



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Fermented *Rosa roxburghii* Tratt juice ameliorates obesity by modulating the composition of gut microbiota in high-fat diet induced obese mice

Shuhong Liu, Jiuchang Li, Xinghua Chen, Huaisheng zheng, Chaobing Huang, Ping Hu*

School of Liquor and Food Engineering, Guizhou University, Guiyang 550025, China;

ABSTRACT: *Rosa roxburghii* Tratt is abundant in a variety of active ingredients, which exhibit regulatory effects on gut microbiota, hypoglycemic and hypolipidemic activities, and antioxidant properties. However, the impact of probiotic-fermented *Rosa roxburghii* Tratt juice on obesity and related complications remains unclear. Thus, using a high-fat diet (HFD) mouse obesity model and comparing it to the model group after 56 d of consecutive gavage with probiotic fermented *Rosa roxburghii* Tratt juice (FRTJ), *Rosa roxburghii* Tratt juice (RTJ), saline, and orlistat, respectively, we investigated the effect of FRTJ on obesity and related complications in this study. In HFD-induced obese mice, FRTJ supplementation significantly reduced fat accumulation and improved dyslipidemia, liver inflammation, endotoxemia, and colonic mucosal damage compared to the model group (MC). Moreover, FRTJ treatment was superior to RTJ treatment. FRTJ intervention dramatically improved the diversity and changed the composition of gut microbiota for the better. Compared with the MC group, the ratio of *Firmicutes* to *Bacteroidetes* at least decreased by 40.93% after FRTJ supplementation. Additionally, the FRTJ intervention led to a significant enhancement in the presence of beneficial bacteria, including *Bacteroidetes*, *Lactobacillaceae*, *Lachnospiraceae*, *Corynebacteriaceae*, *Lactobacillus*, *Akkermansia*, and *Clostridium_XIVa*. Conversely, the relative abundance of harmful bacteria such as *Firmicutes*, *Porphyromonadaceae*, *Desulfovibrionaceae*, *Erysipelotrichaceae*, *Allobaculum*, *Barnesiella*, and *Desulfovibrio* was observed to decrease. The results of the correlation analysis revealed that the following bacteria had different relationships with body weight: *Bacteroidetes*, *Acidobacteria*, *Proteobacteria*, *Lactobacillaceae*, *Lactobacillus*, *Tenericutes*, and *Saccharibacteria* had negative correlations with body weight ($P < 0.05$), while *Allobaculum*, *Paenalcigenes*, *Barnesiella*, *SRI*, *Porphyromonadaceae*, *Firmicutes*, and *Deferribacteres* were positively correlated with body weight ($P < 0.05$). In addition, we conducted antibiotic intervention experiments to assess the effects of FRTJ on the gut microbiota. The results showed that antibiotic treatment eliminated all metabolic improvements mediated by FRTJ, suggesting that the beneficial effects of FRTJ on obesity symptoms and associated metabolic disorders are exerted by regulating gut microbiota composition.

Keywords: Probiotic fermented; *Rosa roxburghii* Tratt juice; Improvement; Obesity symptoms; Gut microbiota

1. Introduction

In addition to the hereditary factors, the majority of obesity cases stem from the frequent intake of high-fat and high-sugar diets, coupled with detrimental lifestyle choices such as sedentary behavior and irregular work and rest. Nowadays, obesity is becoming a widespread and growing global public health

*Corresponding author
phul@gzu.edu.cn

Received 30 October 2024
Received in revised form 16 December 2024
Accepted 16 June 2025

concern. It diminishes the life quality of patients and intensifies the progression of a spectrum of metabolic syndromes, including heart disease, insulin resistance, hyperlipidemia, hyperglycemia, and liver steatosis, all of which pose substantial risks to human health. Extensive research has confirmed that obesity, frequently triggered by a diet rich in fats, is accompanied by liver fat accumulation, dyslipidemia, intestinal mucosal damage, and intestinal microbiota imbalance^[1–3]. However, weight loss through exercise demands a lot of time and effort, making it challenging to sustain over the long term. Additionally, certain anti-obesity drugs have a high incidence of harmful side effects, which people are afraid to consume due to concerns about their safety and efficacy^[4]. Consequently, there is an increasingly pressing demand for the development of alternative, safe, innovative, and efficacious strategies to address the multifaceted challenges posed by obesity.

The gut microbiota is increasingly acknowledged as a promising therapeutic target for obesity and its associated metabolic diseases. Researchers observed that obese individuals tend to exhibit a lower diversity of gut microbiota compared to their non-obese counterparts^[5]. Transplanting the gut microbiota from obese mice into germ-free mice resulted in the transmission of the obesity phenotype^[6]. Recent studies have shown that transplanting gut microbiota from treated obese mice to high-fat diet mice can alleviate obesity^[7,8]. Besides, an elevated *Firmicutes* to *Bacteroidetes* (F/B) ratio has been consistently linked to obesity and intestinal inflammation across rodent models and human clinical studies^[9]. Moreover, an increasing body of research indicates that gut microbiota dysbiosis may drive obesity, diabetes, atherosclerosis, cancer, liver steatosis, and chronic inflammation^[6,10,11]. Some articles have pointed out that probiotics can affect food intake, appetite, body weight and metabolic function through gastrointestinal pathways and regulating intestinal bacterial communities, thereby alleviating obesity^[12]. In a meta-analysis study by Shirvani-Rad et al.^[13], probiotic foods showed good results in managing obesity by reducing body mass index (BMI), body weight (BW) or body fat mass (BFM) in obese patients to control obesity. Probiotics have become the most widely used intervention for the gut microbiome and its metabolites in clinical practice. These findings all pointed to the possibility that regulating the intestinal microbiota could be a key strategy for preventing and treating obesity and the metabolic syndrome that goes along with it.

Rosa roxburghii Tratt (RRT) is a valuable plant resource in China. It is rich in nutrients and functional components such as vitamin C (VC) (2000–3000 mg/100 g fresh weight), superoxide dismutase (SOD), polysaccharides, phenolic compounds, organic acids, minerals and flavone. These components offer significant health benefits, including immunity enhancement, anticancer, antioxidant, and regulation of intestinal microbiota^[14,15]. Nowadays, research on the health aspects of fermented foods receives increasing attention, primarily because fermented foods have the ability to enhance intestinal barrier function and regulate host metabolic pathways, which gives them the potential to prevent and alleviate obesity and other related metabolic diseases^[16]. Probiotic-fermented fruit and vegetable raw materials have been shown in numerous studies that they can not only improve flavor, extend shelf life, and remove anti-nutritional factors^[17], but also produce a range of functional bioactive compounds with

health-promoting properties. These bioactives help maintain intestinal microbial homeostasis^[18], lower lipid levels^[19], reduce cardiovascular risks^[20], and exert antioxidant^[21] and antibacterial activities^[22], making them highly desirable to consumers. Hu et al.^[18] discovered that fermented carrot juice promotes beneficial bacteria growth and modifies gut microbiota. Zhong et al.^[10] found that gavage of fermented blueberry juice to HFD mice resulted in a noteworthy enhancement of the gut microbiota's diversity and community richness. Additionally, the animals showed signs of reduced obesity. Wang et al.^[23] demonstrated that feeding mice fermented litchi juice could effectively regulate intestinal microbiota disorders and reduce obesity. Furthermore, our team previously proved that *Rosa roxburghii* Tratt fruit juice fermented by *Lactobacillus paracasei* SR10-1 had a mitigation effect on mice with colitis^[24].

Probiotic fermentation is a complex process that creates an ideal environment for probiotic growth. Metabolites like short-chain fatty acids (SCFAs) generated during fermentation assist probiotics in colonizing the gut and stimulate immune cells to produce cytokines. These effects are of great significance for regulating the body's immunity and avoiding inflammatory responses^[25]. Fermentation also enhances the bioavailability and activity of compounds like flavonoids, improving their absorption and utilization^[26]. The information presented above showed the health benefits of probiotic-fermented fruit and vegetable juices. However, research has not been done on how probiotic-fermented *Rosa roxburghii* Tratt juice affects the intestinal microbiota and reduces obesity in HFD mice. Thus, this study aims to determine whether fermented *Rosa roxburghii* Tratt juice can improve obesity and its related consequences by modulating the gut microbiota, offering new strategies for obesity management.

2. Materials and methods

2.1 Dietary supplementation and FRTJ preparation

Normal diet (ND) and HFD (78.8% normal diet, 10% egg yolk powder, 10% fat, 1% cholesterol, 0.2% salt of cholate) for experimental mice were purchased from Tengxin Biotechnology Co., Ltd. (Chongqing, China). Fresh RRT with moderate ripeness was picked, selected, cleaned, pulped, filtered, and sterilized by ultra-high temperature instantaneous sterilization (UHT) and then aseptically filled to obtain RTJ. *Lactobacillus paracasei* SR10-1 (CCTCC NO: M2016527) was isolated from the fermented sour meat products of the Dong nationality in China, and *Lactobacillus fermentum* GZSC-1 (CCTCC NO: M2017847) was isolated from the fermented sauerkraut made by villagers of the Bouyei nationality in Guizhou Province, China. RTJ was mixed with sterile water (1:2, v/v) and sugar (6%, v/m). After pasteurization, 3% *L. paracasei* SR10-1 (8 log CFU/mL), *L. fermentum* GZSC-1 (8 log CFU/mL) (in a 1:1 ratio) and 0.3% of aroma-producing yeast (Angel Yeast, Hubei, China) were added. Following 96 hours of fermentation at 32 °C, the mixture was centrifuged and the supernatant obtained by centrifugation is FRTJ.

2.2 Determination of chemical functional components

In this study, the content of VC was determined by the Chinese National Standard 2,6-Dichloro Indophenoltitration Method (GB 5009.86-2016). SOD content was measured using a SOD kit purchased

from Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd. (Jiangsu, China) according to the kit instructions. Total polyphenols content was determined using the Folin–Ciocalteu Method^[27]. Total flavonoid content was measured by $\text{NaNO}_2\text{-Al}(\text{NO}_3)_3\text{-NaOH}$ reagent using rutin as a standard^[27]. Total acid was determined by the Chinese National Standard Titration Method (GB/T 12456-2008).

Organic acids were analyzed by HPLC, following the procedure outlined in the literature^[28] with some minor modifications. The separation was performed on an Agilent Z0 RBAX SB-Aq column (250 mm×4.6 mm, 5 μm , American Agilent Corporation (Santa Clara, CA, USA) with an Agilent 1260 VWD detector. The mobile phase conditions were configured as follows: Isocratic elution was performed with 0.02 mol/L, pH=2 monopotassium phosphate buffer solution, and methanol (95:5, v/v) at a flow rate of 0.8 mL/min, the injection volume was 10 μL , the column temperature was 35 $^{\circ}\text{C}$, and the spectrum was recorded at 210 nm. Three experiments were performed for each sample.

2.3 Animal experiment design

All the animal experiments were allowed and performed according to the “Administrative Regulations on Laboratory Animals”, and were reviewed and approved by the Laboratory Animal Ethics Sub-Committee of Guizhou University (No. EAE-GZU-2022-E011). In total, 117 healthy Kunming male mice (body weight $20\text{ g} \pm 2\text{ g}$, 4 weeks old) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). These mice were accommodated under specific pathogen-free (SPF) standards ($23 \pm 2\text{ }^{\circ}\text{C}$ at $50\% \pm 5\%$ relative humidity), ensuring unrestricted access to both water and food, and with a 12 h light/dark cycle. After 2-week of acclimatization, mice were randomly divided into 9 groups ($n = 12$ per group) based on their body weight and the number of groups. The grouping was as follows: (1) normal group (NC): normal feed, 0.9% saline; (2) model group (MC): HFD; 0.9% saline; (3) positive group (PC): HFD, 50 mg/kg orlistat; (4) RTJ control group (RC): HFD, 50% RTJ (RTJ: sterile water = 1:1); (5) FRTJ low dose group (LD): HFD, 10% FRTJ; (6) FRTJ medium dose group (MD): HFD, 50% FRTJ; (7) FRTJ high dose group (HD): HFD, 90% FRTJ; (8) antibiotic control group (HFD+A): HFD, antibiotic (ampicillin 1 g, metronidazole 1 g, gentamycin sulfate 1 g and guillamycin 1 g, dissolved in 1L of water); (9) antibiotic dose group (HFD+A+R): HFD, antibiotic, 50% FRTJ. The gavage dose was performed according to previous reports^[29], the gavage volume of each mouse was 1% of its body weight (0.1 mL/10 g), and the maximum dose of gavage did not exceed 0.4 mL. During this period, the mice were gavaged daily, and their body weight and intake were measured once a week. The FRTJ intervention was discontinued when the average body weight of the mice in the model group was 10% higher than the average body weight of the mice in the normal group. Finally, Lee’s index, adipose coefficient, and organ index were calculated. The whole experiment lasted for 56 d. After the 8th week, mice were fasted overnight and sacrificed to obtain organs, tissues, and serum samples, which were stored at $-80\text{ }^{\circ}\text{C}$ until use. The Lee's index, adipose coefficient, and organ index were calculated according to the following formula^[30]: Lee’s index= $[\text{body weight (g)} \times 1000]^{1/3}/\text{length (cm)}$, adipose coefficient (%)= $\text{perirenal and epididymal adipose tissues (g)}/\text{body weight (g)}$, organ index (%) = $\text{organ weight (g)}/\text{body weight (g)} \times 100\%$.

2.4 Biochemical analysis

The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) in serum were measured using a commercial kit (Raysun Biotechnology Co., Ltd, Guangzhou, China). The serum concentrations of tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6), interferon- γ (IFN- γ), and lipopolysaccharide (LPS) were quantified using commercial ELISA kits (Raysun Biotechnology Co., Ltd, Guangzhou, China).

2.5 Histopathological analysis

The liver, colon, perirenal adipose, and epididymal adipose tissues of mice were fixed in 4% neutral formaldehyde solution for 24 h. Tissues were embedded in paraffin and sectioned into 5- μ m thick sections via the RM2016 pathology sectioning machine (Leica Instrument Co., Ltd, Shanghai, China). All tissues were stained with hematoxylin and eosin (H&E), and pathological changes were observed using a light microscope (Nikon Eclipse E100, Japan Nikon).

2.6 Gut microbiota analysis

After dissecting the mice, the cecum contents were collected under sterile conditions, placed in sterile freezing tubes, and then transferred to -80 °C for microbiota analysis. Total DNA was extracted from cecum contents using a MagPure Stool DNA KF kit B (Magen, Guangzhou, China) according to the manufacturer's instructions. The fragment ranges and concentrations of the libraries were evaluated using an Agilent 2100 Bioanalyzer. For the sequencing of the bacterial 16S rRNA gene, the primers of choice were 338 F and 806 R (fwd 5' -ACTCCTACGGGGAGGCAGCA-3' and rev 5' -GGACTACHVGGGTWTCTAAT-3'). Sequencing was based on the Illumina NovaSeq Sequencing platform, and then the CCS (Cyclic Consensus Sequencing) sequences were filtered, clustered or denoised, divided into OTUs, and species annotated. Community diversity and richness were evaluated using the α -diversity index (Sobs, Chao 1, Shannon index, Simpson index), and further β -diversity analysis was used to evaluate the similarity of the species composition of each sample.

2.7 Statistical analysis

All data were presented as mean $\pm$ standard deviation (SD), and all statistical analyses were performed using one-way analysis of variance (ANOVA). Origin 2021 was used for graphical processing. $P < 0.05$ (*) indicated a significant difference, $P < 0.01$ (**) and $P < 0.001$ (***) indicated a highly significant difference.

3. Results

3.1 Bioactive substances in FRTJ

At the same dilution factor, we measured the bioactive substances in RTJ and FRTJ, and the results showed that after the fermentation of *Rosa roxburghii* Tratt juice by probiotics, the nutrients such as VC,

SOD, organic acids, total polyphenols, and total flavonoids were reduced to a certain extent, but their contents were still at a high level (Table 1). FRTJ has higher VC (417.22 ± 1.37) mg/100 mL and SOD (897.17 ± 41.30) U/mL levels than the documented fermented celery juice^[29]. The total organic acid concentration was (43.89 ± 1.22) mg/mL, significantly exceeding the values reported for fermented mango slurries^[31]. In addition, compared to fermented papaya juice^[32], the contents of total polyphenols (308.91 ± 8.44) mg /100mL and total flavonoids (183.84 ± 4.97) mg /100mL were significantly higher. Numerous studies have demonstrated the potential of foods high in VC, flavonoids, polyphenols, and other active ingredients to mitigate the symptoms of chronic illnesses, including obesity^[33,34]. Thus, the wealth of nutrients in FRTJ provides a strong foundation for further investigation into its potential to lower obesity.

Table 1. Nutritional composition of RTJ and FRTJ.

Items	The content of RTJ	The content of FRTJ
acetic acid	-	5.50 ± 1.28 mg/mL
malic acid	44.14 ± 0.17 mg/mL	26.95 ± 6.37 mg/mL
lactic acid	6.75 ± 0.11 mg/mL	6.17 ± 0.18 mg/mL
tartaric acid	3.21 ± 0.09 mg/mL	2.05 ± 0.74 mg/mL
citric acid	3.77 ± 0.09 mg/mL	0.68 ± 0.05 mg/mL
succinic acid	2.21 ± 0.22 mg/mL	2.35 ± 1.21 mg/mL
fumaric acid	-	0.20 ± 0.08 mg/mL
oxalic acid	0.08 ± 0.01 mg/mL	-
total organic acid	62.16 ± 1.32 mg/mL	43.89 ± 1.22 mg/mL
total polyphenols	356.35 ± 8.61 mg /100mL	308.91 ± 8.44 mg /100mL
total flavonoids	217.24 ± 3.19 mg /100mL	183.84 ± 4.97 mg /100mL
VC	448.38 ± 1.33 mg /100mL	417.22 ± 1.37 mg /100mL
SOD	1959.86 ± 50.11 U/mL	897.17 ± 41.30 U/mL
pH	3.51 ± 0.01	3.45 ± 0.01
soluble solids	9.03 ± 0.06 °Brix	4.07 ± 0.06 °Brix

Data were mean $\pm$ SD (n = 3), and statistical analyses were performed using one-way analysis of variance (ANOVA). “-” indicated that this component was not present.

3.2 FRTJ can inhibit HFD-induced obesity in mice

As shown in Table 2, feeding HFD significantly increased the body weight of mice by 10.82% ($P < 0.05$) compared with the NC group, indicating that feeding mice on an HFD for an extended period of time may cause obesity in mice. However, FRTJ supplementation significantly inhibited body weight gain in mice. The mice's final body weight did not differ significantly from the NC group's after 8 weeks of continuous FRTJ gavage ($P > 0.05$), suggesting that FRTJ can effectively inhibit mice from gaining weight due to a high-fat diet. The interventional effect of RTJ and orlistat on body weight was not as substantial as that of FRTJ. Besides, the NC group consumed the least amount of food out of all the groups, and the weekly food intakes of all other groups exceeded the NC group's, indicating that the influence of FRTJ on the body weight of mice is not attributable to lower food intake.

Table 2. Effects of different treatment groups on body weight and weekly food intake of HFD mice.

Group	Initial weight (g)	Final weight (g)	Weight gain (g)	Food intake (g/w/ per mouse)
NC	36.18 ± 2.32^a	50.5 ± 1.15^b	14.32 ± 1.16^c	17.72 ± 2.01^b
MC	36.97 ± 2.02^a	55.95 ± 2.51^a	18.98 ± 1.24^a	19.16 ± 1.37^{ab}
PC	36.45 ± 1.55^a	49.89 ± 1.44^b	13.44 ± 1.21^d	20.41 ± 4.01^a

RC	36.96 ± 2.01 ^a	51.91 ± 2.01 ^b	14.95 ± 1.43 ^b	19.00 ± 3.11 ^{ab}
LD	36.63 ± 2.63 ^a	51.02 ± 2.12 ^b	14.39 ± 1.17 ^b	18.54 ± 2.19 ^{ab}
MD	36.88 ± 1.41 ^a	49.54 ± 1.86 ^b	12.66 ± 1.24 ^c	18.44 ± 2.56 ^{ab}
HD	36.73 ± 1.66 ^a	49.53 ± 1.31 ^b	12.80 ± 1.20 ^c	20.45 ± 3.74 ^a

Data were mean ± SD (n = 3), and statistical analyses were performed using one-way analysis of variance (ANOVA). Different superscript lowercase letters in the same column indicate significant differences ($P < 0.05$).

In general, weight gain is usually accompanied by fat accumulation. As shown in Table 3, the MC group had the largest adipose coefficient (3.56 ± 0.23), which differed significantly from the other groups ($P < 0.05$), indicating that obesity contributes to the accumulation of both perirenal and epididymal fat. The adipose coefficients of all the groups following the FRTJ intervention were lower than those of the MC group, suggesting that FRTJ treatment is effective in preventing the buildup of fat. Additionally, the mice in the model group exhibited the highest Lee's index, but this did not differ substantially from the rest of the groups ($P > 0.05$), indicating that the high-fat diet had no discernible impact on the mice's Lee's index. The liver, kidneys, and spleen index of the MC group were not significantly different from the other groups ($P > 0.05$), suggesting that feeding HFD to mice did not have a significant effect on the accumulation of fat in these organs as much as it did in their perirenal and epididymal tissues.

Table 3. Organ index in mice fed experimental diets for 56 days.

Group	Lee's Index	Adipose coefficient	Liver Index	Kidneys Index	Spleen Index
NC	3.36 ± 0.21 ^a	2.47 ± 0.06 ^b	3.40 ± 0.31 ^a	1.34 ± 0.08 ^a	0.26 ± 0.032 ^a
MC	3.83 ± 0.46 ^a	3.56 ± 0.23 ^a	3.57 ± 0.25 ^a	1.23 ± 0.36 ^a	0.23 ± 0.045 ^a
PC	3.68 ± 0.41 ^a	2.89 ± 0.21 ^b	3.53 ± 0.34 ^a	1.38 ± 0.20 ^a	0.24 ± 0.044 ^a
RC	3.73 ± 0.53 ^a	2.9 ± 0.46 ^b	3.42 ± 0.20 ^a	1.32 ± 0.25 ^a	0.24 ± 0.034 ^a
LD	3.71 ± 0.33 ^a	2.94 ± 0.13 ^b	3.43 ± 0.29 ^a	1.33 ± 0.20 ^a	0.24 ± 0.036 ^a
MD	3.67 ± 0.69 ^a	2.56 ± 0.22 ^b	3.55 ± 0.31 ^a	1.37 ± 0.26 ^a	0.25 ± 0.046 ^a
HD	3.67 ± 0.54 ^a	2.81 ± 0.42 ^b	3.45 ± 0.17 ^a	1.37 ± 0.21 ^a	0.25 ± 0.059 ^a

Data were mean ± SD (n = 3), and statistical analyses were performed using one-way analysis of variance (ANOVA). Different superscript lowercase letters in the same column indicate significant differences ($P < 0.05$).

3.3 Effect of FRTJ on dyslipidemia, serum inflammation and endotoxemia in obese mice

HFD induces dyslipidemia, which is one of the characteristics of obesity, and is usually accompanied by increased blood levels of TC, TG, and LDL-C and decreased levels of HDL-C^[33,35]. As shown in Figs. 1, there was a significant rise in the levels of TC, TG, and LDL-C ($P < 0.05$), along with a significant reduction in HDL-C ($P < 0.05$) in the MC group when contrasted with the NC group. This suggested that a high-fat diet has the potential to trigger dyslipidemia in obese mice. In the PC, LD, MD, and HD groups, there was a notable increase ($P < 0.05$) in HDL-C and a notable decrease ($P < 0.05$) in TC, TG, and LDL-C as compared to the MC group. TC was remarkably reduced to below (4.06 ± 0.68) mmol/L after FRTJ supplementation, outperforming the RC group with levels of (5.67 ± 0.15) mmol/L and the PC group with (4.26 ± 0.86) mmol/L (Fig. 1 A); TG levels were notably reduced to a level below (1.26 ± 0.09) mmol/L, demonstrating a superior outcome in comparison to the RC group (1.36 ± 0.18) mmol/L (Fig. 1B); LDL-C was reduced by 45.96%, 36.06%, 41.63%, 42.55%, and 43.92% in the PC, RC, LD, MD, and HD groups, respectively, as compared with the MC group; The HDL-C content in the MD group was not significantly

different from that in the NC group ($P > 0.05$) (Fig. 1C). According to the aforementioned findings, FRTJ treatment significantly modifies dyslipidemia in obese mice. In addition, research has demonstrated that the ratio of TG to HDL-C (TG/HDL-C) is a major risk factor for cardiovascular disease, insulin resistance, and metabolic syndrome. It has also been utilized as a predictor of patients with adverse cardiometabolic risk^[36]. As shown in Fig. 1E, TG/HDL-C was reduced by 58.33%, 24.36%, 40.38%, 50.64%, and 35.26% in the PC, RC, LD, MD, and HD groups, respectively, when compared with the MC group, suggesting that FRTJ has a preventive effect against adverse cardiometabolism induced by a high-fat diet.

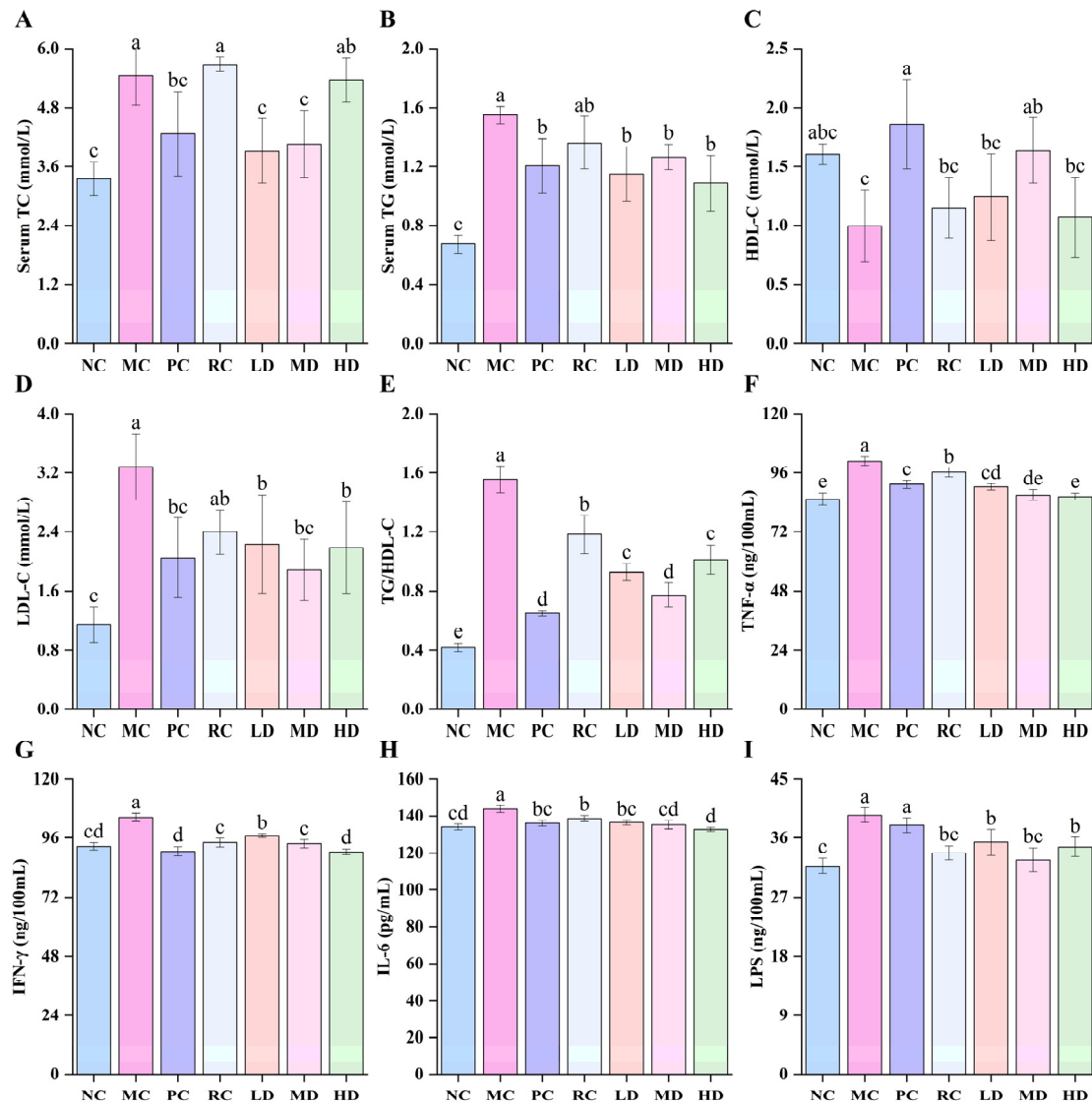


Fig. 1 Serum TC (A); TG (B); HDL-C (C); LDL-C (D); TG/HDL-C (E); TNF- α (F); IFN- γ (G); IL-6 (H); LPS (I) levels in mice fed the experimental diet for 8 weeks. Bars with different letters are significantly different at $P < 0.05$. NC: normal group (normal feed, 0.9% saline); MC: model group (HFD, 0.9% saline); PC: positive group (HFD, 50 mg/kg orlistat); RC: RTJ control group (HFD, 50% RTJ); LD: FRTJ low dose group (HFD, 10% FRTJ); MD: FRTJ medium dose group (HFD, 50% FRTJ); HD: FRTJ high dose group (HFD, 90% FRTJ).

Moreover, HFD-induced obesity is usually accompanied by higher concentrations of serum inflammatory cytokines and LPS^[37]. Endotoxins are large, heat-stable LPS, and elevated endotoxin stimulates an inflammatory response and the secretion of proinflammatory cytokines, triggering metabolic endotoxemia^[38]. As shown in Fig. 1F-I, mice fed HFD had greater levels of TNF- α , IFN- γ , IL-6, and LPS than mice fed a normal diet ($P < 0.05$). This suggested that feeding mice HFD promoted inflammation and

endotoxemia. The levels of TNF- α , IFN- γ , IL-6, and LPS were significantly reduced after FRTJ gavage; the medium dose of FRTJ had the best overall intervention effect, indicating that fermented *Rosa roxburghii* Tratt juice could be used to effectively alleviate inflammation in obese mice.

3.4 FRTJ has the ability to ameliorate oxidative stress in the liver of obese mice

Figs. 2 shows the level of oxidative stress in the liver. Mice's livers after HFD induction showed lower SOD, GSH, and CAT levels and elevated MDA, ALT, and AST levels in comparison to the NC group. Compared with the MC group, FRTJ treatment resulted in a significant decrease in MDA, ALT, and AST and a significant increase in SOD, GSH, and CAT ($P < 0.05$). The levels of SOD, GSH, and CAT were increased by at least 73.2%, 42.2%, and 49.7%, while the levels of MDA, ALT, and AST were decreased by at least 29.7%, 8.6%, and 6.2%, respectively. And the overall effect of medium-dose fermented *Rosa roxburghii* Tratt juice was better. The MDA, ALT, AST, SOD, GSH, and CAT indices were effectively improved by FRTJ supplementation, indicating that FRTJ had a positive effect on improving the hepatic oxidative stress capacity of HFD mice.

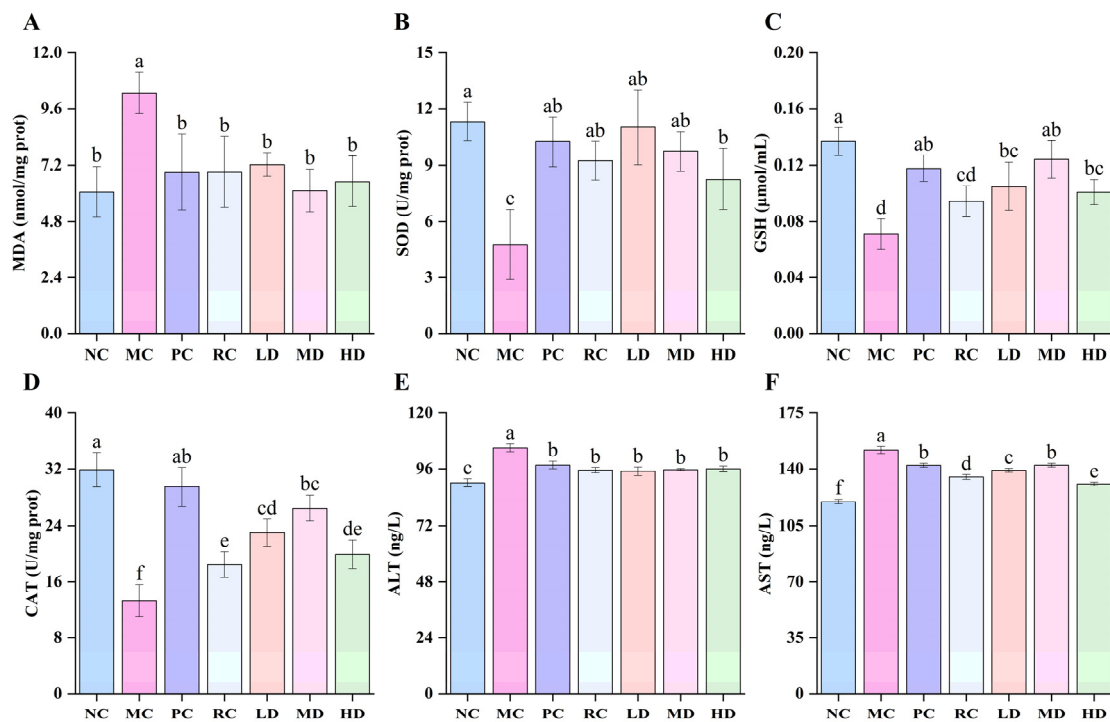


Fig. 2 Oxidative stress in the liver of mice fed an experimental diet for 8 weeks. (A) MDA; (B) SOD; (C) GSH; (D) CAT; (E) ALT; (F) AST. Bars with different letters are significantly different at $P < 0.05$. NC: normal group (normal feed, 0.9% saline); MC: model group (HFD, 0.9% saline); PC: positive group (HFD, 50 mg/kg orlistat); RC: RTJ control group (HFD, 50% RTJ); LD: FRTJ low dose group (HFD, 10% FRTJ); MD: FRTJ medium dose group (HFD, 50% FRTJ); HD: FRTJ high dose group (HFD, 90% FRTJ).

3.5 Effects of FRTJ on the liver, adipocytes and colonic mucosa

HFD-induced obesity can cause liver inflammation, hepatocyte damage, and steatosis, which can lead to the development of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)^[39]. As shown in Fig. 3A, the stained nuclei in NC group were more numerous and more uniform in size, and the distribution of hepatocytes was well arranged. In the MC group, the number of stained nuclei

was lower, the size was not uniform, and a large number of rounded vacuoles appeared in the cytoplasm, and the hepatocytes showed edematous lesions and lipid droplet accumulation. Compared with the MC group, hepatocyte damage was not completely improved in the PC and RC groups, while round vacuoles in the cytoplasm were basically absent, the number of cell nuclei coloring increased, and the phenomenon of hepatocyte edema and lesions was significantly improved after FRTJ supplementation. The MD group among them shown the greatest recovery, indicating that FRTJ may have the ability to reduce lipid droplet accumulation and liver injury.

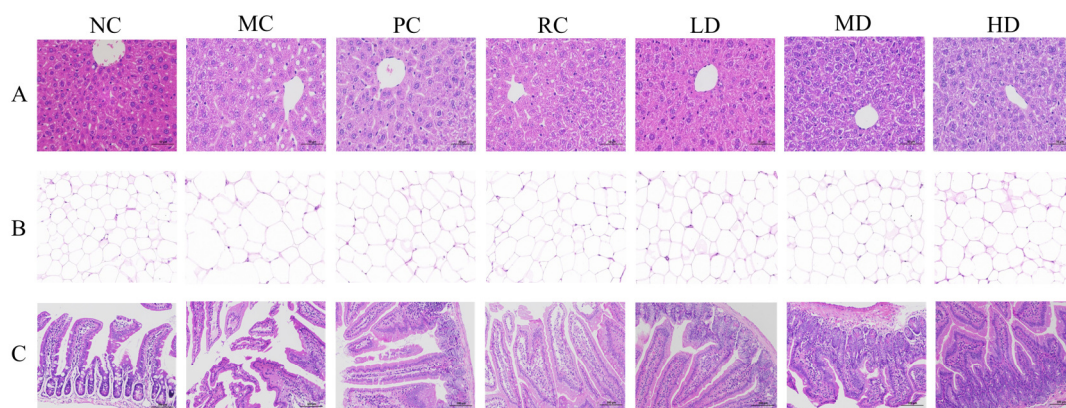


Fig. 3 Effects of FRTJ on the liver, adipocytes and colonic histomorphology. (A) Liver tissue; (B) adipocytes; (C) colon tissue. NC: normal group (normal feed, 0.9% saline); MC: model group (HFD, 0.9% saline); PC: positive group (HFD, 50 mg/kg orlistat); RC: RTJ control group (HFD, 50% RTJ); LD: FRTJ low dose group (HFD, 10% FRTJ); MD: FRTJ medium dose group (HFD, 50% FRTJ); HD: FRTJ high dose group (HFD, 90% FRTJ).

Earlier reports have pointed out that the significance of adipocytes in sensing and managing energy status^[40], and obesity caused by HFD can be characterized on the basis of adipocytes. In addition to storing extra energy in the body, white adipose tissue (WAT) secretes a number of hormones and metabolites that help to maintain the proper balance of body energy^[41]. Therefore, blocking WAT growth can help prevent obesity. As can be seen in Fig. 3B, the number of adipocytes in the NC group was larger and more uniform in size. In contrast, the epididymal adipocytes in the MC group were hypertrophied, with a significant increase in the area of adipocytes, a decrease in their number, and an irregular arrangement. These findings suggested that the HFD was responsible for the adipocytes' increased size and disorderly arrangement. After orlistat, FRTJ or RTJ interventions, there was a notable decrease in cell volume, an increase in cell count, and the morphology of adipocytes in the MD group was closer to that of the NC group. These findings imply that FRTJ inhibited the proliferation of WAT in the epididymis.

HFD-induced obesity would lead to obvious changes in intestinal morphology, which is an important indicator of the integrity of the intestinal mucosa^[42]. Sections of colonic tissues in the NC group showed normal colonic morphology, whereas sections in the MC group showed edema in the mucosal layer. In the MC group, there was infiltration of inflammatory cells, local gaps between the mucosal epithelium and lamina propria, accompanied by acidic substance exudation, and connective tissues were loosely arranged in many places (Fig. 3B). These observations indicated that the high-fat diet caused damage to the colonic mucosa of mice. Compared with the MC group, there was autolysis at the tip of the intestinal villi in the PC

group, and there was a gap between the mucosal epithelium and the lamina propria in the RC group, but eosinophilic exudation was improved. After gavage of FRTJ, the morphology of colon tissue was significantly restored, with intestinal villi distributed on the surface, goblet cells distributed between epithelial cells, and the colonic tissues showed the normal features of crypt (Fig. 3B), which illustrated that the FRTJ intervention could effectively protect mice from damaged colonic mucosa.

3.6 Effect of FRTJ on gut microbiota in obese mice

Growing evidence of a direct causal relationship between gut microbiota and obesity and its associated complications^[43]. Therefore, after 8 weeks of feeding the experimental diet, we sequenced samples of cecum contents to elucidate the effect of FRTJ on the structure of the gut microbiota. The number of OTUs per sample was obtained at 97% similarity. The NC, MC, PC, RC, LD, MD, and HD groups collectively possessed 421 OUT counts, with each group displaying 53, 48, 51, 52, 53, 56, and 61 unique OUTs (Fig. 4 A). The dilution curves were used to assess whether the sequencing data were reasonable and reflected the diversity and abundance of species in samples. As can be seen from the curves in Fig. 4B, the dilution curves of the samples leveled off when the sequencing number exceeded 40,000, indicating that the sequencing number covered almost all the species in the samples. When the sequencing number reaches more than 10000, two groups in the MC group always have the smallest number of species at the same sequencing number, while one group in the NC group has the largest number of species. After the intervention of FRTJ, RTJ and orlistat, the number of OUTs of the samples was between the minimum of the MC group and the maximum of the NC group. The above analysis showed that FRTJ was able to regulate the number of OTUs in the intestinal microbiota of mice and had a reversal effect on the reduction of intestinal microbiota species induced by HFD. Sobs, Chao1, Shannon and Simpson indices are commonly used to measure the microbial diversity and richness of the samples, The larger the Sobs, Chao1 and Shannon indices, the greater the microbial richness and diversity of the samples, whereas Simpson index was negatively correlated with microbial diversity^[44]. As shown in Table 4, the Sobs, Chao1, and Shannon indices of the MC group were significantly reduced ($P < 0.05$) compared with those of the NC group, illustrating that the HFD caused a decrease in the abundance and diversity of gut microbiota in mice. After gavage of FRTJ and RTJ, Sobs, Chao1, and Shannon indices were elevated, with the most significant elevation of the three indices in the HD group and the smallest change in the relevant indices in the PC group, suggesting that high-dose FRTJ has a good regulating effect on both the diversity and abundance of gut microbiota in obese mice. To further understand the effects of HFD and FRTJ supplementation on the structure and composition of the gut microbiota, β -diversity analysis was performed. As shown in Fig. 4 C, D, the MC group differed from the NC group, And from Fig. 4C, we can see that the distance between the minimum and maximum of MC group is bigger, which indicated that the difference within MC group was bigger, indicating that the high-fat diet had caused a significant effect on the species of gut microorganisms in mice. After FRTJ supplementation, the LD and MD groups were distributed on the left and right sides of the NC group, and the branch lengths of the LD, MD, and HD groups were shorter than that of the MC

group, especially the MD group, which was closer to the NC group. It is concluded that FRTJ has a good regulatory effect on the gut microbiota disorder induced by high-fat diet. While the RC and PC groups were farther away from the NC group, indicating that the intervention effect was not as significant as FRTJ.

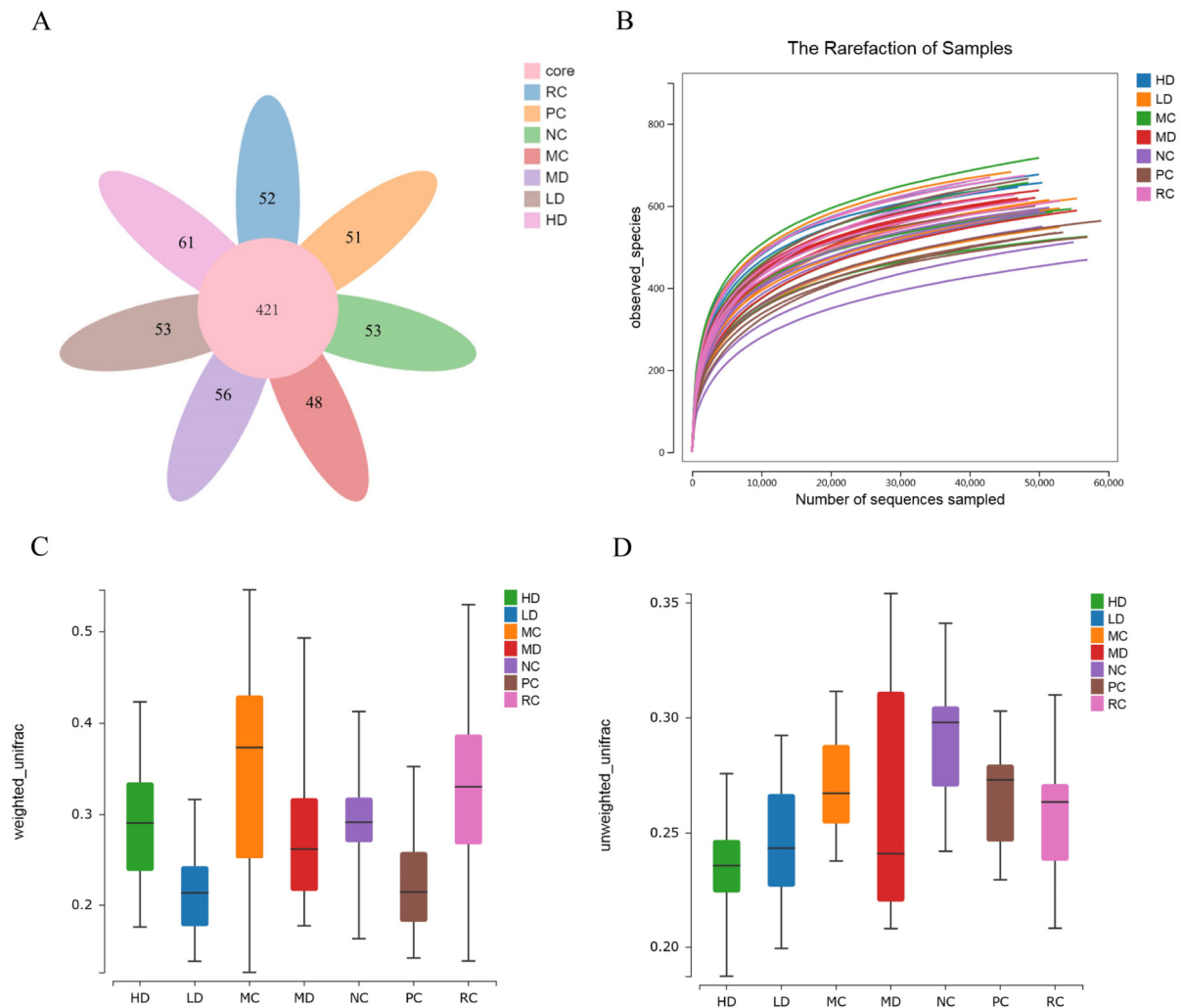


Fig.4 Analysis of the diversity of gut microbiota in obese mice. (A) Venn diagram of sample OTU between different treatment groups; (B) dilution curve; (C) weighted box diagram; (D) unweighted box diagram. NC: normal group (normal feed, 0.9% saline); MC: model group (HFD, 0.9% saline); PC: positive group (HFD, 50 mg/kg orlistat); RC: RTJ control group (HFD, 50% RTJ); LD: FRTJ low dose group (HFD, 10% FRTJ); MD: FRTJ medium dose group (HFD, 50% FRTJ); HD: FRTJ high dose group (HFD, 90% FRTJ).

Table 4. Trends in α -diversity of samples from different treatment groups.

Group	Sobs	Chao1	Shannon	Simpson
NC	603.25 $\pm$ 10.37 ^c	706.61 $\pm$ 9.74 ^{bc}	4.35 $\pm$ 0.11 ^{cd}	0.40 $\pm$ 0.05 ^{ab}
MC	559.00 $\pm$ 9.31 ^d	684.66 $\pm$ 26.07 ^d	4.17 $\pm$ 0.05 ^e	0.42 $\pm$ 0.05 ^a
PC	559.25 $\pm$ 10.75 ^d	663.27 $\pm$ 24.07 ^e	4.26 $\pm$ 0.06 ^{de}	0.32 $\pm$ 0.05 ^{abc}
RC	639.25 $\pm$ 9.70 ^a	722.17 $\pm$ 8.54 ^{ab}	4.40 $\pm$ 0.05 ^{bc}	0.39 $\pm$ 0.03 ^{ab}
LD	603.00 $\pm$ 10.71 ^c	696.00 $\pm$ 10.94 ^{cd}	4.32 $\pm$ 0.07 ^{cd}	0.27 $\pm$ 0.12 ^c
MD	617.50 $\pm$ 3.51 ^b	717.13 $\pm$ 3.05 ^b	4.46 $\pm$ 0.05 ^b	0.31 $\pm$ 0.03 ^{bc}
HD	650.50 $\pm$ 9.29 ^a	738.99 $\pm$ 8.37 ^a	4.56 $\pm$ 0.06 ^a	0.35 $\pm$ 0.03 ^{abc}

Data were mean $\pm$ SD (n = 3), and statistical analyses were performed using one-way analysis of variance (ANOVA). Different superscript lowercase letters in the same column indicate significant differences ($P < 0.05$).

In order to explore the differences in the species composition among groups, this study mainly analysed the distribution of species with higher abundance in phylum, family and genus classifications. As shown in

Fig. 5A, *Verrucomicrobia*, *Tenericutes*, *SRI*, *Acidobacteria*, *Deferribacteres*, *Candidatus Saccharibacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* were the dominant species at the phylum level in all the groups, with relative proportions exceeding 90%. Among them, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* dominated, which was consistent with past studies findings^[8,42]. Compared with the NC group, the relative abundance of *Actinobacteria* and *Firmicutes* in the MC group was dramatically increased ($P < 0.01$), whereas the relative abundance of *Proteobacteria* and *Bacteroidetes* was significantly decreased ($P < 0.01$), making the F/B ratio out of balance. However, FRTJ reversed this situation, reducing the F/B ratio by 40.93%, 52.53%, and 42.21% in the LD, MD, and HD groups, respectively, with the MD group showing the most significant effect, and the improvement of the gut microbiota by each FRTJ dosage group did not show a dose-dependent effect.

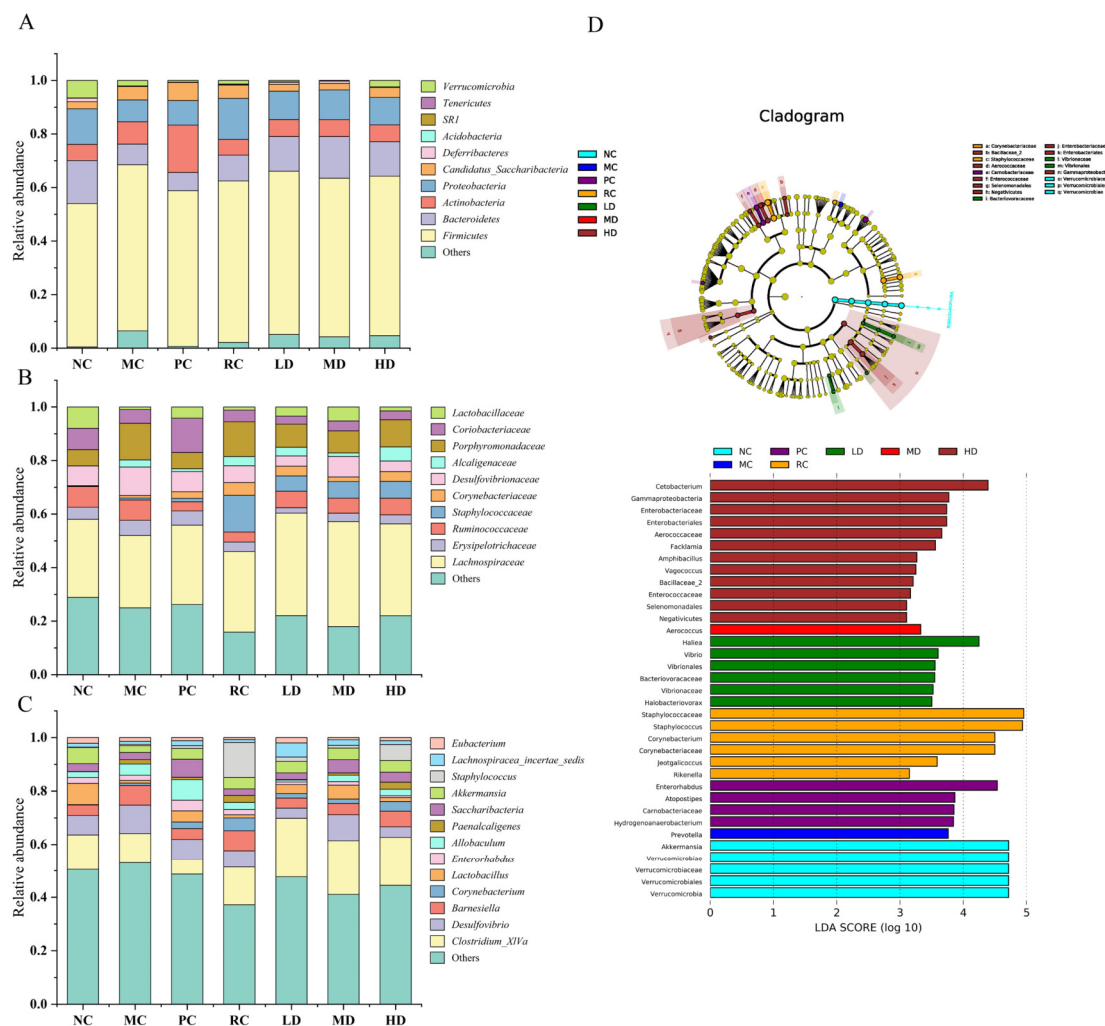


Fig. 5 Effect of FRTJ on gut microbiota of mice and LEfSe analysis of gut microbiota. (A) relative abundance of gut microbiota at the phylum level; (B) relative abundance of gut microbiota at the family level; (C) relative abundance of gut microbiota at the genus level; (D) LEfSe analysis of key genera of mice gut microbiota. In the Cladogram, dots of different colors indicate microbiota that play an important role in the group represented by that color, and yellow dots indicate microbial taxa that do not play an important role in any groups. In the LDA score diagram, the higher the score is, the more important the role is. NC: normal group (normal feed, 0.9% saline); MC: model group (HFD, 0.9% saline); PC: positive group (HFD, 50 mg/kg orlistat); RC: RTJ control group (HFD, 50% RTJ); LD: FRTJ low dose group (HFD, 10% FRTJ); MD: FRTJ medium dose group (HFD, 50% FRTJ); HD: FRTJ high dose group (HFD, 90% FRTJ).

The top 10 bacteria in relative abundance at the family level were shown in Fig. 5B. Compared to the NC group, *Lactobacillaceae* and *Lachnospiraceae* were significantly lower in the MC group ($P < 0.01$),

while *Porphyromonadaceae*, *Alcaligenaceae*, *Desulfovibrionaceae*, and *Erysipelotrichaceae* significantly increased ($P < 0.01$). The relative abundance of *Lactobacillaceae*, *Corynebacteriaceae*, *Staphylococcaceae*, and *Lachnospiraceae* showed a significant increase ($P < 0.01$), and *Porphyromonadaceae*, *Desulfovibrionaceae*, and *Erysipelotrichaceae* showed a significant decrease ($P < 0.01$) after FRTJ gavage compared to the MC group. Interestingly, *Alcaligenaceae* decreased significantly in the MD group, while there was a tendency to increase in the LD and HD groups. Overall, the modulatory effect of medium-dose FRTJ on HFD mice at the family level was more significant. Although RTJ and orlistat also modulated the relative abundance of intestinal microbiota in obese mice, the overall effect was not as effective as that of FRTJ.

Species with a relative abundance higher than 1% at the genus level are shown in Fig. 5C. When compared to the NC group, the MC group exhibited a significant increase in the relative abundance of *Allobaculum*, *Barnesiella*, and *Desulfovibrio* ($P < 0.01$), and a significant decrease in *Akkermansia*, *Lactobacillus*, and *Clostridium_XIVa* ($P < 0.01$). As anticipated, the supplementation with FRTJ led to a substantial reduction in the relative abundance of *Allobaculum*, *Barnesiella*, and *Desulfovibrio* by at least 40.06%, 20.52%, and 9.17%, respectively. Conversely, the relative abundance of *Akkermansia*, *Lactobacillus*, and *Clostridium_XIVa* was notably increased by at least 68.53%, 46.99%, and 63.95%, respectively, following FRTJ supplementation. However, it is interesting to note that *Allobaculum* increased significantly by 83.48% after orlistat treatment instead, whereas *Barnesiella* did not improve after RTJ treatment.

The above comparative analysis at the phylum, family, and genus levels showed that the modulating effect of FRTJ on the structure and relative abundance of gut microbiota was significantly better than that of RTJ and orlistat, and the effect of medium-dose FRTJ was the best, demonstrating that the gavage of FRTJ has a significant effect on the regulation of obesity-induced gut microbiota disorders.

To further identify specific bacterial taxa that responded to the different treatments, a LEfSe analysis ($\text{LDA} > 2$) was performed to compare the microbial community among the seven groups. As shown in Fig. 5D, a total of 35 OUTs were selected as iconic bacteria, with *Akkermansia*, *Verrucomicrobia*, *Verrucomicrobiales*, *Verrucomicrobiaceae*, and *Verrucomicrobiae* showing higher LDA scores in the NC group, indicating that the abundance of these bacteria was significant in the NC group. In contrast, only *Prevotella* was present in the MC group. Past findings revealed that *Prevotella* is associated with metabolic disease and low-grade systemic inflammation, which can promote chronic inflammation^[45], suggesting that HFD can alter the balance of gut microorganisms and the abundance of associated bacteria, causing an enrichment of *Prevotella*. And significant control of *Prevotella* after gavage of FRTJ. The number of significantly different species in the LD and HD groups was 6 and 12, respectively, which was significantly higher than that in the MC group. And there was also only one signature bacterium: *Aerococcus*, in the MD group. This suggests that the effects of different doses of FRTJ on the intestinal microbiota of HFD mice

were somewhat different, but the overall effect was helpful in improving the disorder of the intestinal microbiota.

3.7 Correlation between gut microbiota and obesity-related parameters in obese mice

The top 15 bacterial species with high correlation at the phylum, family, and genus level were selected for correlation analysis with obesity-related parameters. As shown in Fig. 6, *Allobaculum*, *Paenalcaligenes*, *Barnesiella*, *SR1*, *Porphyromonadaceae*, *Firmicutes* and *Deferribacteres* were positively correlated with body weight, among them, *Barnesiella*, *Porphyromonadaceae* and *Firmicutes* were positively correlated with ALT, LPS, AST, IFN- γ , TNF- α , TC, TG, LDL-C, MDA, IL-6 indexes, and *Paenalcaligenes*, *Barnesiella*, *Porphyromonadaceae* and *Firmicutes* were negatively correlated with HDL-C, SOD, CAT and GSH; *Bacteroidetes*, *Acidobacteria*, *Proteobacteria*, *Lactobacillaceae*, *Lactobacillus*, *Tenericutes* and *Saccharibacteria* were negatively correlated with body weight, IFN- γ , TNF- α , TC, TG, LDL-C, MDA, IL-6, and positively correlated with GSH, CAT, HDL-C indexes, these bacteria may exert a positive influence on preventing obesity caused by HFD. In short, these bacteria were linked to weight gain, dyslipidaemia, and oxidative stress in mice. The above studies showed that FRTJ treatment can improve gut microbiota disorders, enhance immunity, and control weight gain by reducing harmful bacteria (*SR1*, *Firmicutes*, *Porphyromonadaceae*, *Barnesiella*, etc.) and modifying beneficial bacteria (*Saccharibacteria*, *Lactobacillus*, *Lactobacillaceae*, *Bacteroidetes*, etc.).

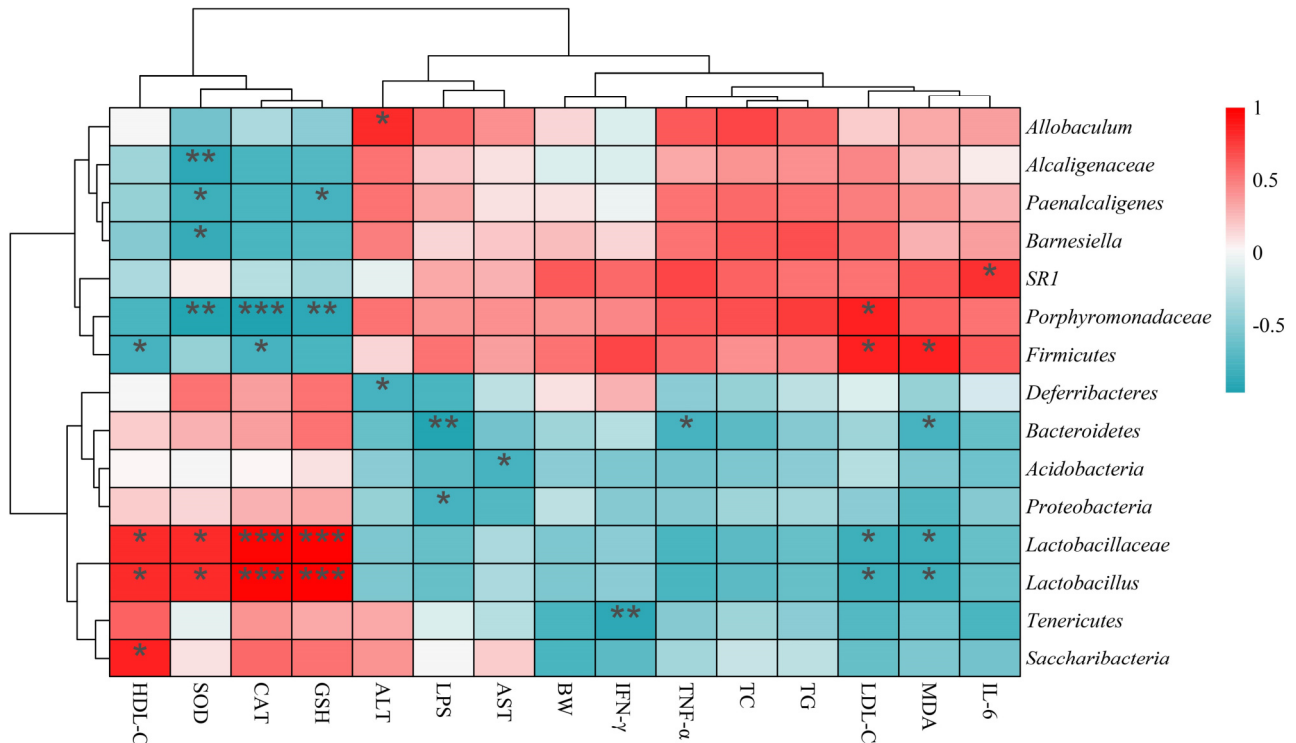


Fig. 6 Heatmap of Spearman's correlation between gut microbiota and obesity-related parameters. Blue indicates a negative correlation, while red indicates a positive correlation. *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$). HDL-C: high-density lipoprotein cholesterol; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; ALT: alanine aminotransferase; LPS: lipopolysaccharide; AST: aspartate aminotransferase; BW: body weight; IFN- γ : interferon- γ ; TNF- α : tumour necrosis factor- α ; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; MDA: malondialdehyde; IL-6: interleukin 6.

3. 8 Effect of FRTJ on antibiotic intervention in obese mice

In order to verify the potential mechanism of FRTJ against obesity, a comparative antibiotic experiment was conducted to eliminate the majority of microorganisms from the obese mice's guts in order to investigate whether the gut microbiota would affect the therapeutic efficacy of FRTJ. We treated the animals in the HFD + A and HFD+ A+ R groups with antibiotics to lower their gut microbiota. Compared to the MC group, antibiotic treatment caused a dramatic change in the composition of the gut microbiota of obese mice. At the phylum level, the abundance of *Proteobacteria* was significantly increased ($P < 0.01$) in the HFD+A and HFD+A+R groups, but the abundance of *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* was significantly decreased ($P < 0.01$) (Fig. 7G). At the family level, the abundance of the *Enterococcaceae* and *Enterobacteriaceae* had an anomalously higher relative abundance, while *Coriobacteriaceae*, *Erysipelotrichaceae*, *Porphyromonadaceae*, *Lactobacillaceae*, *Desulfovibrionaceae*, *Lachnospiraceae*, and *Ruminococcaceae* had a significantly lower abundance (Fig. 7H). At the genus level, the relative abundance of *Klebsiella*, *Enterococcus*, *Vagococcus*, and *Escherichia* was significantly increased, while that of *Barnesiella*, *Clostridium_XIVa*, *Lactobacillus*, and *Desulfovibrio* was significantly reduced (Fig. 7I). However, after antibiotic treatment and then consumption of FRTJ, the results showed a failure to repair or adjust the relative abundance and species of gut microbiota, especially some beneficial bacteria associated with obesity metabolism (*Lactobacillus*, *Lactobacillaceae*, *Clostridium_XIVa*, *Saccharibacteria*, *Bacteroidetes*, *Tenericutes*, *Acidobacteria*, and *Proteobacteria*), which were not improved.

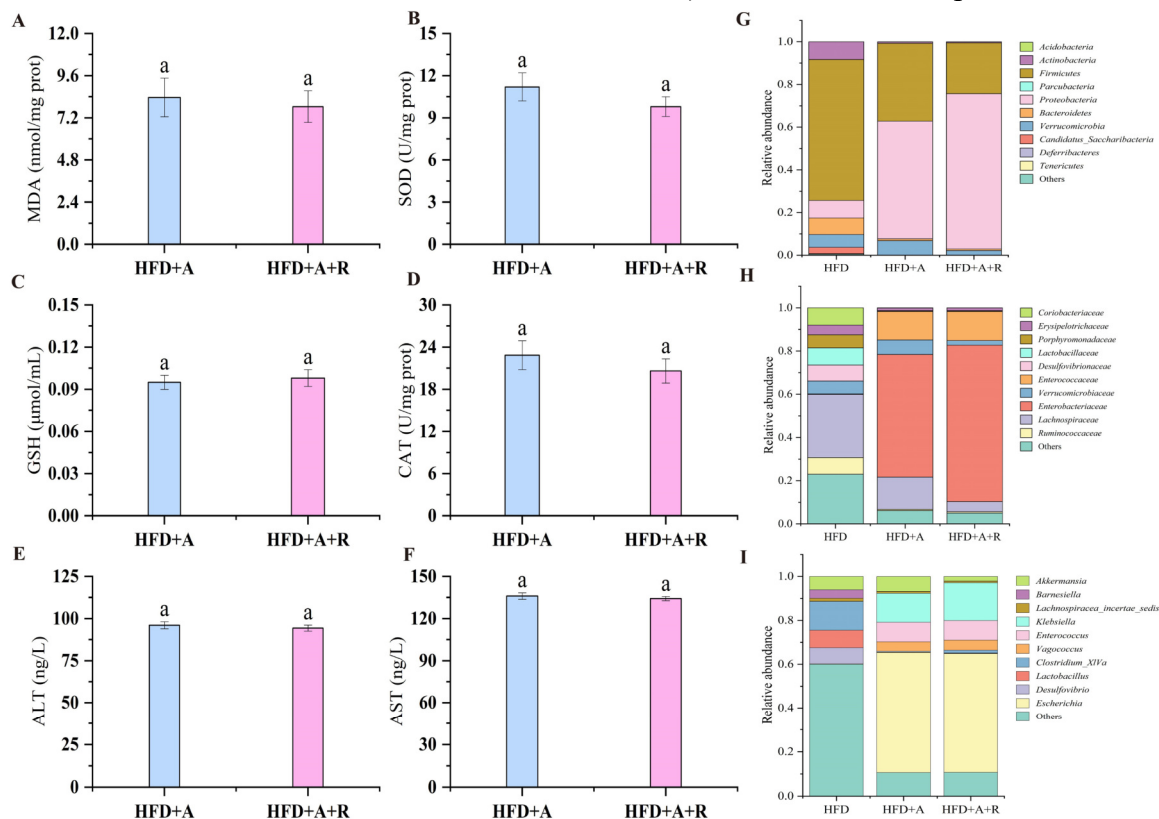


Fig. 7 Physicochemical indices of mice liver and the relative abundance of intestinal microbiota at phylum, family and genus levels. MC: model group (HFD, 0.9% saline); HFD+A: antibiotic control group (HFD, antibiotic); HFD+A+R: antibiotic dose group (HFD, antibiotic, 50% FRTJ).

Besides, FRTJ supplementation had no effect on HFD-induced weight gain in mice after antibiotic treatment, and the body weight of mice in the HFD+A group was slightly higher than that in the HFD+A+R group, and the difference was not significant ($P > 0.05$) (Figs. 8). In antibiotic-treated obese mice, gavage of FRTJ did not improve serum indices (Fig. 9A-D), inflammatory factors (Figure 9E-H), and hepatic oxidative stress indices (Fig. 7A-F). Additionally, histological examination of liver sections, epididymal fat, and colonic mucosa showed that antibiotics blocked the mitigation effects of FRTJ on hepatic steatosis, adipocyte hypertrophy, and colonic mucosal damage in mice fed a high-fat diet (Figs. 8). In summary, depletion of the gut microbiota eliminated the obesity-preventing properties of FRTJ, suggesting that FRTJ's capacity to alleviate obesity triggered by a high-fat diet is mainly achieved by modulating the gut microbiota.

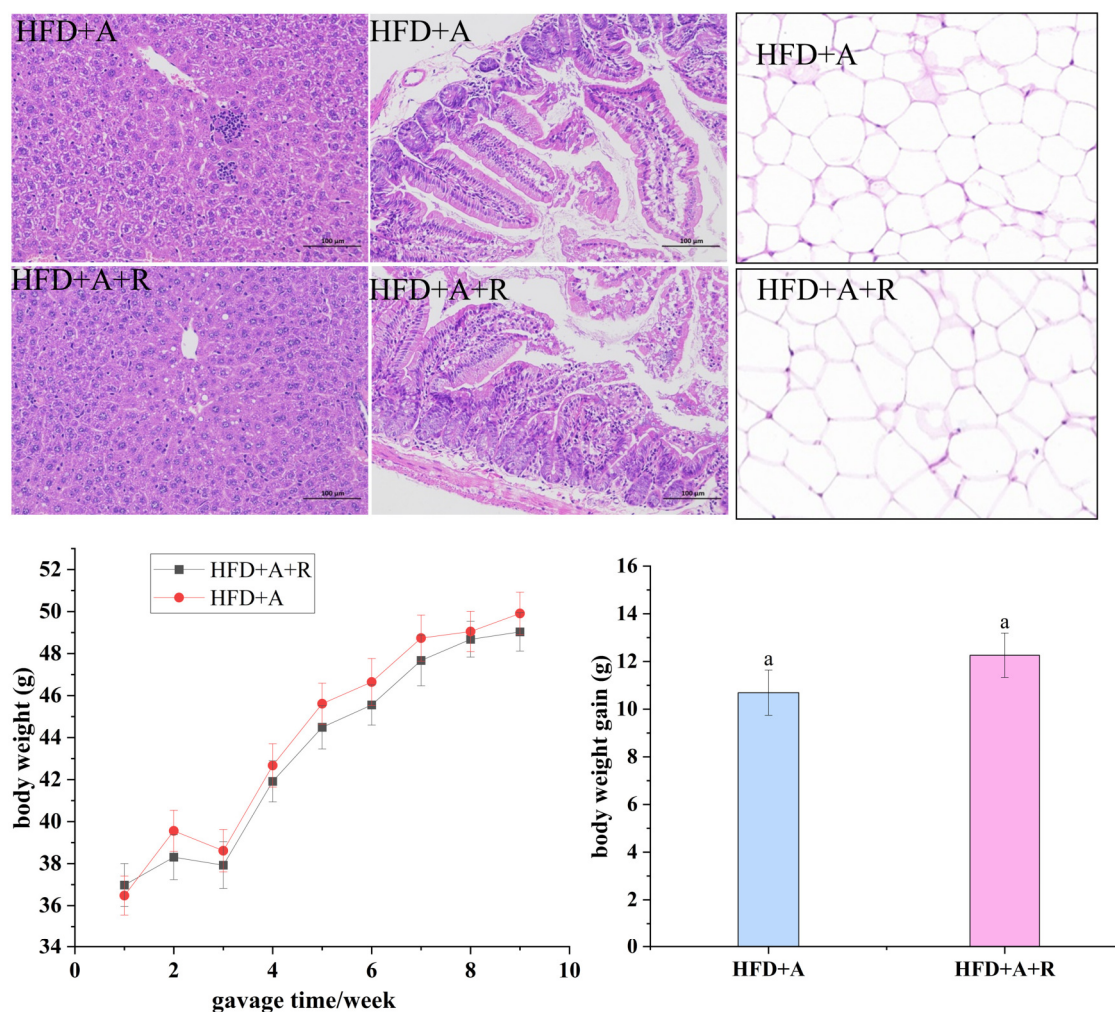


Fig. 8 Tissue sections of liver, colon and epididymis and body weight growth curves of mice. HFD+A: antibiotic control group (HFD, antibiotic); HFD+A+R: antibiotic dose group (HFD, antibiotic, 50% FRTJ).

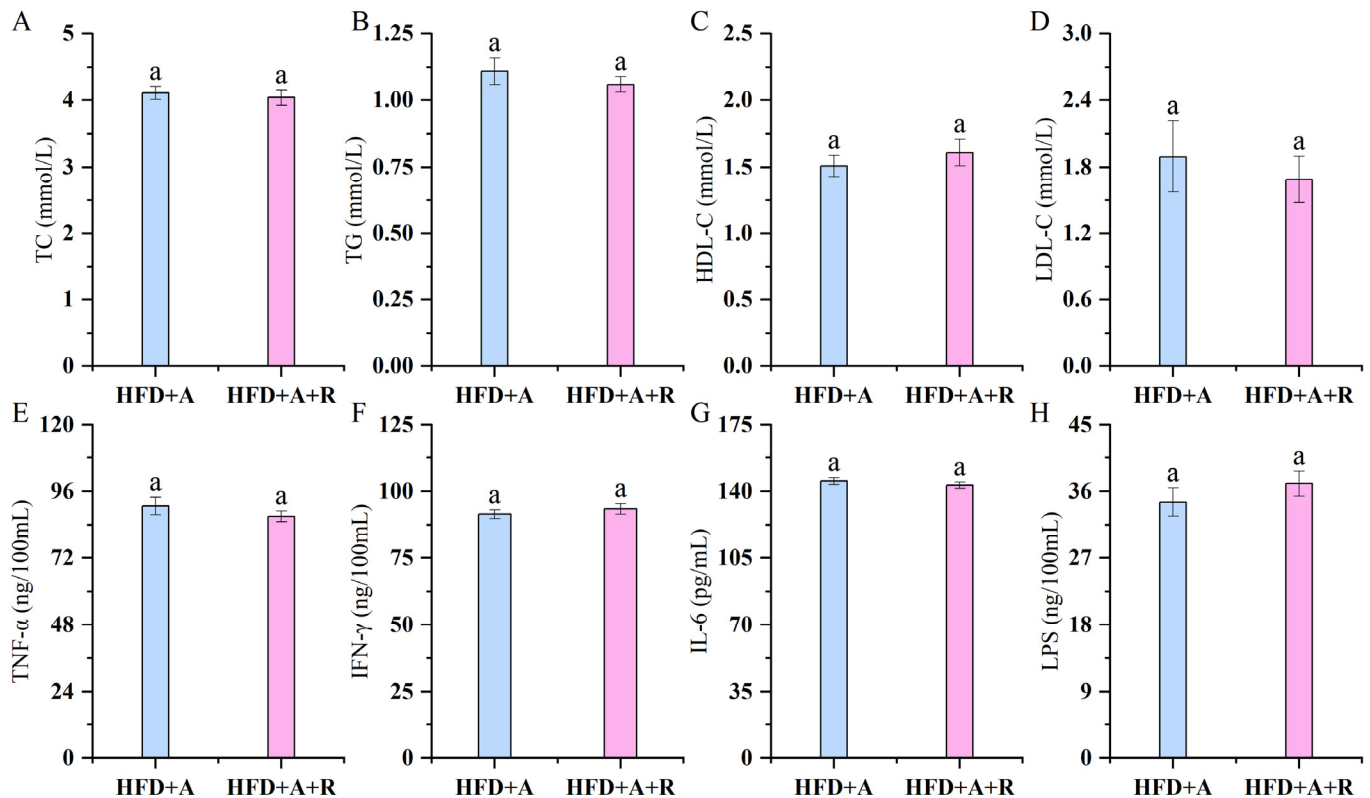


Fig. 9 Serum indices of mice. HFD+A: antibiotic control group (HFD, antibiotic); HFD+A+R: antibiotic dose group (HFD, antibiotic, 50% FRTJ).

4. Discussion

Obesity is a global metabolic epidemic linked to various diseases like type II diabetes, cardiovascular disease, and NAFLD. The gut microbiota and its metabolites play a crucial role in the development of obesity [3,46]. Nutrient-rich fermented fruit juices can regulate gut microbiota and promote weight loss [10,11]. The FRTJ used in this study also contained bioactive compounds like VC, SOD, polyphenols, flavonoids, and organic acids. Fermentation improved the nutrient balance in *Rosa roxburghii* Tratt juice and added acetic acid (5.50 ± 1.28) mg/mL (Table 1). Yamashita et al. [34] proved that acetic acid had a significant function in ameliorating obesity in rats. Based on the above studies, FRTJ may play a role in controlling the gut microbiota and reducing obesity. Thus, we established an HFD-induced obesity mouse model to study FRTJ's effects on obesity and associated metabolic syndrome.

Overconsumption of high-fat foods causes obesity and related metabolic disorders, which are typically manifested as weight gain and dramatic changes in physiological and biochemical parameters in the serum. Our study's findings showed that, in comparison to the NC group, the HFD led to fat accumulation, dyslipidemia, and liver injury in the mice. Nevertheless, following an 8-week FRTJ intervention, the obese mice's final body weights did not differ substantially from the NC group's ($P > 0.05$) (Table 2), suggesting that the mice's weight increase was successfully managed. Feeding HFD significantly increased serum levels of TC, TG, and LDL-C and markedly reduced serum levels of HDL-C in mice (Figs. 1), which was consistent with previous studies [35]. In this study, FRTJ intake not only improved the serum levels of TC, TG, HDL-C, LDL-C, and TG/HDL-C in obese mice but also significantly improved the abnormal levels of

TNF- α , IFN- γ , IL-6, LPS, MDA, ALT, AST, SOD, GSH, and CAT (Figs. 1, 2), and FRTJ was significantly better than RTJ, especially in the MD group. These data illustrated that fermentation had endowed FRTJ with better activity, and FRTJ had the potential to improve hepatic oxidative stress, reduce obesity, and mitigate dyslipidemia.

H&E staining results showed that hepatocyte degeneration and lipid droplet aggregation were improved after FRTJ supplementation and were most significant in the MD group (Fig. 3A), while the RTJ group was not effectively improved, indicating that medium-dose FRTJ has a potential role in preventing liver injury and lipid droplet accumulation. The morphology of adipocytes in the MD group was closer to that of the NC group (Fig. 3B), which suggests that the FRTJ intervention can inhibit the proliferation of WAT in obese mice. Studies have shown that villus length and crypt depth can reflect the health of the gut^[47]. In this experiment, we observed that after gavage of FRTJ, intestinal villi and crypt were visible on the surface of the intestinal surface, and the morphology of the colonic mucosal tissue was basically restored to normal (Fig. 3C), and the overall effect was still better than that of RTJ.

In this study, since HFD-induced obesity is associated with gut microbiota dysbiosis and the anti-obesity effect of FRTJ may be closely related to gut microbiota modulation, we used 16S rRNA sequencing to analyze the intestinal flora of each group of mice. The results showed that at the phylum level, the relative abundance of the *Actinobacteria* and *Firmicutes* in the MC group was significantly increased ($P < 0.01$), while the relative abundance of the *Proteobacteria* and *Bacteroidetes* was significantly decreased ($P < 0.01$)(Fig. 5A), resulting in an increase in the F/B ratio. Research has demonstrated that *Firmicutes* possess the ability to ferment and metabolize carbohydrates and lipids, which can predispose the host to obesity^[52], while *Bacteroidetes* produce SCFAs during metabolism, promoting energy supply for intestinal epithelial cells and suppressing inflammatory responses in the gut^[49]. Furthermore, an elevated F/B ratio is associated with obesity, and the restoration of the imbalanced F/B ratio through dietary supplementation can serve as a therapeutic or preventive strategy for obesity^[9]. Additionally, it has been found that *Actinobacteria* is positively associated with obesity, while *Proteobacteria* is negatively associated with weight gain in mice^[2,48]. This study showed that FRTJ intervention significantly altered the relative abundance of *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* and reduced the F/B value, indicating that FRTJ has a positive effect on regulating the gut microbiota to normal levels.

At the family level, we found that FRTJ intervention significantly increased the relative abundance of *Lactobacillaceae*, *Corynebacteriaceae*, *Staphylococcaceae*, and *Lachnospiraceae* and decreased the relative abundance of *Porphyromonadaceae*, *Desulfovibrionaceae*, and *Erysipelotrichaceae* in the intestines of obese mice. It was interesting to note that *Alcaligenaceae* showed a tendency to increase in the LD and HD groups but significantly decreased in the MD group (Fig. 5B). According to Liu et al.^[49], *Lactobacillaceae* in the intestines of mice significantly reduced after a 12-week high-fat diet, which is consistent with our study. *Corynebacteriaceae* were found to be significantly reduced in the mouse model of diabetes and negatively correlated with hyperglycaemia^[50]. Wang et al.^[51] found decreased levels of

Staphylococcaceae and increased levels of *Alcaligenaceae* in colitis mice. Besides, *Lachnospiraceae* can produce or modulate butyrate to maintain the integrity of the intestinal barrier, and its relative abundance is negatively correlated with serum lipid levels^[52]. *Alcaligenaceae* is found in greater abundance in the guts of obese and NASH patients than in healthy people, according to research by Zhu et al.^[53]. According to reports, *Porphyromonadaceae* was increased in people suffering from spondyloarthropathy and induced associated inflammation^[54]. Moreover, *Desulfovibrionaceae* belongs to sulphate-reducing bacteria and can produce endotoxins. It can damage intestinal mucosa, disrupt intestinal barrier function and trigger inflammation^[55]. Cheng et al.^[56] reported that *Erysipelotrichaceae* showed higher abundance in HFD-induced obese mice, enhancing the host's ability to extract energy from the diet and thereby promoting the development of obesity-related phenotypes and lipid metabolism disorders. Our research revealed that, in contrast to the MC group, FRTJ consumption effectively up-regulates *Lactobacillaceae*, *Lachnospiraceae*, *Staphylococcaceae*, and *Corynebacteriaceae*, which have an important role in reducing obesity at the family level.

At the genus level, the relative abundance of *Allobaculum*, *Barnesiella* and *Desulfovibrio* decreased significantly after FRTJ supplementation, whereas the relative abundance of *Akkermansia*, *Lactobacillus* and *Clostridium_XIVa* increased significantly (Fig. 5C). The relative abundance of *Allobaculum* in the intestines of obese mice was significantly higher than that of mice in the normal diet group^[2], and is involved in the development of NASH^[57]. Recent findings showed that *Barnesiella* is positively associated with liver fat accumulation in mice on a high-fat diet^[58]. According to Hu et al.^[59], *Desulfovibrio* is a Gram-negative bacterium capable of releasing LPS into the bloodstream, thereby contributing to obesity and inflammation. In addition, *Akkermansia* and *Lactobacillus* are considered probiotics that help alleviate obesity by regulating lipid metabolism, glucose uptake, and immune responses^[59,60]. The relative abundance of *Clostridium XIVa* has been reported to be significantly negatively correlated with body weight, serum TG, TC and LDL-C in obese mice^[46]. In summary, our results illustrated that FRTJ was indeed effective in alleviating the imbalance of gut microbiota and obesity in obese mice.

Furthermore, the relative balance of *Allobaculum*, *Paenicaligenes*, *Barnesiella*, *SRI*, *Porphyromonadaceae*, *Firmicutes*, *Deferribacteres*, *Bacteroidetes*, *Acidobacteria*, *Proteobacteria*, *Lactobacillaceae*, *Lactobacillus*, *Tenericutes*, and *Saccharibacteria* had a significant impact on body weight and related chronic diseases in mice, according to the Spearman's correlation heatmap. It was noteworthy that *Porphyromonadaceae* showed highly significant differences with SOD ($P < 0.01$), CAT ($P < 0.001$), and GSH ($P < 0.01$), *Lactobacillaceae* and *Lactobacillus* showed highly significant differences with CAT and GSH ($P < 0.001$), indicating that these three bacteria have a great influence on whether they can restore the level of hepatic oxidative stress in mice. In brief, the correlation analysis provided a reference for further exploration of the direct relationship between gut microbiota and obesity and its metabolic syndrome.

In this experiment, we also performed antibiotic comparison experiments to illustrate the direct causal relationship between obesity and the intestinal microbiota. The results demonstrated that the species and

balance of intestinal microbiota in HFD mice were disrupted by antibiotics, and the dominant gut microbiota was significantly altered. *Enterobacteriaceae*, *Enterococcus*, and *Escherichia*, the pathogenic bacteria associated with intestinal inflammation, increased significantly after antibiotic treatment (Figs. 7). Pathogenic bacteria such as *Enterobacteriaceae* and *Enterococcus* can cause inflammatory diseases by promoting the release of LPS^[61]. *Escherichia* has been reported to be a possible risk factor for the progression of the disease from obesity to NASH^[53]. FRTJ gavage, however, was found to have no significant ameliorative effect on body weight, serum parameters, hepatic steatosis, adipocyte hypertrophy, and colonic mucosal damage in obese mice treated with antibiotics. Therefore, this experiment proved that FRTJ can improve obesity induced by HFD by modulating the composition of intestinal microbiota.

In this study, FRTJ showed better effects than RTJ, which may be due to the secondary metabolites produced by the microorganisms that utilize the nutrients (total polyphenols or total flavonoids) after fermentation, as well as some enzymes produced by probiotic fermentation, which help to break down the macromolecules in the juice so that they can be more easily absorbed and utilized by the mice^[62]. In addition, acetic acid, a small bioactive molecule, was produced after fermentation. At the genus level, *Barnesiella* is a specific strain altered by FRTJ compared to RTJ. In summary, it can be stated that FRTJ showed better effects than RTJ, and it has great potential as a functional food for the prevention of obesity and related complications. Although our study demonstrates the potential of FRTJ in alleviating obesity in a high-fat diet-induced mouse model, further human clinical trials and mechanistic investigations are necessary to confirm its efficacy and safety as a functional food for obesity prevention, thereby providing stronger evidence for its potential applications in human health. Notably, FRTJ contains a variety of bioactive components, such as flavonoids and organic acids, which may have a mitigating effect on obesity independently of the gut microbiota and deserve further investigation.

5. Conclusion

This study found that RTJ and FRTJ improved body weight, serum indices, liver indices, and intestinal microbiota diversity in obese mice, with FRTJ showing superior effects. These results demonstrated that probiotic fermentation enhances the health benefits of *Rosa roxburghii* Tratt and provides new insights into its weight loss effects. In conclusion, this research proved that *Rosa roxburghii* Tratt juice fermented by *Lactobacillus paracasei* SR10-1 and *Lactobacillus fermentum* GZSC-1 can prevent HFD-induced obesity and related complications by up-regulating beneficial bacteria and down-regulating harmful bacteria of the gut microbiota, and it has great potential as a functional food to reduce obesity and related complications. However, exactly which functional metabolites produced by which microbial taxa play a role remains unclear. Future studies could employ in-depth analyses using metagenomics and metabolomics to identify the key microbial species and their metabolic pathways involved in the amelioration of obesity by FRTJ, which would contribute to a more comprehensive understanding of FRTJ's mechanisms of action.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgments

This work was supported by *Rosa roxburghii* industry development program of Guizhou Province, China (QCN2019-261), the National Natural Science Foundation of China (31960485) and the National Natural Science Foundation of China (32460606).

References

- [1] Y. Ding, Z. Song, H. Li, et al., Honokiol ameliorates high-fat-diet-induced obesity of different sexes of mice by modulating the composition of the gut microbiota, *Front. Immunol.* 10 (2019) 2800. <https://doi.org/10.3389/fimmu.2019.02800>.
- [2] B. Li, Q. Mao, R. Xiong, et al., Preventive effects of different black and dark teas on obesity and non-alcoholic fatty liver disease and modulate gut microbiota in high-fat diet fed mice, *Foods* 11 (2022) 3457. <https://doi.org/10.3390/foods11213457>.
- [3] Y. Zhao, J. Bi, J. Yi, et al., Dose-dependent effects of apple pectin on alleviating high fat-induced obesity modulated by gut microbiota and SCFAs, *Food Science and Human Wellness* 11 (2022) 143–154. <https://doi.org/10.1016/j.fshw.2021.07.015>.
- [4] D.H. Bessesen, L.F. Van Gaal, Progress and challenges in anti-obesity pharmacotherapy, *The Lancet Diabetes & Endocrinology* 6 (2018) 237–248. [https://doi.org/10.1016/S2213-8587\(17\)30236-X](https://doi.org/10.1016/S2213-8587(17)30236-X).
- [5] E. Le Chatelier, T. Nielsen, J. Qin, et al., Richness of human gut microbiome correlates with metabolic markers, *Nature* 500 (2013) 541–546. <https://doi.org/10.1038/nature12506>.
- [6] M.J. Villanueva-Millán, P. Pérez-Matute, J.A. Oteo, Gut microbiota: A key player in health and disease. A review focused on obesity, *J Physiol Biochem* 71 (2015) 509–525. <https://doi.org/10.1007/s13105-015-0390-3>.
- [7] P. Liu, W. Zhou, W. Xu, et al., The main anthocyanin monomer from lycium ruthenicum murray fruit mediates obesity via modulating the gut microbiota and improving the intestinal barrier, *Foods* 11 (2021) 98. <https://doi.org/10.3390/foods11010098>.
- [8] Z. Liu, N. Wang, Y. Ma, et al., Hydroxytyrosol improves obesity and insulin resistance by modulating gut microbiota in high-fat diet-induced obese mice, *Front. Microbiol.* 10 (2019) 390. <https://doi.org/10.3389/fmicb.2019.00390>.
- [9] S. Stojanov, A. Berlec, B. Štrukelj, The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease, *Microorganisms* 8 (2020) 1715. <https://doi.org/10.3390/microorganisms8111715>.
- [10] H. Zhong, Abdullah, L. Deng, et al., Probiotic-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota, *Food Funct.* 11 (2020) 9192–9207. <https://doi.org/10.1039/D0FO00334D>.
- [11] Q. Guan, T. Xiong, M. Xie, Influence of probiotic fermented fruit and vegetables on human health and the related industrial development trend, *Engineering* 7 (2021) 212–218. <https://doi.org/10.1016/j.eng.2020.03.018>.
- [12] N. Kobyliak, C. Conte, G. Cammarota, et al., Probiotics in prevention and treatment of obesity: A critical view, *Nutr Metab (Lond)* 13 (2016) 14. <https://doi.org/10.1186/s12986-016-0067-0>.
- [13] S. Shirvani-Rad, O. Tabatabaei-Malazy, S. Mohseni, et al., Probiotics as a complementary therapy for management of obesity: A systematic review, *Evidence-Based Complementary and Alternative Medicine* 2021 (2021) 1–11. <https://doi.org/10.1155/2021/6688450>.
- [14] L.-T. Wang, M.-J. Lv, J.-Y. An, et al., Botanical characteristics, phytochemistry and related biological activities of *rosa roxburghii* tratt fruit, and its potential use in functional foods: A review, *Food Funct.* 12 (2021) 1432–1451. <https://doi.org/10.1039/D0FO02603D>.
- [15] G. Chen, J. Kan, Characterization of a novel polysaccharide isolated from *rosa roxburghii* tratt fruit and assessment of its antioxidant in vitro and in vivo, *International Journal of Biological Macromolecules* 107 (2018) 166–174. <https://doi.org/10.1016/j.ijbiomac.2017.08.160>.

- [16] M.L. Marco, M.E. Sanders, M. Gänzle, et al., The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on fermented foods, *Nat Rev Gastroenterol Hepatol* 18 (2021) 196–208. <https://doi.org/10.1038/s41575-020-00390-5>.
- [17] Y. Feng, M. Zhang, A.S. Mujumdar, et al., Recent research process of fermented plant extract: A review, *Trends in Food Science & Technology* 65 (2017) 40–48. <https://doi.org/10.1016/j.tifs.2017.04.006>.
- [18] R. Hu, F. Zeng, L. Wu, et al., Fermented carrot juice attenuates type 2 diabetes by mediating gut microbiota in rats, *Food Funct.* 10 (2019) 2935–2946. <https://doi.org/10.1039/C9FO00475K>.
- [19] Y. Wang, H. Li, Y. Ren, et al., Preparation, model construction and efficacy lipid-lowering evaluation of kiwifruit juice fermented by probiotics, *Food Bioscience* 47 (2022) 101710. <https://doi.org/10.1016/j.fbio.2022.101710>.
- [20] B. Escudero-López, G. Berná, Á. Ortega, et al., Consumption of orange fermented beverage reduces cardiovascular risk factors in healthy mice, *Food and Chemical Toxicology* 78 (2015) 78–85. <https://doi.org/10.1016/j.fct.2015.02.006>.
- [21] Z.-P. Zhang, J. Ma, Y.-Y. He, et al., Antioxidant and hypoglycemic effects of diospyros lotus fruit fermented with microbacterium flavum and lactobacillus plantarum, *Journal of Bioscience and Bioengineering* 125 (2018) 682–687. <https://doi.org/10.1016/j.jbiosc.2018.01.005>.
- [22] S.M.B. Hashemi, A. Mousavi Khaneghah, F.J. Barba, et al., Fermented sweet lemon juice (citrus limetta) using lactobacillus plantarum LS5: Chemical composition, antioxidant and antibacterial activities, *Journal of Functional Foods* 38 (2017) 409–414. <https://doi.org/10.1016/j.jff.2017.09.040>.
- [23] D. Wang, Y. Deng, L. Zhao, et al., GABA and fermented litchi juice enriched with GABA promote the beneficial effects in ameliorating obesity by regulating the gut microbiota in HFD-induced mice, *Food Funct.* 14 (2023) 8170–8185. <https://doi.org/10.1039/D2FO04038G>.
- [24] Y. Zuo, H. Xu, D. Feng, et al., Effect of fermented *rosa roxburghii* tratt fruit juice on gut microbiota in a dextran sulfate sodium (DSS)-induced colitis mouse model, *Food Science and Human Wellness* 13 (2024) 2234–2243. <https://doi.org/10.26599/FSHW.2022.9250186>.
- [25] T. Ma, X. Shen, X. Shi, et al., Targeting gut microbiota and metabolism as the major probiotic mechanism - an evidence-based review, *Trends in Food Science & Technology* 138 (2023) 178–198. <https://doi.org/10.1016/j.tifs.2023.06.013>.
- [26] S. Shi, Y. Wei, X. Lin, et al., Microbial metabolic transformation and antioxidant activity evaluation of polyphenols in kombucha, *Food Bioscience* 51 (2023) 102287. <https://doi.org/10.1016/j.fbio.2022.102287>.
- [27] J. Li, J. Zhang, Y. Zhang, et al., Effect and correlation of rosa roxburghii tratt fruit vinegar on obesity, dyslipidemia and intestinal microbiota disorder in high-fat diet mice, *Foods* 11 (2022) 4108. <https://doi.org/10.3390/foods11244108>.
- [28] Q. Liu, G.-Y. Tang, C.-N. Zhao, et al., Antioxidant activities, phenolic profiles, and organic acid contents of fruit vinegars, *Antioxidants* 8 (2019) 78. <https://doi.org/10.3390/antiox8040078>.
- [29] D. Zhao, J. Cao, H. Jin, et al., Beneficial impacts of fermented celery (*apium graveolens* L.) juice on obesity prevention and gut microbiota modulation in high-fat diet fed mice, *Food Funct.* 12 (2021) 9151–9164. <https://doi.org/10.1039/D1FO00560J>.
- [30] C. Sun, A. Li, H. Wang, et al., Positive regulation of acetate in adipocyte differentiation and lipid deposition in obese mice, *Nutrients* 15 (2023) 3736. <https://doi.org/10.3390/nu15173736>.
- [31] X. Jin, W. Chen, H. Chen, et al., Comparative evaluation of the antioxidant capacities and organic acid and volatile contents of mango slurries fermented with six different probiotic microorganisms, *Journal of Food Science* 83 (2018) 3059–3068. <https://doi.org/10.1111/1750-3841.14373>.
- [32] R. Chen, W. Chen, H. Chen, et al., Comparative evaluation of the antioxidant capacities, organic acids, and volatiles of papaya juices fermented by *lactobacillus acidophilus* and *lactobacillus plantarum*, *Journal of Food Quality* 2018 (2018) 1–12. <https://doi.org/10.1155/2018/9490435>.
- [33] H.E. Verón, P. Gauffin Cano, E. Fabersani, et al., Cactus pear (*opuntia ficus-indica*) juice fermented with autochthonous *lactobacillus plantarum* S-811, *Food Funct.* 10 (2019) 1085–1097. <https://doi.org/10.1039/C8FO01591K>.
- [34] H. Yamashita, Biological function of acetic acid—improvement in obesity and glucose tolerance by acetic acid in type 2 diabetic rats, *Critical Reviews in Food Science and Nutrition* 56 (2016) S171–S175. <https://doi.org/10.1080/10408398.2015.1045966>.
- [35] Q. Lin, L. Yang, L. Han, et al., Effects of soy hull polysaccharide on dyslipidemia and pathoglycemia in rats induced by a high-fat-high-sucrose diet, *Food Science and Human Wellness* 11 (2022) 49–57. <https://doi.org/10.1016/j.fshw.2021.07.006>.

- [36] C. Chen, J.-L. Dai, Triglyceride to high-density lipoprotein cholesterol (HDL-C) ratio and arterial stiffness in Japanese population: A secondary analysis based on a cross-sectional study, *Lipids Health Dis* 17 (2018) 130. <https://doi.org/10.1186/s12944-018-0776-7>.
- [37] J. Ye, Y. Zhao, X. Chen, et al., Pu-erh tea ameliorates obesity and modulates gut microbiota in high fat diet fed mice, *Food Research International* 144 (2021) 110360. <https://doi.org/10.1016/j.foodres.2021.110360>.
- [38] N.E. Boutagy, R.P. McMillan, M.I. Frisard, et al., Metabolic endotoxemia with obesity: Is it real and is it relevant?, *Biochimie* 124 (2016) 11–20. <https://doi.org/10.1016/j.biochi.2015.06.020>.
- [39] S.L. Glisan, C. Ryan, A.P. Neilson, et al., Cranberry extract attenuates hepatic inflammation in high-fat-fed obese mice, *The Journal of Nutritional Biochemistry* 37 (2016) 60–66. <https://doi.org/10.1016/j.jnutbio.2016.07.009>.
- [40] S.M. Reilly, A.R. Saltiel, Adapting to obesity with adipose tissue inflammation, *Nat Rev Endocrinol* 13 (2017) 633–643. <https://doi.org/10.1038/nrendo.2017.90>.
- [41] S. Heinonen, R. Jokinen, A. Rissanen, et al., White adipose tissue mitochondrial metabolism in health and in obesity, *Obesity Reviews* 21 (2020) e12958. <https://doi.org/10.1111/obr.12958>.
- [42] X. Deng, N. Zhang, Q. Wang, et al., Theabrownin of raw and ripened pu-erh tea varies in the alleviation of HFD-induced obesity via the regulation of gut microbiota, *Eur J Nutr* 62 (2023) 2177–2194. <https://doi.org/10.1007/s00394-023-03089-w>.
- [43] M.J. Dalby, A.W. Ross, A.W. Walker, et al., Dietary uncoupling of gut microbiota and energy harvesting from obesity and glucose tolerance in mice, *Cell Reports* 21 (2017) 1521–1533. <https://doi.org/10.1016/j.celrep.2017.10.056>.
- [44] Y. Liu, X. Chen, F. Li, et al., Analysis of microbial diversity and metabolites in sauerkraut products with and without microorganism addition, *Foods* 12 (2023) 1164. <https://doi.org/10.3390/foods12061164>.
- [45] J.M. Larsen, The immune response to *prevotella* bacteria in chronic inflammatory disease, *Immunology* 151 (2017) 363–374. <https://doi.org/10.1111/imm.12760>.
- [46] Y. Li, D. Bai, Y. Lu, et al., The crude guava polysaccharides ameliorate high-fat diet-induced obesity in mice via reshaping gut microbiota, *International Journal of Biological Macromolecules* 213 (2022) 234–246. <https://doi.org/10.1016/j.ijbiomac.2022.05.130>.
- [47] X. Shi, X. Zhou, X. Chu, et al., Allicin improves metabolism in high-fat diet-induced obese mice by modulating the gut microbiota, *Nutrients* 11 (2019) 2909. <https://doi.org/10.3390/nu11122909>.
- [48] X. Jiao, Y. Wang, Y. Lin, et al., Blueberry polyphenols extract as a potential prebiotic with anti-obesity effects on C57BL/6 J mice by modulating the gut microbiota, *The Journal of Nutritional Biochemistry* 64 (2019) 88–100. <https://doi.org/10.1016/j.jnutbio.2018.07.008>.
- [49] Y. Liu, Y. Gao, F. Ma, et al., The ameliorative effect of *lactobacillus plantarum* Y44 oral administration on inflammation and lipid metabolism in obese mice fed with a high fat diet, *Food Funct.* 11 (2020) 5024–5039. <https://doi.org/10.1039/D0FO00439A>.
- [50] P. Zhuang, H. Li, W. Jia, et al., Eicosapentaenoic and docosahexaenoic acids attenuate hyperglycemia through the microbiome-gut-organs axis in db/db mice, *Microbiome* 9 (2021) 185. <https://doi.org/10.1186/s40168-021-01126-6>.
- [51] Y. Wang, N. Zhang, J. Kan, et al., Structural characterization of water-soluble polysaccharide from arctium lappa and its effects on colitis mice, *Carbohydrate Polymers* 213 (2019) 89–99. <https://doi.org/10.1016/j.carbpol.2019.02.090>.
- [52] H. Mu, Q. Zhou, R. Yang, et al., Naringin attenuates high fat diet induced non-alcoholic fatty liver disease and gut bacterial dysbiosis in mice, *Front. Microbiol.* 11 (2020) 585066. <https://doi.org/10.3389/fmicb.2020.585066>.
- [53] L. Zhu, S.S. Baker, C. Gill, et al., Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH, *Hepatology* 57 (2013) 601–609. <https://doi.org/10.1002/hep.26093>.
- [54] L.M. Rehaume, N. Matigian, A.M. Mehdi, et al., IL-23 favours outgrowth of spondyloarthritis-associated pathobionts and suppresses host support for homeostatic microbiota, *Ann Rheum Dis* 78 (2019) 494–503. <https://doi.org/10.1136/annrheumdis-2018-214381>.
- [55] Y. Zhang, H. Pan, X. Ye, et al., Proanthocyanidins from Chinese bayberry leaves reduce obesity and associated metabolic disorders in high-fat diet-induced obese mice through a combination of AMPK activation and an alteration in gut microbiota, *Food Funct.* 13 (2022) 2295–2305. <https://doi.org/10.1039/D1FO04147A>.

- [56] L. Cheng, Y. Wei, L. Xu, et al., Gut microbiota differentially mediated by qingmao tea and qingzhuan tea alleviated high-fat-induced obesity and associated metabolic disorders: The impact of microbial fermentation, *Foods* 11 (2022) 3210. <https://doi.org/10.3390/foods11203210>.
- [57] Y. Jung, I. Kim, M. Manna, et al., Effect of kombucha on gut-microbiota in mouse having non-alcoholic fatty liver disease, *Food Sci Biotechnol* 28 (2019) 261–267. <https://doi.org/10.1007/s10068-018-0433-y>.
- [58] J. Rodriguez, S. Hiel, A.M. Neyrinck, et al., Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients, *Gut* 69 (2020) 1975–1987. <https://doi.org/10.1136/gutjnl-2019-319726>.
- [59] S. Hu, H. Yang, X. Gao, et al., Egg oil from *portunus trituberculatus* alleviated obesity and regulated gut microbiota in mice, *Sci Rep* 10 (2020) 8454. <https://doi.org/10.1038/s41598-020-65199-3>.
- [60] J. Du, P. Zhang, J. Luo, et al., Dietary betaine prevents obesity through gut microbiota-driven microRNA-378a family, *Gut Microbes* 13 (2021) 1862612. <https://doi.org/10.1080/19490976.2020.1862612>.
- [61] B. Chen, J. Luo, Y. Han, et al., Dietary tangeretin alleviated dextran sulfate sodium-induced colitis in mice via inhibiting inflammatory response, restoring intestinal barrier function, and modulating gut microbiota, *J. Agric. Food Chem.* 69 (2021) 7663–7674. <https://doi.org/10.1021/acs.jafc.1c03046>.
- [62] Y.-H. Yuan, D.-D. Mu, L. Guo, et al., From flavor to function: A review of fermented fruit drinks, their microbial profiles and health benefits, *Food Research International* 196 (2024) 115095. <https://doi.org/10.1016/j.foodres.2024.115095>.