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Tea polyphenols combined with *Lactobacillus rhamnosus* R5 ameliorate obesity, and alter gut microbiota composition in high-fat-diet-fed mice

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Abstract

Both tea polyphenols (TP) and *Lactobacillus rhamnosus* have been shown to alleviate obesity, and regulate lipid metabolism. However, the combined effects and their underlying mechanisms of action remain elusive. In the present work, the effects of TP, *Lactobacillus rhamnosus* R5 (R5), and TP+R5 on blood lipids and the gut microbiota of mice fed with a high-fat diet were compared. Results showed that the combination of TP and R5 effectively increased the serum levels of high-density lipoprotein cholesterol (HDL-C), and decreased total cholesterol (TC) levels, low-density lipoprotein cholesterol (LDL-C) levels, and the atherosclerosis index (AI) in mice fed with a high-fat diet. The combination treatment resulted in a modification of the structure of the gut microbiota in mice, as evidenced by a decrease in the F/B ratio, and an increase in the abundance of beneficial genera, such as *Akkermansia muciniphila*, *Faecalibaculum rodentium*, and *Ruminococcus_uncultured* bacterium. Additionally, the contents of SCFAs (acetic, propionic, and butyric acids) in faeces also increased. These could provide new ideas for anti-obesity methods, and a theoretical basis for the development of foods combining probiotics and tea polyphenols.

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Introduction

A high-fat diet initiates oxidative stress, inflammation, and gut microbiome disorders, thus increasing the risk of hyperlipidaemia, cardiovascular disease, insulin resistance, diabetes, and even cancer (Kim and Choi, 2010). Hyperlipidaemia is a disease caused by abnormal metabolism that mainly manifests as an unusual increase in the content of low-density lipoprotein cholesterol (LDL-C) in the blood (Rinella, 2015). At present, most of the therapeutic drugs for hyperlipidaemia are statins. The targets of these drugs focus on inhibiting the synthesis of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGR), and increasing the expression of low-density lipoprotein receptor (LDLR). Admittedly, statins have a substantial effect on the treatment of cardiovascular diseases. However, the long-term intake of high doses of statins results in adverse effects such as liver toxicity and diabetes sensitivity (Sessa *et al*., 2018). Therefore, healthy treatment methods without side effects will become a new research direction for the treatment of hyperlipidaemia.

Gut microbiota is an important "organ" for maintaining host health. The balance of gut microbiota has become a key factor affecting the development of hyperlipidaemia and metabolic syndrome (Yatsunenko *et al*., 2012; Moossavi and Bishehsari, 2019; Sun *et al*., 2020).

Increasing studies have focused on probiotics to treat certain diseases, and promote human health (Ren *et al*., 2018; Zhang *et al*., 2020; Puttarat *et al*., 2021; Levit *et al*., 2021). Lactic acid bacteria as a typical representative of probiotics (Tao *et al*., 2021) have been demonstrated to lower cholesterol levels *in vitro* and *in vivo*, and to effectively prevent obesity and its associated metabolic disorders and intestinal dysbiosis. Tjandrawinata *et al*. (2022) isolated 11 strains of lactic acid bacteria from breast milk, mango, and goat colostrum. The ability of *Lactobacillus* isolates to remove cholesterol ranged

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from 21.72 to 84.67%. Studies have revealed that *Lactobacillus rhamnosus* GG and *Lactobacillus plantarum* Uruma-SU4 could modulate the structure and abundance of gut microbiota in mice, thus regulating blood lipid levels, and alleviating high-fat diet-induced obesity (Zhang *et al*., 2015; Shikano *et al*., 2019). Park *et al*. (2018) found that the use of *Lactobacillus* BFE5264 could decrease the number of *Helicobacter* and *Desulfovibrio* in the gut of mice fed with a high-fat diet, and increase the abundance of beneficial bacteria such as *Lactobacillus*. Moreover, probiotics have the advantages of low price and few side effects.

Tea polyphenols (TP), a class of polyhydroxyphenolic compounds found in tea, also exert cholesterol-lowering and antioxidant effects (Li, 2013). The consumption of TP is negatively correlated with the incidence and development of hyperlipidaemia according to previous studies (Miyata *et al*., 2011; Wang *et al*., 2015). The digestion and absorption of TP mainly occurs in the intestinal tract, which is broken down and absorbed under the action of the gut microbiota and their enzymes to exert its effect. Based on PCR-DGGE technology, Zhang *et al*. (2014) found that TP could promote the reproduction of intestinal probiotics, and improve the imbalance of intestinal flora caused by antibiotics. Since TP provides health benefits similar to those of traditional oligosaccharide prebiotics, it can be considered a prebiotic (Liu *et al*., 2022).

Synbiotics are dietary supplements that combine probiotics and prebiotics (Sergeev *et al*., 2020; Mahmoud *et al*., 2022). Multiple studies have shown the promising effect of both probiotics and prebiotics, their combination in the promotion of gut health, and the alleviation of obesity-associated metabolic disorders (Ke *et al*., 2019; Oh *et al*., 2021). Cho *et al*. (2018) investigated the combined effects of polyphenol-rich wine grape seed flour and lactic acid bacteria derived from kefir on obesity-related metabolic disease in high-fat diet-induced obese mice. However, there are few reports on the synergistic action of TP with probiotics. Therefore, the present work combined TP with *Lactobacillus rhamnosus* R5 to jointly treat high-fat diet-fed mice to detect changes in blood lipid metabolism and intestinal microorganisms in mice. It is hoped that the combination of the two can enhance their effects, complement each other, and provide a basis for new methods of treatment of hyperlipidaemia.

Materials and methods

Materials and reagents

TP (purity > 98%) was purchased from Yuanye Biological Technology Co., Ltd. (Shanghai, China). *Lactobacillus rhamnosus* R5 was deposited in the School of Food and Bioengineering, Xihua University, China. R5 was isolated from Sichuan traditional kimchi with a strong acid and bile salt resistance. Its cholesterol degradation rate *in vitro* was 66.35 ± 9.41%. Preliminary safety experiments have shown that R5 did not have the toxic effects of producing amines and haemolysis. The basic feed was obtained from Dashuo Animal Experiment Centre (Chengdu, China), and the approximate nutrient content was as follows: crude protein, 18.5%; crude fat, 5%; crude fibre, 4%; calcium, 1.0 - 1.5%; and phosphorus, 0.6 - 1.2%. The high-fat feed formula was developed following the literature (Bao *et al*., 2019) as follows: lard, 10%; egg yolk powder, 5%; cholesterol, 1.5%, bile salt, 0.3%; and basic feed, 83.2%.

Preparation of R5 bacterial solution

R5 was activated by MRS medium at 37°C for 24 h for two generations. Then, the precipitate was obtained after centrifugation, and added to sterilised skim milk with ampoules. Vacuum freeze-dried milk tubes were stored at -20°C. The cultured bacterial suspension was washed by centrifugation with saline under aseptic conditions (3,000 rpm, 3 min). After three repetitions, the suspension of R5 at a concentration of 10^9 CFU/mL was prepared by resuspension in sterile saline.

Animals and experimental protocols

Fifty C57BL/6 male mice (6-w old, 16 ± 2 g) were purchased from Dashuo Animal Experimental Centre (Chengdu, China). All animal experiments were performed in accordance with the guidelines for the care and use of laboratory animals.

The mice were housed in individual cages under a 12 h light/dark cycle at a constant room temperature of 23 ± 2°C, and relative humidity of 50 ± 5%. After acclimation for 7 d, 50 mice were randomly divided into five groups (n = 10 per group): LFD (normal mice treated with sterile saline), HFD (high-fat-diet-fed mice treated with sterile saline), R5 (high-fat-diet-fed mice treated with 10^9 CFU/mL R5 suspension), TP (high-fat-diet-fed mice treated with

TP at a dose of 400 mg/kg body weight), and TP+R5 (high-fat-diet-fed mice treated with R5 and TP at a combined dose).

During the feeding period, each group of mice received an oral gavage of 0.4 mL of their respective treatment at 9:00 am daily. The mice were weighed and recorded once a week. The gavage amount was adjusted based on the weight of the mice every week.

Serum and faeces collection

After 4 w of feeding, all mice were fasted for 12 h. Blood samples were collected from the orbit aseptically and centrifuged (4°C, 3,000 rpm, 10 min) to obtain the separated serum, which was used for biochemical analyses. Fresh faecal samples of mice from each group were collected and placed in sterile EP tubes. They were stored at -80°C until intestinal flora analysis.

Determination of blood biochemical indices

A fully automated biochemical analyser was used to measure serum TC, TG, HDL-C, and LDL-C levels.

Third-generation high-throughput sequencing

Microbial DNA was extracted from stool samples using the E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, USA) following manufacturer's instructions. The V1 - V9 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95°C for 2 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and a final extension at 72°C for 5 min) using the primers 27F 5'-AGRGTTYGA TYMTGGCTCAG-3' and 1492R 5'-RGYTACCTTGTTAC GACTT-3', where the barcode was an eight-base sequence unique to each sample. PCRs were performed in triplicate in a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of DNA template. Amplicons were extracted from 2% agarose gels, and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following manufacturer's instructions.

Bioinformatics analysis

PacBio raw reads were processed using SMRT Link Analysis software version 6.0 to screen for the length and quality of the sequences. Further filtering was performed by removing barcodes, primer sequences, chimaeras, and sequences containing ten consecutive identical bases.

OTUs were clustered with a 98.65% similarity cut-off using UPARSE, and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analysed by RDP Classifier against the silva (SSU132) 16S rRNA database using a confidence threshold of 70% (Amato *et al*., 2013).

Alpha- and beta-diversity analyses

Rarefaction analysis based on Mothur v.1.21.1 was conducted to reveal the diversity indices, including Chao, ACE, and Shannon diversity indices (Schloss *et al*., 2009). Beta diversity analysis was performed using UniFrac (Lozupone *et al*., 2011) matrix comparisons to compare the results of community differences. Variance analysis (MANOVA) was conducted to further confirm the observed differences. R (pheatmap package) and Cytoscape were applied to visualise the relationships through correlation heatmaps and network diagrams, respectively. One-way analysis of variance (ANOVA) was performed to assess the statistically significant differences in diversity indices between samples. Differences were considered significant at $p < 0.05$.

LEFse analysis

To identify biomarkers that distinguish two or more biological conditions, linear discriminant analysis effect size (LEFse) analysis was performed (Ijaz *et al*., 2018). The Kruskal-Wallis sum-rank test was performed to examine the changes and dissimilarities among classes. This was followed by LDA analysis to determine the size effect of each distinctively abundant taxon (Shang *et al*., 2016).

Determination of short-chain fatty acids in mouse faeces

Referring to Chen *et al*. (2022) method with slight changes, 0.2 g of faecal sample was placed into a 2 mL centrifuge tube, and dissolved in 1 mL of pure water. After shaking for 2.0 min, the sample was centrifuged at 10,000 rpm for 10 min. The supernatant was removed, filtered through a 0.22 μm filter, and poured into a 2 mL centrifuge tube. Then, 0.5 mL of the supernatant was mixed with 0.05 mL of 50% sulphuric acid solution and 0.5 mL of ether. It was then shaken well, centrifuged at 10,000 rpm for

5 min, and placed in a refrigerator (4°C) for 30 min. The upper ether layer was obtained for GC analysis.

The GC conditions were as follows = WAX capillary column: 30 m × 0.25 mm × 0.25 μm; heating program: 100°C for 1 min → 5°C/min → 150°C for 5 min; carrier gas: high-purity nitrogen, purity ≥ 99.999%; carrier gas flow rate: 2 mL/min; injection port temperature: 270°C; injection mode: spitless injection; the injection volume: 1 μL; and detector temperature: 280°C.

Results and discussion

TP and/or R5 regulated body weight in HFD-fed mice

As shown in FigureA, there was no significant difference in body weight between the groups after adaptive feeding. Following 4 w intervention, mice in the HFD group had significantly higher body weights than those in the LFD group. Compared with the LFD and HFD groups, the TP and/or R5 groups demonstrated significant weight loss. This suggested that HFD could cause metabolic disorders in mice that lead to weight gain and obesity, and TP and/or R5 treatment reduced obesity in mice.

TP and/or R5 regulated serum lipid levels and arteriosclerosis index in HFD-fed mice

The levels of blood lipids (TG, TC, HDL, and LDL) reflect whether the whole-body lipid metabolism is normal. As shown in FigureB, the contents of TC and LDL-C in the HFD group were significantly higher than those in the LFD group, indicating that the high-fat mouse model was successfully established. Compared with the those in the HFD group, the serum lipid levels in the TP and/or R5 groups were effectively regulated. The contents of TC and LDL-C in the TP and/or R5 groups were significantly decreased, and the content of HDL-C was increased. Moreover, the contents of TC, LDL-C, and HDL-C in the TP+R5 group were significantly different from those in the TP and R5 groups alone.

The arteriosclerosis index (AI) is commonly used in medicine to indicate the risk of atherosclerosis. The serum AI of the HFD group was significantly increased (FigureC). The AI was decreased in all three intervention methods of feeding TP and/or R5, and it demonstrated the most robust reduction in the TP+R5 treatment.

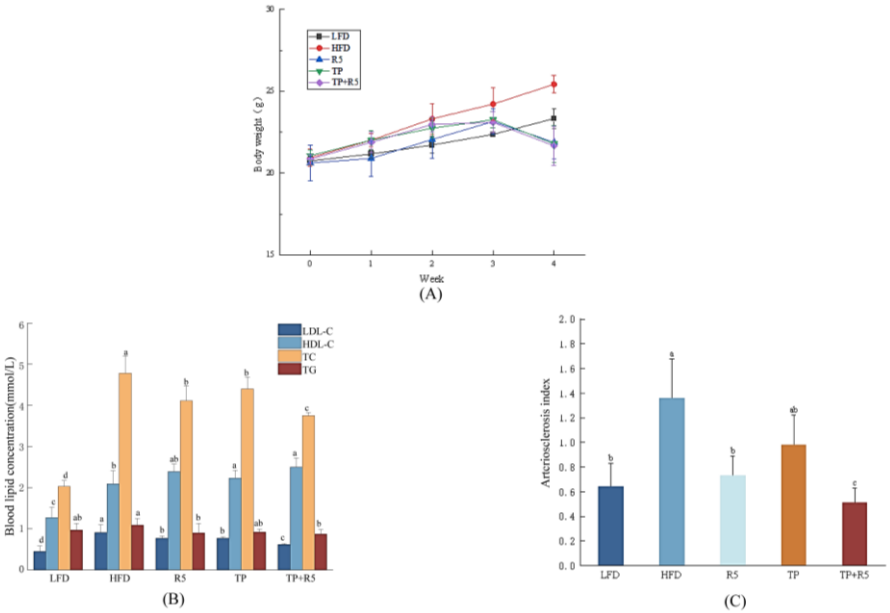

Figure 1. TP and/or R5 ameliorated HFD-induced obesity and abnormal blood metabolic parameters. **(A)** Body weight change, **(B)** blood lipid concentration, and **(C)** arteriosclerosis index.

TP and/or R5 influenced intestinal alpha diversity in high-fat diet mice

The alpha diversity index of a single sample was used to analyse the species abundance and diversity of microbial communities. The indices of community richness mainly include the Chao and ACE indices. The indices of community diversity include the Shannon and Simpson indices. Based on Table, TP and/or R5 could improve the mouse intestinal alpha diversity to some extent. The Chao and ACE indices were noticeably improved by the TP+R5 feeding regimen compared to the HFD-fed mice.

Table 1. Alpha diversity index of samples in each group.

Group	Chao	Shannon	Simpson	ACE	Coverage
LFD	2,529.25 ± 315.66^{b}	7.96 ± 0.63^{a}	0.022 ± 0.0046^{b}	3,106.31 ± 112.96^{a}	0.91 ± 0.044^{a}
HFD	2,472.64 ± 400.37^{b}	8.47 ± 0.96^{a}	0.024 ± 0.0023^{b}	2,215.21 ± 130.53^{b}	0.88 ± 0.030^{a}
R5	3,084.02 ± 436.44^{ab}	8.83 ± 0.17^{a}	0.050 ± 0.014^{a}	3,458.66 ± 284.67^{ab}	0.87 ± 0.022^{a}
TP	3,318.32 ± 484.39^{a}	7.86 ± 0.50^{a}	0.030 ± 0.013^{b}	3,987.88 ± 96.20^{a}	0.90 ± 0.063^{a}
TP+R5	3,736.10 ± 95.79^{a}	8.68 ± 0.38^{a}	0.019 ± 0.012^{b}	3,904.45 ± 1402.79^{a}	0.86 ± 0.053^{a}

Different lowercase superscripts in the same column indicate significant difference ($p < 0.05$).

TP and/or R5 influenced the composition of intestinal flora in high-fat diet mice

As shown in FigureA, the relative abundances of *Firmicutes*, *Bacteroidota*, *Campylobacterota*, *Desulfobacterota*, *Proteobacteria*, and *Verrucomicrobiota* were dominant, and the proportions of *Firmicutes* and *Bacteroidetes* reached approximately 70%. The relative abundance of *Bacteroidetes* was 40.36% in the LFD group, and 30.46% in the HFD group. Following 4 w of feeding, the relative abundance of *Bacteroidetes* was increased by 12.30, 51.85, and 70.10% in the TP, R5, and TP+R5 groups, respectively. As shown in FigureD, the *Firmicutes*/*Bacteroidetes* ratio (F/B ratio) of the HFD group reached 1.40, which was significantly higher than that of the LFD group. Moreover, the F/B ratio was significantly lower in the TP+R5 group than in the HFD group.

At the genus level (FigureB), the relative abundance of *Desulfovibrionaceae* in the HFD group increased by 70.44-fold compared to that in the LFD group, and the relative abundance of *Akkermansia* decreased by 79.12%. After TP and/or R5 treatment, the abnormalities were recovered. The intake of R5, TP, and TP+R5 decreased the relative abundance of *Desulfovibrionaceae* by 65.77, 70.24, and 84.18%, respectively, compared to the HFD group. The relative abundance of *Akkermansia* was increased by 60.56% in the TP+R5 group compared to that in the HFD group. In addition, the relative abundances of *Ruminococcus* and *Roseburia* were markedly increased in the TP+R5 group.

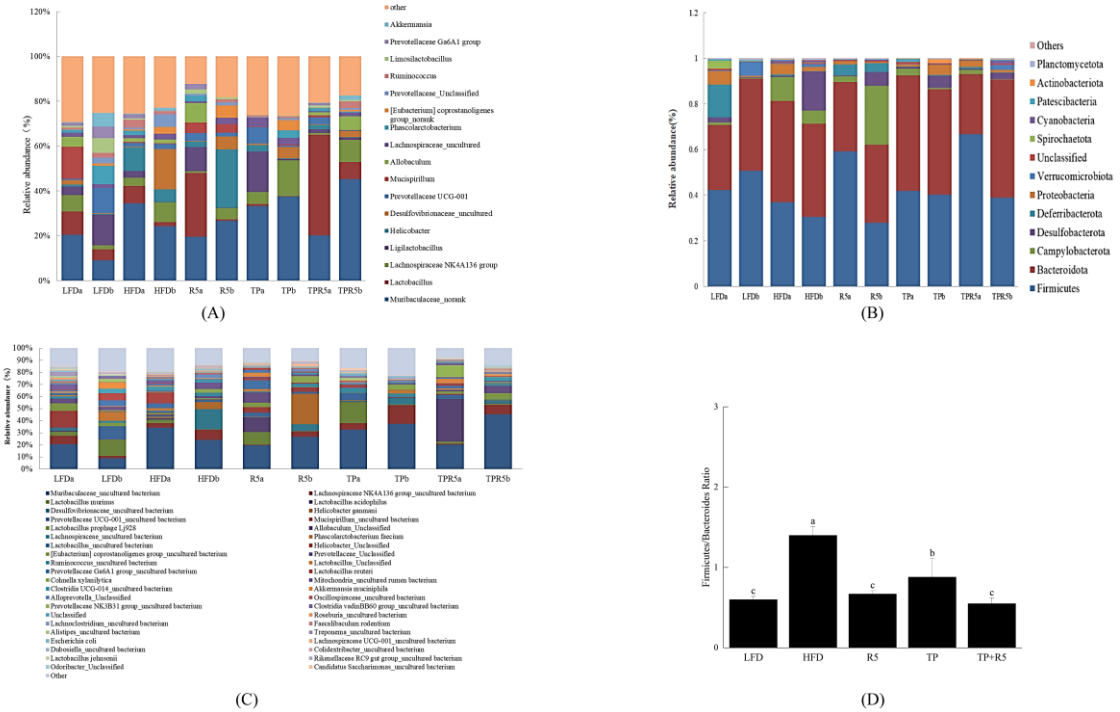

Figure 2. Changes in the abundance of gut microbiota in mice, before and after, R5 and TP intervention and co-intervention. **(A)** Phylum level, **(B)** genus level, **(C)** species level, and **(D)** Firmicutes/Bacteroides (F/B) ratio. a: initial gut microbiota; and b: end gut microbiota.

At the species level (FigureC), a total of 72 microorganisms were identified. Compared with those in the LFD group, the relative abundances of *Akkermansia muciniphila*, *Lactobacillus murinus*, and *Faecalibaculum rodentium* in the HFD group were decreased, but the relative abundances of *Desulfovibrionaceae_uncultured bacterium*, *Helicobacter ganmani*, *Oscillospiraceae_uncultured*, and *Escherichia coli* were increased. All of these changes were recovered by taking TP and/or R5.

LEfSe analysis

To further accurately assess the effect of TP and/or R5 on the composition of the gut microbiota, species with significant variability among different groups were obtained by LEfSe analysis (Figure), and we found almost consistent results. The different circles represent seven taxonomic levels (kingdom, phylum, class, order, family, genus, and species) from the centre outwards. Each node represents a species classification below that level, and the greater the abundance of that species is, the larger the node. By comparing the LFD and HFD groups, and the HFD and three intervention groups, it became clear that the regulation of beneficial bacterial genera (*Bacteroidota*, *Ruminococcus*, *Roseburia*, *Akkermansia*, *Phascolarctobacterium*, and *Lactobacillus*) and harmful bacterial genera (*Firmicutes*, *Oscillobacte*r, and *Desulfovibrionaceae*) in the gut was particularly evident in the TP+R5 group.

TP and/or R5 changed the intestinal short-chain fatty acid content in high-fat diet mice

As shown in Figure, the contents of acetic, propionic, and butyric acids in the faeces of mice in the HFD group were significantly decreased compared with those in the LFD group. TP and/or R5 restored the reduction in short-chain fatty acids induced by HFD to normal levels. Moreover, the contents of acetic, propionic, and butyric acids in the TP+R5 group were significantly higher than those in the LFD group.

Discussion

Previous studies have shown good anti-obesity effects of TP by regulating enzyme activity, inhibiting fat accumulation, promoting fat oxidation, stimulating body energy consumption, and regulating intestinal flora disorders (Gondoin *et al*., 2010; Türközü and Tek, 2017; Sun *et al*., 2018; Tan *et al*., 2018). Moreover, lactic acid bacteria have become a hot spot for functional food research and development because of their physiological effects, such as enhancing the immune response, balancing intestinal flora, lowering serum cholesterol, and inhibiting *Helicobacter pylori* (Liu *et al*., 2020). In the present work, we found that the synbiotic effects of TP and R5 significantly decreased body weight, and improved dyslipidaemia and gut microbiota disorders in mice. Furthermore, the synbiotic effect produced the highest amount of SCFAs compared to each supplementation alone.

A high-fat diet causes significant increases in serum TC, LDL-C, HDL-C, and AI in animals (Chen *et al*., 2022). The increase in HDL-C content may be related to the stress response. The intake of large amounts of cholesterol causes mice to produce large amounts of HDL-C to clear it (Al-Sheraji *et al*., 2012). Consistent with these previous studies (Miyata *et al*., 2011; Tan *et al*., 2018; Shikano *et al*., 2019), this study found that the blood lipid level of mice on a HFD could be adjusted to different degrees by TP and/or R5 treatment, and TP+R5 cointervention showed the best effect on blood lipid regulation.

A high-fat diet not only increases body's total cholesterol levels but also leads to disordered gut microbiota (Cani *et al*., 2007; Yan *et al*., 2015). Changes in the gut microbiota may be an important factor in obesity. Some of these genera and species can play a key role in the overall regulatory process. The findings demonstrated that HFD decreased the gut microbiota diversity of mice to a certain extent, and TP+R5 effectively improved the gut microbiota richness.

The abundance of *Bacteroides* in the gut of mice fed with HFD decreased, while the abundance of *Firmicutes* increased (Wang *et al*., 2020). In 2005, Ley *et al*. (2005) first proposed the effect of the ratio of *Firmicutes* to *Bacteroides* on obese animals. To date, the F/B ratio is considered to be a key factor in maintaining intestinal homeostasis. An increase or decrease in the F/B ratio represents intestinal flora imbalance, which may cause various pathologies (Stojanov *et al*., 2020; Chen *et al*., 2021). Guo *et al*. (2017) investigated the regulation of green tea polyphenols on the gut microbiota in obese mice, and found that in the HFD group, the diversity of total bacteria was decreased, and significantly lower than that in the HFD-green tea polyphenols group. Moreover, the relative abundance of *Bacteroidetes* in

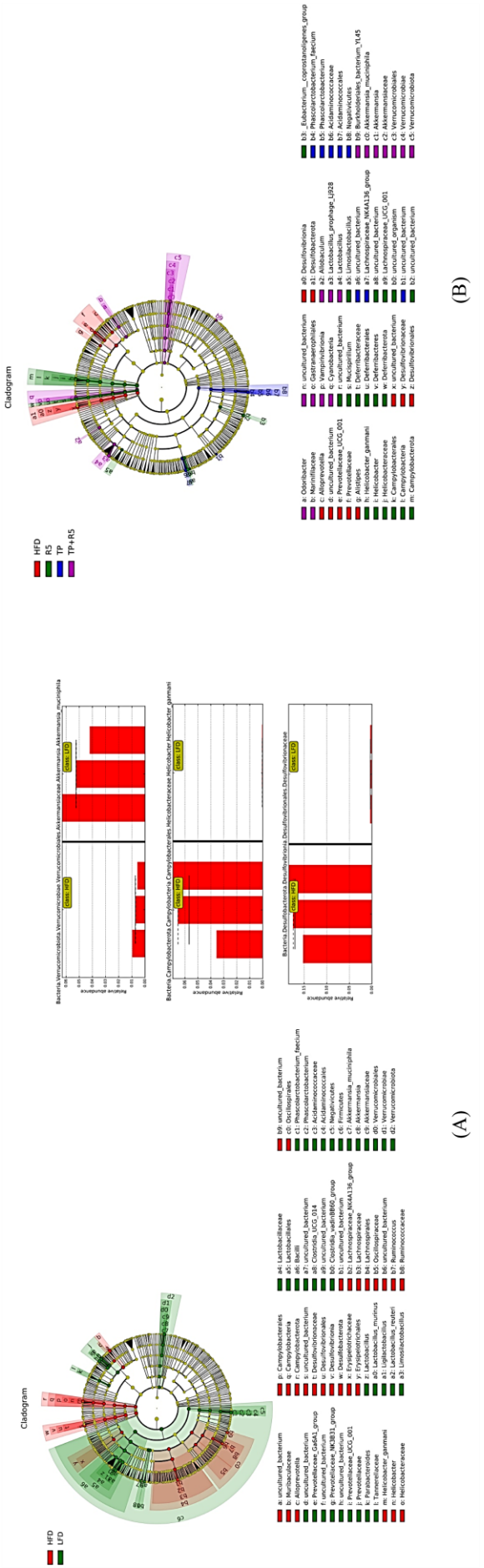

Figure 3. Evolutionary branch diagram between the LFD and HFD group **(A)**, and the HFD group and TP and/or R5 groups **(B)**.

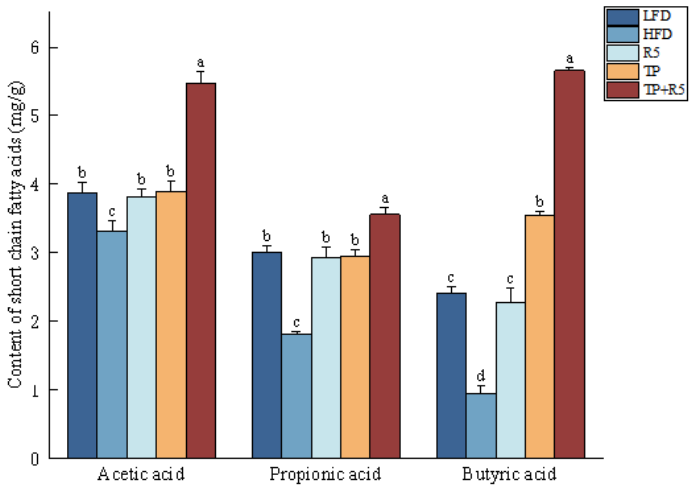

Figure 4. Contents of short-chain fatty acids in different groups of mice.

the HFD group increased from 0.56 ± 0.06 (week 1) to 0.60 ± 0.05 (week 3), while the relative abundance of *Firmicutes* decreased from 0.42 ± 0.06 to 0.37 ± 0.02.

At the genus level, we observed that TP+R5 significantly increased the relative abundances of *Ruminococcus*, *Roseburia*, *Akkermansia*, and *PhascolarctobacteriIum*, and decreased the relative abundance of *Oscillobacter* and *Desulfovibrionaceae* compared with the HFD group. *Ruminococcus* can promote the degradation of various polysaccharides and fibres to produce SCFAs, the abundance of which is significantly negatively correlated with increasing intestinal permeability (Zhang *et al*., 2020). It is also a butyrate-producing bacterium that can maintain the stability of intestinal flora structure and function. *Roseburia* has been reported as a prominent gut-associated butyrate-producing bacterial genus that is inversely correlated with atherosclerotic lesion development (Kasahara *et al*., 2018). In addition, *Akkermansia* can exert an anti-obesity effect by inhibiting metabolic disorders caused by HFD (Cao *et al*., 2019). *Akkermansia muciniphila* is the only strain of *Akkermansia* present in the intestine, and has been shown to inhibit cholesterol synthesis by using mucins to produce acetate and propionate. Everard *et al*. (2013) found that HFD-induced metabolic disturbances, including increased adiposity, endotoxemia, adipose tissue inflammation, and insulin resistance, were restored after a gavage of this strain in obese and type 2 diabetic mice. Other studies have found that its relative abundance was negatively correlated with diseases such as inflammatory bowel disease (IBD), appendicitis (Png *et al*., 2010), and adolescent autism (Wang *et al*., 2011). *Phascolarctobacterium* is an obligately anaerobic and Gram-negative bacterium that produces SCFAs, including acetate and propionate, and it colonises the human gut in large numbers. *Phascolarctobacterium* have been reported as major utilisers of succinic acid to produce propionic acid. *Phascolarctobacterium faecium* (*P. faecium*) is the first strain isolated from koalas (Ikeyama *et al*., 2020). Sanz Herranz *et al*. (2013) found that *Phascolarctobacterium faecium* can be used to prevent and treat obesity-related metabolic diseases and immune disorders, including dyslipidaemia (hyperlipidaemia and hypercholesterolemia) and diabetes. In contrast, *Desulfovibrionaceae*, a sulphate-reducing bacterium, can degrade SCFAs, amino acids, and other nutrients to produce toxic H_2S. This destroys the intestinal mucosal barrier, and causes endotoxins to enter the circulatory system and produce an inflammatory response (Weglarz *et al*., 2003). The abundance of *Faecalibaculum rodentium* in the TP+R5 group was 5.37 times higher than that in the HFD group. A recent study published in Nature Microbiology found that *Faecalibaculum rodentium* can exert an antitumour effect by producing SCFAs in a mouse model of colorectal cancer (Zagato *et al*., 2020).

In short, HFD alters the living environment of intestinal microorganisms, resulting in the inhibition of the growth of beneficial bacteria, and the proliferation of harmful bacteria, thus increasing the risk of disease. TP and/or R5 have a certain recovery effect on the intestinal flora imbalance. TP+R5 protected mice from obesity by most effectively modulating the composition of the gut microbiota. Thus, the number of harmful and beneficial bacteria that were altered in HFD-fed mice were regulated, and lipid metabolism disorders were improved. Gao *et al*. (2018) obtained a similar pattern of flora

changes that used Pu-erh tea rich in TP and caffeine to modify the HFD-induced intestinal flora imbalance to improve diet-induced metabolic syndrome.

SCFAs are the main energy source for cells in the small intestine and colon mucosa, and play an extremely important role in maintaining normal intestinal function. The quantity and relative proportions of SCFAs depend on the substrate, microbiota composition, and intestinal transit time (Lu *et al*., 2013). In addition to the effect on the composition of the gut microbiota, we also verified that TP+R5 could produce more SCFAs. Consistent with our results, studies have shown that HFD could reduce the SCFAs content in the gut (Ayesh *et al*., 1999). However, the contents of SCFAs in the TP, R5, and TP+R5 groups were increased compared with those in the HFD group, and the content of butyric acid increased by 2.38, 3.74, and 5.96 times, respectively. The contents of SCFAs corresponded to the abundance of probiotics that produce SCFAs in the gut microbiota. A previous study found that supplementation with Liupao tea, *Lactobacillus rhamnosus*, and other substances can effectively increase the contents of intestinal SCFAs in mice fed with HFD, and maintain the stability of the intestinal environment (Huang *et al*., 2019; Ye *et al*., 2019). According to a report in the journal of Modern Food Technology, an increase in SCFAs can decrease serum TC and LDL-C levels (Ju and Wang, 2016). Studies have also shown that SCFAs could inhibit the synthesis of cholesterol, and reduce its concentration in plasma (Feng *et al*., 2022). This was consistent with the trend of TG content in blood lipids and total cholesterol in mice observed in the present work.

Conclusion

The present work is the first to explore the synbiotic effect of TP and R5 on blood lipids and gut microbiota. The results obtained could provide a certain experimental basis for the combination of TP and R5 as an intervention treatment for high-fat-diet-fed mice, and lay a theoretical foundation for the development and application of R5 in functional food.

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