indicating their potential advantage in alleviating obesity (Garrison et al., 2024; Osborn et al., 2022; Wang et al., 2021). In addition, in the vitexin group of this study, the relative abundance of *Muribaculum* and *Duncaniella* in Bacteroidetes was higher, but there are few literature reports. *Muribaculum* may indirectly alleviate obesity-related chronic inflammation by competitively inhibiting the growth of pro-inflammatory bacteria (such as some members of *Proteobacteria*) (Zhao et al., 2018). Currently, there are very limited direct studies on *Duncaniella*, and its classification and function have not been fully clarified. According to some metagenomic analyses, *Duncaniella* may belong to butyrate-producing bacteria (such as those related to *Ruminococcaceae*) or be related to dietary fiber metabolism. In the gut microbiota of obese patients, the abundance of some butyrate-producing bacteria (such as *Ruminococcaceae*) is usually reduced, and the supplementation of prebiotics or dietary fiber may restore their abundance and improve metabolic health. The high abundance of *Duncaniella* in the vitexin treatment group in this study suggests its potential as a key target for dietary treatment of obesity. In this study, the harmful bacteria Romboutsia still had a high abundance in the Vitexin30 group, indicating that the inhibition of obesity by vitexin is not complete, and vitexin may need to work in combination with other anti-inflammatory and anti-obesity factors to fully exert its effects (García-Barrado et al., 2020; Lee et al., 2019; Luo et al., 2024; Oliveira et al., 2022). In the vitexin group, the correlation between *Anaerotignum* and body weight was significantly opposite to that in the HFD group. This bacterium belongs to the Firmicutes phylum, and can degrade a variety of carbohydrates, producing SCFAs such as acetate, propionate, and butyrate. These SCFAs are important for gut health and energy metabolism (Blüher, 2019). Additionally, at the phylum level, Bacteroidota and Bacillota showed a positive correlation with many non-obese phenotypes in the vitexin group, which may also be an important phylum-level factor in its ability to inhibit obesity.

It has been reported that testosterone propionate can induce dyslipidemia, and the effects of formoterol in preventing adipogenesis and IPP in promoting adipocyte differentiation were also been demonstrated (Ogbu et al., 2021; Zhang et al., 2022; Chakrabarti and Wu, 2015). In this study, the fecal contents of testosterone propionate, formoterol and IPP in the HFD group were significantly lower than those in other groups. Interestingly, the testosterone propionate content in the Vitexin30 group was only 1.47% lower than that in the Control group. Moreover, Trolox exerts protective effects against lipid peroxidation by scavenging reactive oxygen species and stabilizing cellular membranes (Horizumi et al., 2025). In the HFD group, the content of Trolox was higher than that in other groups. Specifically, it was 11.42, 9.71, 15.25 and 9.96 times higher than that in the HL100, HL500, Vitexin6 and Vitexin 30 groups, respectively. These results suggest that hawthorn leaves and vitexin treatments may alleviate obesity-related symptoms by increasing the fecal content of testosterone propionate, formoterol, and IPP while decreasing that of Trolox.

However, this study has certain limitations. Although animal models can replicate human diseases to some extent, there may be various differences between animals and humans, such as in physiology and immune systems. Therefore, the key confirmation of the therapeutic effects of hawthorn leaves and their extract, vitexin, on obesity requires future clinical trials. Additionally, although hawthorn leaves and vitexin treatment may be beneficial for obesity, their dosage and safety need careful consideration. Different dosages and treatment durations may produce different effects and potentially induce adverse reactions. Therefore, future safety research on hawthorn leaves and vitexin is

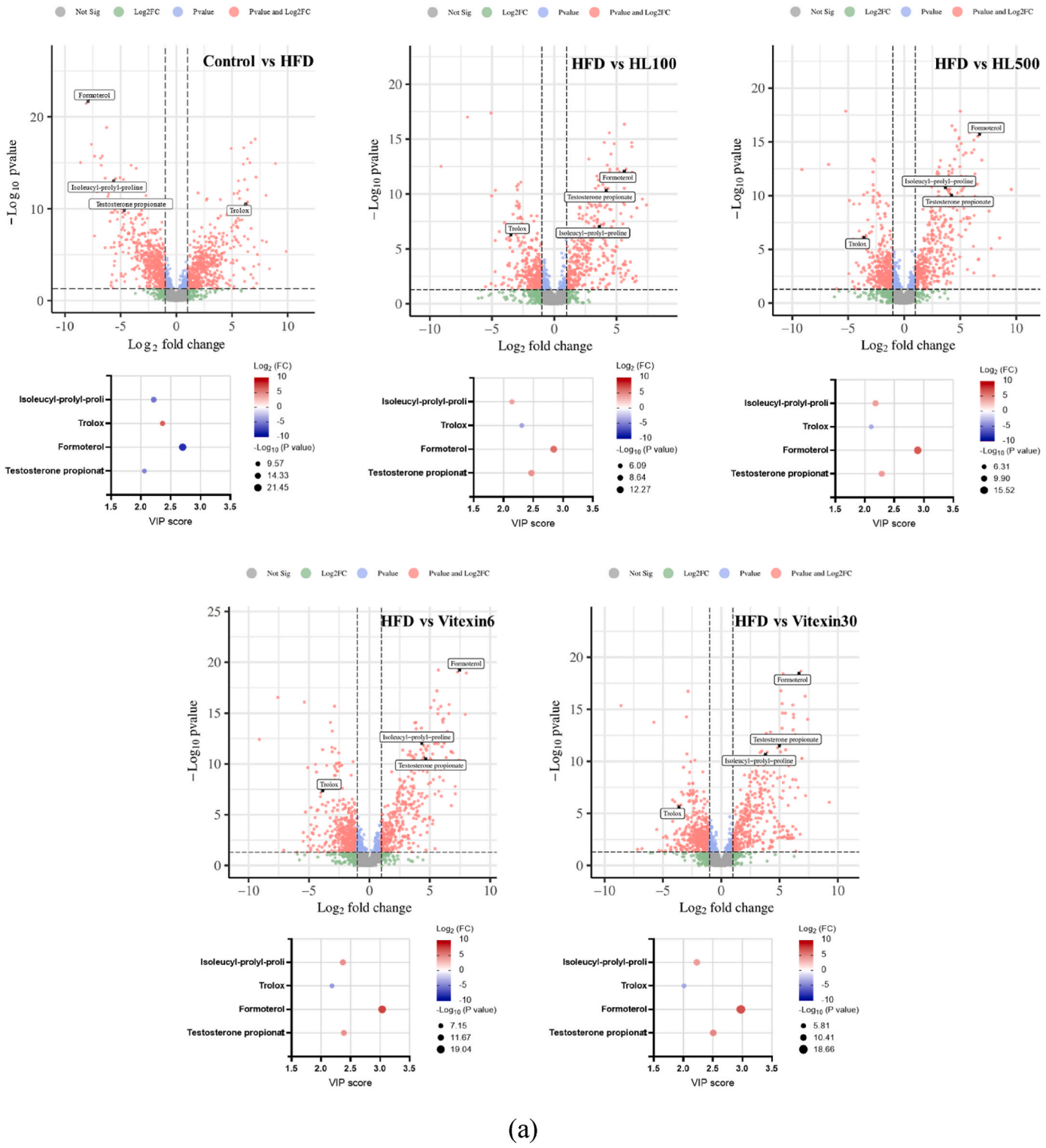

Fig. 5. Hawthorn leaves and vitexin treatments modified the content and composition of fecal metabolites. (**a**) Number of differentially abundant metabolites (DAMs) compared with HFD group; (**b**) The cluster analysis of Control, HFD and Hawthorn leaves treatment (HL100 and HL500) groups; (**c**) The cluster analysis of Control, HFD and vitexin treatment (Vitexin6 and Vitexin30) groups; (**d**) The enrichment pathway of DAMs of Hawthorn leaves treatment groups compared with HFD group; (**e**) The enrichment pathway of DAMs of Vitexin treatment groups compared with HFD group. Control, low fat diet with sterile water control; HFD, high fat diet control with sterile water control; HL100, high fat diet with 100 mg/kg bw/day Hawthorn leaves treatment; HL500, high fat diet with 500 mg/kg bw/day Hawthorn leaves treatment; Vitexin6, high fat diet with 6 mg/kg bw/day vitexin treatment; Vitexin6, high fat diet with 30 mg/kg bw/day vitexin treatment.

essential.

In conclusion, our study indicates that hawthorn leaves and their extract, vitexin, exert a protective effect against HFD-induced obesity through a combined response of their anti-inflammatory and prebiotic capabilities. We have identified specific gut microbiota changes associated with vitexin-mediated alleviation of obesity. Our research further

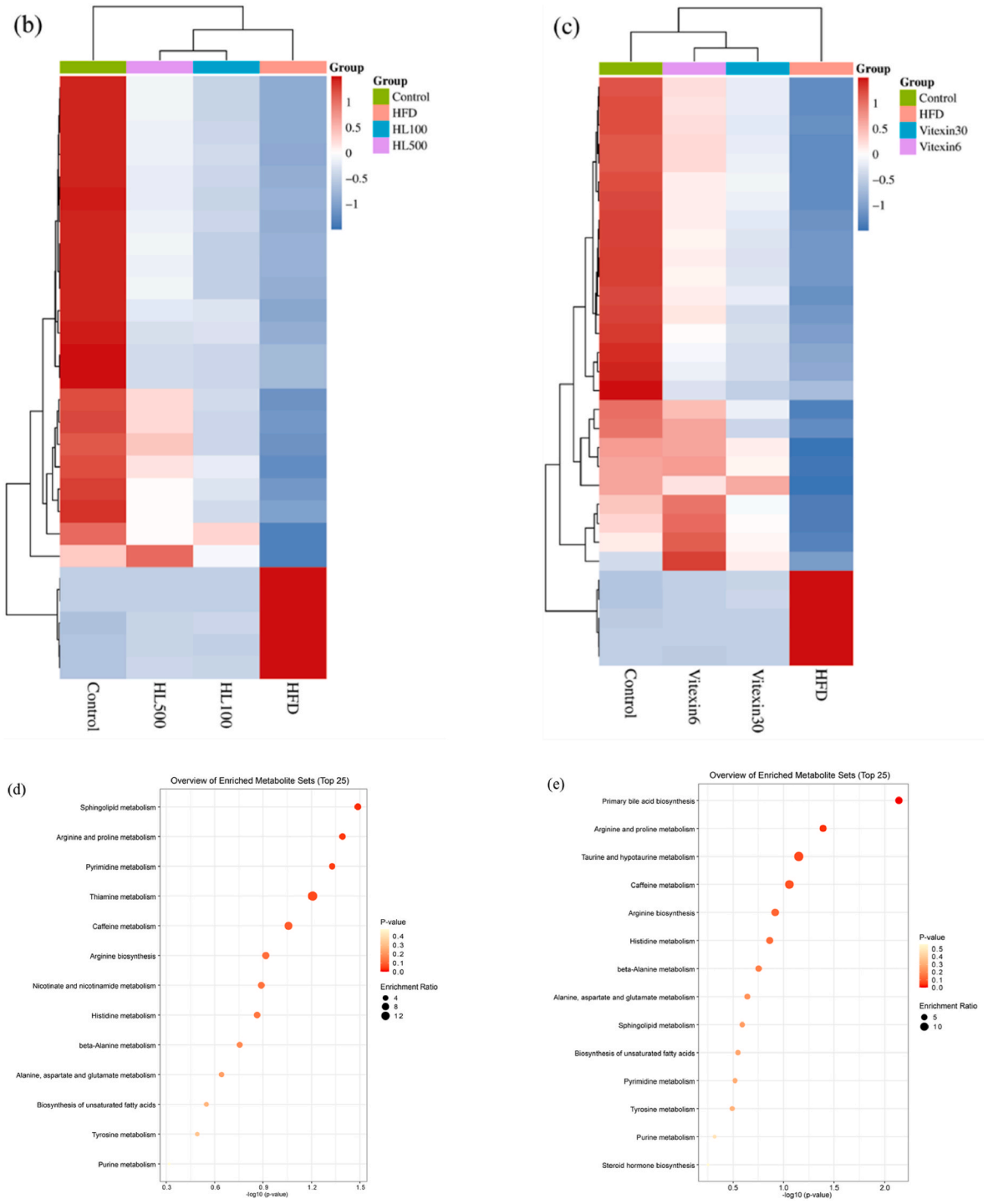

Fig. 5. (*continued*).

confirms the possibility that a diet rich in vitexin or supplementation with this flavonoid may have protective effects and could reduce the risk of obesity. This is also a promising direction for the development of dietary therapies for the treatment of obesity-related metabolic syndrome.

CRediT authorship contribution statement

Ziqi Liu: conducted experiments, Writing – original draft. **Tianrui Gao:** conducted experiments, Writing – original draft. **Haoyu Chang:** conducted the animal experiments. **Yuqing Xu:** conducted the animal experiments. **Letao Wang:** conducted the animal experiments. **Xiangyi Wang:** conducted the animal experiments. **Jiayin Lang:** conducted the animal experiments. **Yingxing Yu:** Writing – original draft, Funding acquisition. **Ying Xiao:** Supervision. **Ye Peng:** Supervision, All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work, ensuring integrity and accuracy.

Consent for publication

The authors declare that they have no competing interests.

Declaration of competing interest

The authors declare no conflict of interest associated with this manuscript.

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Glossary

SCFAs: Short-chain fatty acids; DNA: Deoxyribonucleic acid; HFD: High-fat diet; GTT: Glucose tolerance test; AUC: area under the curve; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; GPO-PAP: glycerol phosphate oxidase-phenol aminophenazone peroxidase; COD-PAP: cholesterol oxidase-phenol aminophenazone peroxidase; PCR: Polymerase chain reaction; OTUs: Operational taxonomic units; FC: fold change; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; DAMs: differentially abundant metabolite; PLS-DA: Partial Least Squares Discriminant Analysis; eWAT: Epididymal white adipose tissue; PCoA: Principal coordinate analysis; Lefse: Linear discriminant analysis effect size; IPP: isoleucyl-prolyl-proline.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101025.

Data availability

Data will be made available on request.

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