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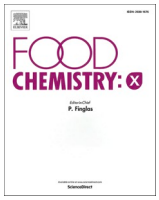

Potential mechanism of probiotic fermentation of *Auricularia cornea* var. Li./blueberry to reduce obesity induced by a high-fat diet

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ABSTRACT

The primary objective of this research was to investigate the effects of fermented *Auricularia cornea* var. Li./blueberry (FACB) on the gut microbiota of these super-large mouse models. The study, found that the groups who were given different amounts of FACB saw a significant reduction in their triglyceride and total cholesterol levels. There was a noteworthy increase in the ranks of high-density lipoprotein cholesterol (HDL-C) ($P < 0.05$). Furthermore, it was noted that FACB influenced the gut microbiota of the obese rats, improving in both the variety and quantity of short-chain fatty acids present in their intestines. This research provided the inaugural evidence of FACB's potential as an effective anti-obesity agent in a high-fat diet model, implying it could serve as a preventive measure against obesity.

Introduction

With the accelerated pace of life, unreasonable lifestyles and eating habits have brought many adverse effects to people. Statistics show that over 1 billion adults globally are overweight (Sonnenburg & Bäckhed, 2016). This is mainly due to the imbalance between the amount of food consumed and the energy consumed, increasing adipose tissue. Metabolic syndrome is closely linked to obesity, and seriously affects people's health and living standards. Research results in recent years show that obesity can be adjusted by intestinal microbiota (Xiao, Sha, Li, Gan, & Li, 2018).

Fermentation has been demonstrated to be a beneficial factor in human health, as it can enhance the flavor of food and generate a variety of bioactive compounds with antioxidant properties, blood lipid-lowering properties, and other biological activities (Marco et al., 2017). Moreover, it has been demonstrated to have health benefits and reduce the likelihood of numerous illnesses, such as high blood pressure, diabetes, hyperlipidemia, oxidative stress, etc. The microbes in fermented foods also improve people's gut microbiota. So, fermented food is getting more and more attention (Diez-Ozaeta & Astiazaran, 2022).

Auricularia cornea var. Li. is a superb variety of edible and medicinal bacteria, boasting both high quality and high yield, and has been identified as having excellent antioxidant, hypoglycemic, hypolipidemic, anti-inflammatory, antibacterial, and other effects. It has been reported that fermented edible fungi have suitable biological activities (Sun, Chen, Xiang, Hu & Zhao, 2022; Lao, Zhang, Li & Bhandari, 2020). The anthocyanins of blueberries, which are rich in phenolic compounds and nutrient-rich, have antioxidant and hypoglycemic effects. Fermentation can promote the release of bioactive compounds in blueberries, which benefits to human health.

The host's metabolism can be regulated by trillions of gut microbes, which comprise the complex and diverse micro-ecosystem known as the human gut microbiome (Bo, Wen, Zhao, Tian, Zhang & Wang, 2020). Obesity and intestinal microbiota are mutually beneficial and symbiotic relations. The two interact and complement each other. Obesity will affect intestinal microbiota structure, while changes in microbiota species, construction, and abundance will lead to obesity. The relationship between the two is reversible (Cheng et al., 2018). The results show that intestinal microbiota is one of the most important causes of hereditary obesity, which will also help people prevent and treat obesity, even curing metabolic diseases (Ma, Hu, Zhang, Shao, Eugeni & Wang, 2022).

Enzymes are especially favored amongst the many varieties of fermented foods. Enzyme products have the functions of anti-tumor, immunity enhancement, anti-oxidation, blood sugar lowering, and

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intestinal regulation (Gao et al., 2019; Clevers et al., 2020). FACB is a new composite fermentation product prepared by *Auricularia cornea* var. Li., blueberry, and various probiotics. The primary focus of this study was utilizing high-throughput sequencing technology to examine how FACB impacts the gut microbiota of overweight rats. The aim was to gather essential data for the potential anti-obesity impact of fermented food, which holds significant social and economic importance.

Materials and methods

Materials and reagents

The prime approach in our laboratory for preparing fermented probiotic products is based on previously established methods that were already established (Sheng et al., 2021). First, *Auricularia cornea* var. Li. was hydrolyzed with cellulase, and then blueberries were crushed. Distilled water, sucrose, and trehalose were prepared and well-blended. After reaching a temperature of 90 ℃, the mixture was maintained at that level for 40 min until it gradually cooled to the ambient room temperature. Compound probiotics (Contain 9 probiotics: 0.25 mg/g *Lactobacillus acidophilus*, 0.25 mg/g *Lactobacillus rhamnosus*, 0.25 mg/g *Lactobacillus casei*, 0.041 mg/g *Lactobacillus plantarum*, 0.041 mg/g *Bifidobacterium longum*, 0.042 mg/g *Bifidobacterium lactis*, 0.042 mg/g *Bifidobacterium breve*, 0.042 mg/g *Lactobacillus paracasei*, 0.042 mg/g *Streptococcus thermophilus*) were inoculated under aseptic conditions. The mixture was incubated at a temperature of 37 ℃ for a period of 17 days. Following the incubation, the fermented product was obtained by sterilizing the mixture at a temperature range of 65–80 ℃ for 30 min. The main active components of FACB are shown in Table 1.

We acquired freshly obtained *Auricularia cornea* var. Li and blueberries from Huaxin Fungus, located in Jilin, China. The complex probiotics were sourced from Taiwan Subcore Biotechnology in Taiwan, China. Male SD rats, aged five weeks and weighing between 160 and 200 g, were obtained from Changchun Yisi Experimental Animal Technology Co., Ltd. These rats were free from Specific Pathogens (SPF). Triglyceride, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol test kits were procured from Nanjing Jiancheng Bioengineering Institute. Analytical grade reagents and drugs were exclusively used for all experiments conducted in this study.

Instruments and Equipment

TL-18M Desktop High-Speed Frozen Centrifuge (Shanghai Centrifugal Machinery Research Institute); RE52 Rotary Evaporator (Shanghai Qiyao Instrument and Equipment Co., Ltd.); InfiniteM200 microplate reader (Tecan, Switzerland); Mini Seq sequencer (Illumina, USA).

Methodology

Animals and experimental design

The guidelines were followed in conducting the animal experiments in this study outlined in the Care and Use of Laboratory Animals. According to the ARRIVE criteria, they were approved by the Animal Ethics Committee of Jilin Agricultural University in Jilin, China. Initially, a group of fifty male SD rats were given a standardized diet and allowed 7 days to acclimate to their environment. The diet and treatment plan of rats in each group were performed according to Table 2. The conditions during this period included a room temperature of 21 ± 2 ℃ degrees, a relative humidity range of 30 % to 70 %, and a light/dark cycle of 12 h each. Next, the rats were randomly selected to be segmented into 5 groups. A regular diet was given to a control group of 10 rats, while the remaining group of 40 rats was provided with a high-fat diet. All rats, regardless of their assigned group, had access to regular food and drinking water. The procedures and protocols for animal care and diet manipulation were conducted following the "Implementation Manual of Technical Specifications for Hygienic Food Inspection and Evaluation ", which aimed to create rat models that mimic the effects of a high-fat diet. The rats were tested daily. Confirmation of the successful establishment of the obese rat model was achieved when the average body weight of the high-fat model group equaled or surpassed the average body weight of the standard control group (Albrahim & Alonazi, 2021). All rats were provided free water, and their daily food intake and residual food content were documented. They also weighed themselves once a week, collected fresh feces, and changed the padding twice weekly for seven weeks. Upon conclusion of the intervention, Meng et al.'s modified method was applied to the male SD rats, all of whom were employed (Meng et al., 2019). At the end of the intervention period, all rats were put to death by first administering polyurethane anesthesia and then inducing death using CO_2.

Determination of body weight and physiological and biochemical indexes of rats

After the trial, the rats' weights were noted, and 2 mL of blood was collected from each rat's abdominal cavity. This experiment was carried out during the rats' fasting period, but they were given water to drink for 12 h. Afterward, the collected blood samples were placed in a centrifuge and spun at a rate of 3000 r/min for 15 min while maintaining a temperature of 4 ℃. As a result, plasma samples were obtained. These plasma samples were then transferred into centrifuge tubes with a capacity of 2 mL and stored in a refrigerator at a temperature of −20 ℃. The serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using a purchased test kit.

Pathological section observation of rat liver tissue

After dissecting the rat, the liver tissues were quickly taken out, and liver tissue pieces with the size of 0.5 cm^3 were cut, cleaned with normal saline, fixed in a solution containing 4 % paraformaldehyde, sealed, and treated overnight. According to the standard process of slice-making, the operation mainly includes the steps of ethanol dehydration, xylene penetration, wax immersion, paraffin embedding, HE (hematoxylin-eosin) staining, and fixation. A light microscope was used to inspect the pathological alterations of rat liver tissues after the slices had been finished.

Determination of intestinal microbiota in rats

The rat feces collected using the animal experimental design methods designed in this study are sent to Shanghai Parsonoff Biotechnology Co., Ltd. for determination and treatment. The precise determination method is employed to obtain the complete DNA of microbial groups. Performing PCR amplification of the V3-V4 region of 16SrDNA, the approach employs particular bacterial primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTC-TAAT-3′). The resulting amplified products are then purified and subjected to fluorescence quantitative analysis. Subsequently, a sequencing library is generated by utilizing Illumina's TruSeq Nano DNA LT Library Prep Kit. Ultimately, high-throughput sequencing is performed by the computer.

The quantity of short-chain fatty acids ascertained

Measure 20 mg of rat excrement precisely and precisely transfer it into a 2 mL centrifuge tube. Introduce 1 mL of phosphoric acid solution and vigorously mix it. After waiting for 10 min, ensure that the mixture becomes uniform, and then expose it to ultrasound for 5 min. Extract 0.1 mL of the resultant liquid and transfer it into a 1.5 mL centrifuge tube. Add 0.5 mL of MTBE solution and thoroughly mix it for 3 min. Perform ultrasound for 5 min. Conduct centrifugation at a rate of 12,000 r/min for 10 min at 4 °C. After centrifugation, take 0.2 mL of the supernatant and place it in a sample bottle for determination and analysis.

To achieve chromatographic separation, utilize a 30 m × 0.25 mm × 0.25 m silica capillary column, DB-FFAP. Employ helium as the carrier gas for the column. The parameters for GC include utilizing an injection

volume of 2 μL, not employing diversion, and keeping a column flow rate of 1.2 mL/min. Implement a column temperature program with gradient stages: (1) Maintain a temperature of 95 ℃ for one minute. (2) Increase the temperature to 100 ℃ at a rate of 25 ℃/min. (3) Further increase the temperature to 130 ℃ at a rate of 17 ℃/min and sustain for 0.4 min. (4) Continue escalating the temperature to 200 ℃ at 25 ℃/min and sustain for 0.5 min. (5) Allow the system to run for 3 min. Adjust the temperature of the injector to 200 ℃ and the temperature of the transmission line to 230 ℃. Keep the ion source temperature at 230 ℃ and the quadrupole temperature at 150 ℃.

Data analysis

At least three repetitions were performed for the biological experiments and analytical tests. The statistical analysis was conducted utilizing SPSS24.0 Software (IBM Corp, Armonk, NY, USA), and the results were presented as the average value accompanied by the standard deviation. GraphPadPrism7.0 (GraphPad Software, La Jolla, California, USA) was utilized to compute the standard deviation. The error bar in the figure represents the standard deviation. Univariate analysis of variance (ANOVA) was then employed to assess significance, * indicating that $p < 0.05$ is a significant difference, ** indicating $p < 0.01$ is a highly significant difference.

Results

Effect of FACB on the weight of rats

The most straightforward way to determine obesity in rats is by measuring their body weight. Table 3 revealed that the average body weight of rats that were fed a high-fat diet following the modeling process was significantly higher than the NC group. This result confirms the successful establishment of the obese rat model. By closely monitoring the changes in body weight of every mouse throughout the 7-week experiment, confirmation was acquired. The final weight of the L, M, and H groups showed a notable decrease similar to that of the HFD group ($p < 0.01$). Additionally, when contrasted with the HFD group, the H group demonstrated a substantial 18.52 % decrease in weight. This finding suggests that the consumption of FACB could potentially mitigate weight gain in obese rats to some extent. Notably, the H group exhibited the most pronounced effect in this respect.

The physiological and biochemical indices of rats are affected by FACB

Fig. 1 displays the findings indicating a notable decline in TG and TC levels in the L, M, and H groups ($p < 0.05$), as evidenced by the study. Additionally, there was a noteworthy elevation in HDL-C levels ($p < 0.01$), specifically in the H group. Additionally, the HDL-C level in the H group increased by 52 %, comparable to the NC group comparable to the HFD group. These findings suggest that excessive fat in individuals with obesity can result in elevated lipid levels in the bloodstream. It should be pointed out that there were no notable alterations detected in the LDL-C level. Research shows that probiotics can improve hyperlipidemia. It can also be seen from the results that the high-fat diet pattern can lead to dyslipidemia in obese rats, and after the intervention of FACB, the lipid levels of obese rats are improved.

Pathological observation results of rat liver slices

Fat synthesis, lipid metabolism, and energy metabolism are primarily the liver's responsibility. Histopathological supplementation of FACB significantly alleviated hepatocyte balloon formation, hepatic vacuolation, and lipid droplet in HFD-fed mice. The findings depicted in Fig. 2 demonstrate that the hepatocytes in the NC group (Fig. 2A) are expected to be intact in appearance and structure. However, the livers of the rats in each experimental group, which were subjected to a high-fat diet, showed varying degrees of damage. The HFD group (Fig. 2B) exhibits the most severe damage, characterized by round lipid droplets and vacuoles of different sizes in the cytoplasm. This suggests that hepatocytes of obese rats become enlarged, accumulate lipids, and undergo fatty deterioration when fed a high-fat diet. In contrast to the HFD group portrayed in Fig. 2B, the arrangement of liver cells can be observed in each dosage group, as shown in Fig. 2C–E. The FACB is quite regular, the nuclear membrane is transparent, the hepatic sinusoids are not distorted, and the amount of lipid droplets and vacuoles is notably diminished. Among them, the H group (Fig. 2E) has the smallest degree of fatty degeneration. Consuming FACB seems to hinder the accumulation of liver fats and reduce liver cell steatosis, offering a protective effect on the livers of rats.

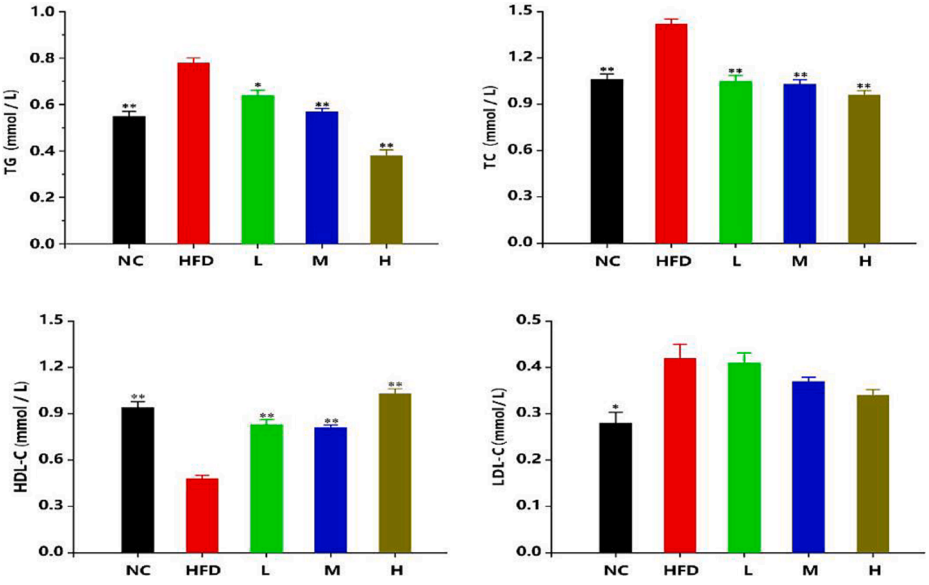

Fig. 1. Effect of FACB on blood lipid level of SD rats. NC: normal diet control group, HFD: high-fat diet group, L: FACB low dose group, M: FACB medium dose group, H: FACB high dose group. Compared with HFD* $p < 0.05$, ** $p < 0.01$.

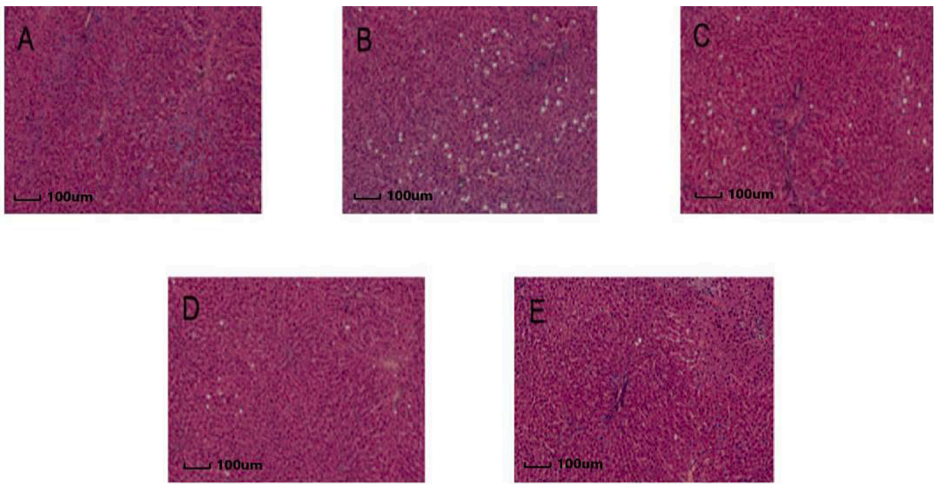

Fig. 2. Photographs showing HE staining of the liver of SD rats in each group (200×). A: normal diet control group (NC), B: high-fat diet group (HFD), C: FACB low dose group (L), D: FACB medium-dose group (M), E: FACB high dose group (H).

An analysis of rat intestinal microbiota's taxonomic composition

Analysis based on phylum level

The dominant bacteria in rats' intestinal microbiota were found to be *Firmicutes*, *Bacteroidetes,* and *Proteobacteria*, with *Firmicutes* accounting for more than 94.4 % of the total proportion (Fig. 3A). The L, M, and H groups had a greater abundance of *Bacteroidetes* than the HFD group, with 0.74 %, 6.49 %, and 1.35 % respectively. At the same time, the relative abundance of *Firmicutes* decreased, with the L and M groups experiencing a decline of 13.13 % and 5.03 %, respectively.

Analysis based on genus level

To perform a more comprehensive analysis of the gut microbiota in rats, the researchers investigated the distribution of microbiota at the genus level. This examination was carried out by creating a histogram displaying the relative abundance of each genus. The results, depicted in Fig. 3(B), revealed that the five groups of samples primarily consisted of *Faecalibacterium*, *Allobaculum*, *Oscillospira*, and *Bacteroides* at the genus level. In each dose group of FACB, the relative abundance of *Allobaculum* and *Oscillospira* was highly compatible with the HFD group. Moreover, the relative abundance of *Allobaculum* in the H group experienced a significant increase of 8.51 % ($p < 0.05$). The L and M groups displayed significantly ($p < 0.05$) higher levels of *Bacteroides* compatible with the HFD group. Additionally, the M and H groups demonstrated a 3.25 % and 2.55 % increase in the relative abundance of *Prevotella* when compatible with the HFD group. Additionally, the H group exhibited a 2.03 % increase in the relative abundance of *Coprococcus*. Furthermore, the L group indicated a significantly lower relative abundance of *Unclassified-Clostridiales* belonging to the *Clostriales* family compared to the HFD group ($p < 0.05$). The implications of these findings suggest that including FACB in our diet can positively impact the diversity of our gut bacteria. By adjusting the levels of certain bacteria like *Allobaculum*, FACB helps maintain a healthy microbial balance in the intestines.

Heatmap analysis of the effect of FACB on intestinal microbiota

Clustering the bacteria with the greatest relative abundance in the top 50 of the genus, R software was employed to ascertain the OTU distribution. The Heatmap map was then created to illustrate the likeness of the microbiota structure between the different groups, and the shift in microbiota distribution could be perceived more intuitively. As can be seen from Fig. 4, under the intervention of FACB, each dosage group of FACB had a higher abundance of *Allobaculum, Parabacteroides, Oxalobacter, Anaerotruncu, Oscillospira, Butyricimonass* than HFD group and had a lower abundance of *Facklamia, Robinsoniella, Providencia, Dorea* than HFD group. The abundance of *Blautia*, *Clostridium*, *Coprococcus*, and *Dehalobacterium* were significantly greater in the H group compatible with the HFD group ($p < 0.05$). The M group had a higher relative abundance of *Phascolarctobacterium*, *Bacteroides*, *Coprobacilus*, and *Alistipes*. Additionally, *Anaerotruncus*, *Odoribacter*, *Bacteroides*, *Coprobacilus*, and *Alistipes* increased in the L group.

Effect of FACB on metabolite short chain fatty acid of intestinal microbiota

According to the information displayed in Fig. 5, it is evident that all five groups of samples contain in considerable quantities of acetic acid, propionic acid, and butyric acid. However, in contrast to the NC group, the HFD group demonstrates significant levels of these short-chain fatty

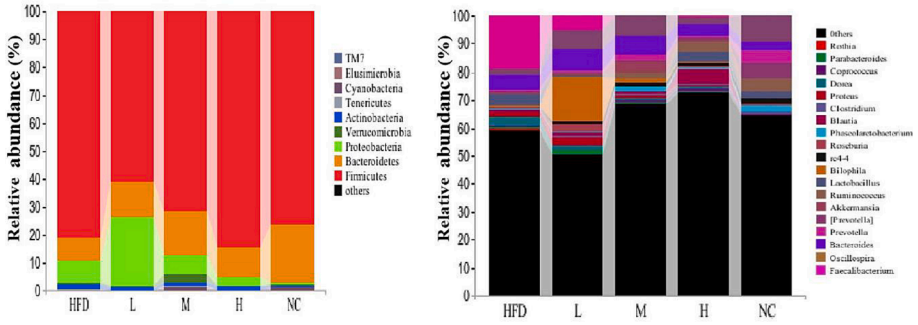

Fig. 3. Relative abundance column chart of five groups of samples at the phylum level (A) and genus level (B). NC: normal diet control group, HFD: high-fat diet group, L: FACB low dose group, M: FACB medium-dose group, H: FACB high dose group.

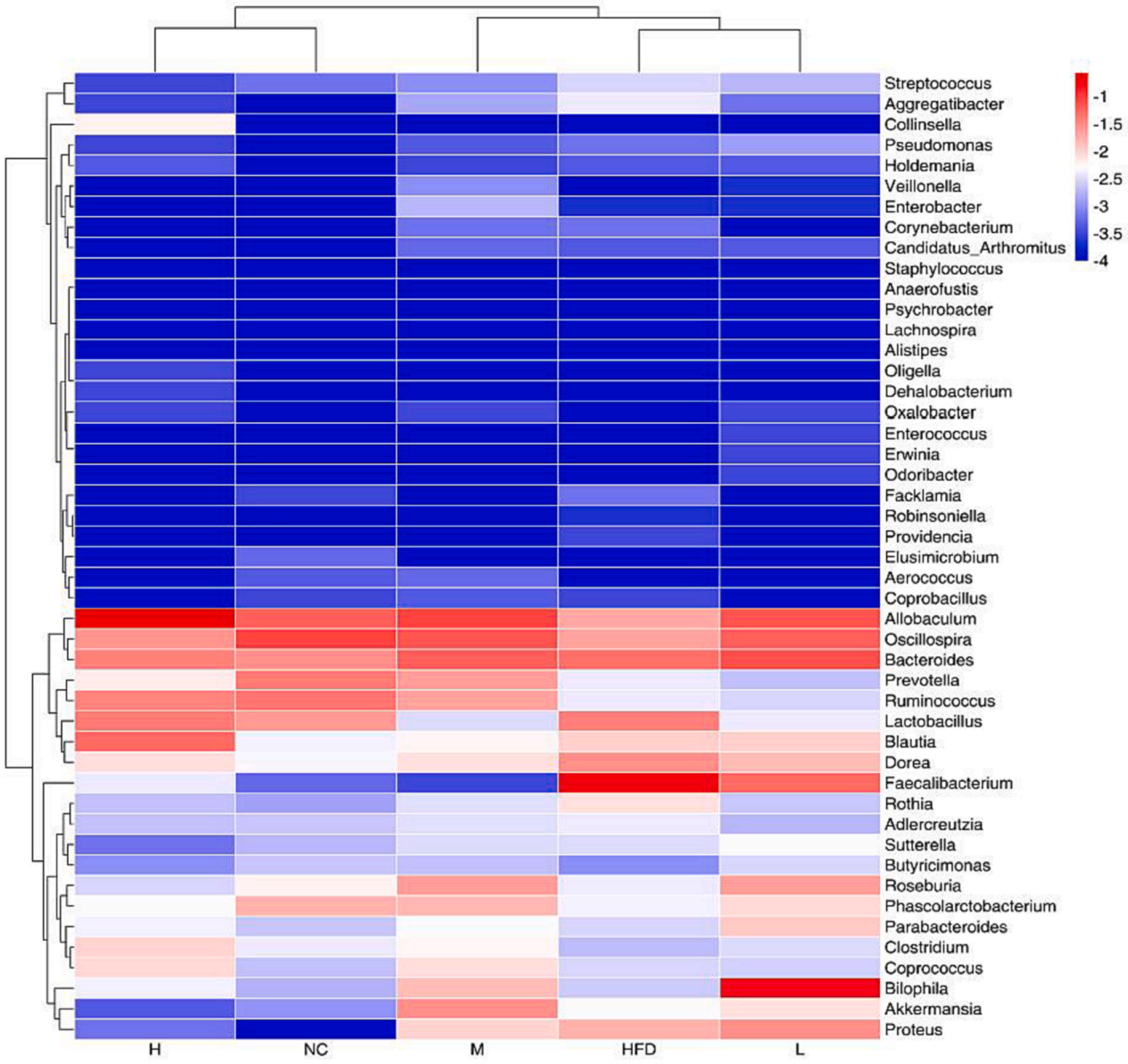

Fig. 4. Heatmap analysis of five groups of samples. The NC: normal diet control group, The HFD: high-fat diet group, The L: FACB low dose group, The M: FACB medium-dose group; The H: FACB high dose group.

acids ($p < 0.05$). After a 7-week FACB intervention, the L, M, and H groups all demonstrate significantly higher concentrations of acetic acid, propionic acid, butyric acid, valeric acid, and isobutyric acid compatible with the HFD group ($p < 0.05$).

Discussion

Presently, the research on fermented foods to prevent and treat obesity has increased (Iñaki & Oihana, 2022). The visual display of body weight results indicated the consumption of fermented products made from *Auricularia cornea* var. Li./blueberry decreased obesity. The impact of this reduction became more pronounced as the dosage of the products increased. The lowest body weight gain induced from the HFD was seen in five-week-old male SD rats, who had been exposed to the most FACB, and the main cause could be the decrease in fat mass while absolute lean mass remained unchanged. This was in agreement with the findings of Johnson (Johnson, Wallig, Vital, & Mejia, 2016), who found no dose–response between the two. The study findings revealed that while the lower amount of FACB (26 mL/kg/d) was successful in blue using blood lipids and promoting weight loss, the higher dosage of FACB yielded even better results. Because obesity is often accompanied by extreme hyperlipidemia, and treating this condition is a relatively effective way to reduce it.

Obese individuals' overabundance of fat can cause a rise in lipid levels in the bloodstream. The FACB effect on the blood lipid levels of SD rats (Fig. 1) revealed that group H's HDL-C level was 52 % higher than the HFD group's, which was almost identical to that of the NC group. There was no notable alteration in LDL-C. FACB is an enzyme product fermented by probiotics, which have been shown to have the ability to improve hyperlipidemia. In addition, *Lactobacillus plantarum*, one of the probiotics used in FACB, has a particularly significant cholesterol-lowering effect (Tjandrawinata, Kartawijaya & Hartanti, 2022). It can also be seen from the results that the high-fat diet pattern will lead to dyslipidemia in obese rats, but after FACB intervention, the lipid level of obese rats improved, consistent with the expected results.

Noticeable histopathological alterations were witnessed in the hepatic tissues of mice fed FACB supplementation while on a high-fat diet. The liver, responsible for fat synthesis, lipid metabolism, and energy

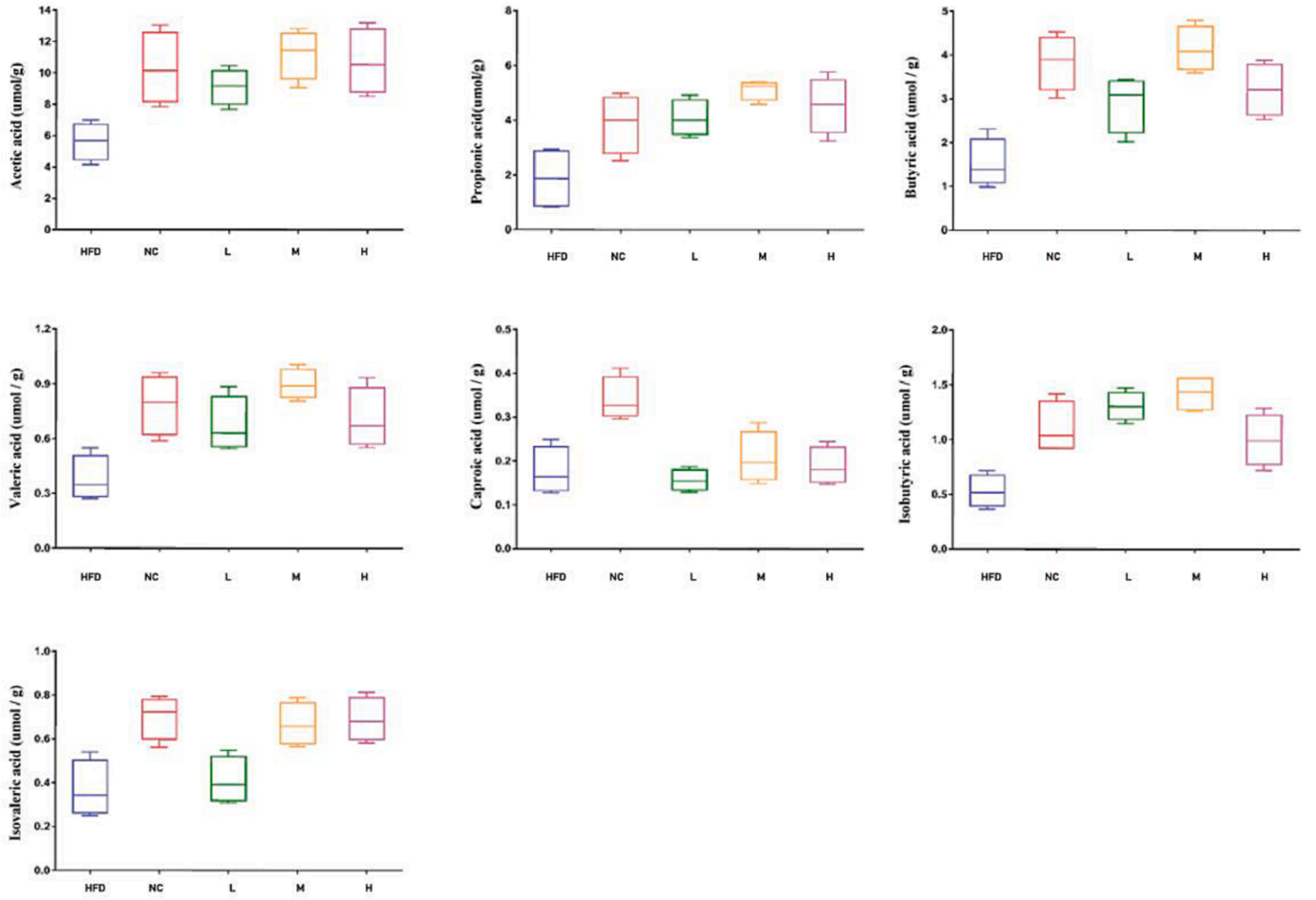

Fig. 5. Effect of FACB on the contents of short-chain fatty acids in SD rats' feces. NC: the normal diet control group, HFD: the high-fat diet group, L: the FACB low dose group, M: the FACB medium-dose group, H: the FACB high dose group.

metabolism, plays an essential role in these processes. These changes led to an election in the formation of hepatocyte balloons, hepatic vacuoles, and lipid droplets. This was evidenced by a comparative analysis of the HE staining photos of the liver of SD rats in each group (Fig. 2). The NC group's liver cells were found to be normal in form and unharmed in structure, whereas the liver of rats in the experimental groups was damaged to varying extents due to the high-fat diet, likely caused by the augmented volume of obese rats' liver cells, lipid buildup, and steatosis. FACB's disruption of the liver cells' arrangement was more consistent. The nuclear membrane was lucid, the hepatic sinuses were not distorted, and the number of lipid droplets and vacuoles was notably diminished. A High dose of FACB will minimize the degree of steatosis. The risk of metabolic diseases is significantly reduced by reducing serum TG and body fat levels, as well as liver fat accumulation (Guo et al., 2020). To sum up, FACB consumption has been shown to reduce the number of liver lipids and the severity of hepatocyte steatosis, thus providing a protective effect on rat liver.

Based on Fig. 3, the findings of a rat intestinal microbiota study have revealed that *Firmicutes* and *Bacteroides* are the principal groups that substantially impact the body's energy metabolism. Yan et al. have demonstrated that the relative abundance of *Firmicutes* is significantly linked to obesity, whereas *Bacteroides* is inversely associated with it (Yan et al., 2018). Increasing the *Proteobacteria/Bacteroidetes* ratio was associated with weight loss and a healthy weight range (Hjorth et al., 2019). Consequently, FACB could be advantageous in treating obesity by altering the composition of intestinal bacteria, encouraging the break down and use of food, and thus controlling the body's energy metabolism. Relevant studies have shown that *Allobaculum* is beneficial to improve human immunity, reducing the occurrence of inflammation, and reducing the risk of metabolic diseases, so it has a certain protective effect on the human intestine (Bai, Zhu & Dong, 2017; Blachier et al., 2019). The production of *Spirulina callosum* was beneficial to increase the content of butyric acid. *Coprococcus* can ferment carbohydrates and has beneficial effects on protecting intestinal health, improving immunity, and enhancing gastrointestinal function (Angelakis, Armougom, Million & Raoult, 2012; Liang, Zhang, Chen, Zhang, Hu & Dai, 2021). *Prevotella* can produce anti-inflammatory metabolites (Iljazovic, Amend, Galvez, Oliveira & Strowig, 2021). From the exhaustive assessment, it can be inferred that the intake of FACB heightens the variety of intestinal microbiota by controlling the number of bacteria and has a certain effect on sustaining the equilibrium of intestinal microbiota.

Studies have shown that *Pseudoides* can produce acetic acid, which is conducive to maintaining the stability stability of intestinal microecology. *Oxalobacterium* is closely related to kindkey stones, which is conducive to oxalic acid degradation and improves effect on digestive tract diseases (Ticinesi, Nouvenne & Meschi, 2019). The sugar metabolism pathway is largely mediated by anaerobic dry, which can generate acetic and butyric acids. Butyrate has certain anti-inflammatory effects and can improve hyperglycemia induced by a high fat (Chai, Luo & Bao, 2021; Batran et al., 2020). At the same time, the increase of *Phascolarctobacterium*, *Bacteroides*, *Anaerotruncus*, and *Odoribacter* can regulate the balance of Intestinal microbiota. In each dose group of FACB, the bacteria mentioned above grew to different extents. In summary, obese rats, upon consuming FACB, modified their gut microbiota, increased the population of beneficial bacteria in their intestines, and improved the consistency of their gut microbiota.

Conversely, the levels of *Robinson*, *Providencia*, and *Dorea*levels decreased in every FACB dose group. *Robinson* and *Providencia* are intestinal pathogens, that are easy to infect and are not beneficial for intestinal health (Puupponen-Pimiä et al., 2005). *Dorea* is the main aerogenic bacteria in the gut. Analysis of heat maps (Fig. 4) revealed that FACB treatment had a diverse effect on intestinal microbiota and that it can generate gas through carbohydrates, potentially resulting in irritable bowel syndrome. After fermentation, the total phenol content, polysaccharide content, and enzyme activity of FACB were significantly increased (Table 1). The findings suggest that using FACB can help partially regulate disruptions in gut microbial balance resulting from a high-fat diet. Notably, fermentation of FACB leads to increased levels of total phenols, polysaccharides, and enzyme activity (as depicted in Table 1). The presence of FACB positively influences the diversity of the gut microbiota. As demonstrated in Table 1, the fermentation process elevates the levels of total phenols, polysaccharides, and enzyme activity in FACB. By employing FACB, it becomes possible to reduce the occurrence of specific harmful bacteria in the gastrointestinal tract while partially improving imbalances in intestinal microbiota resulting from a diet high in fat.

Short-chain fatty acids are a group of organic carboxylic acids that contain between 1 and 6 carbon atoms. This group commonly includes acetic acid, propionic acid, and butyric acid (Silva, Bernardi, & Frozza, 2020). They can regulate lipid metabolism. Research indicates that acetic acid not only supplies energy to intestinal epithelial cells but also restrains the growth of detrimental bacteria (Özcelik, Kuley & Özogul, 2016). Propionic acid is mainly absorbed and utilized in the colon. In addition to cholesterol synthesis inhibition, propionic acid production also stimulates HDL-C production, thereby significantly increasing propionic acid levels in the body. As a result, it effectively prevents atherosclerosis and blood lipid levels. The vital role of butyric acid in maintaining the normal physiological functioning of the intestine is paramount. It is one of the most essential fatty acids in short-chain fatty acids, accelerating lipid metabolism (Thomson, Medina, Ortúzar, Gotteland, & Garrido, 2018). Its content was positively correlated with intestinal homeostasis. This may be because the substances produced by FACB fermentation regulate the intestinal flora to achieve the effect of lipid-lowering. Specifically, what products after fermentation lead to lipid-lowering, and the effects of the types of probiotics on the lipid-lowering of fermentation products remain to be further discussed and studied.

Conclusion

In summary, using FACB reduced in body weight, liver damage, and lipid levels in mice that had become obese due to a high-fat diet. The FACB has caused a significant alteration in the gut microbiota of obese rats, leading to a wider variety of gut bacteria and an observable rise in short-chain fatty acid levels within their digestive system. This change simultaneously aids in regulating the body's energy balance and shows promise in using lipid metabolism disorders caused by the intake of a diet high in fat. This means that fermented *Auricularia cornea* var. Li./blueberry products are promising as natural weight loss products.

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CRediT authorship contribution statement

Xintong Jiang: Data curation, Formal analysis, Investigation, Writing – original draft. **Xue Li:** Investigation, Visualization. **Shuang Li:** Investigation, Visualization. **Minghui Wang:** Investigation, Visualization. **Yunzhu Zhao:** Investigation, Visualization. **SiHan He:** Investigation, Visualization. **Junmei Liu:** Conceptualization, Methodology, Project administration, Resources, Supervision. **Wenguang Fan:** Conceptualization, Methodology, Project administration, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101160.

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