Effects of skim milk fermented with *Lactobacillus plantarum* WW on the constitutions of rats fed a high-fat diet

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ABSTRACT

This study investigated the effects of *Lactobacillus plantarum* WW-fermented skim milk (FSM) on the physiques of rats fed a high-fat diet and the mechanism of lipid lowering. Sprague-Dawley rats were randomly divided into a normal diet group (A), a high-fat diet group (B), a skim milk diet group (C), and an *L. plantarum* WW FSM diet group (D). After 12-wk feeding, we found that treatment with *L. plantarum* WW FSM could significantly alleviate symptoms in the pathological group. Meanwhile, high-throughput sequencing analysis showed that *L. plantarum* WW FSM also had a certain regulatory effect on the intestinal microorganisms in rats, which can increase the number of lactic acid bacteria and *Bacteroides* in the intestine. More importantly, real-time quantitative PCR and Western blot analysis showed that the probiotic was also involved in the expression of genes related to fat metabolism, especially the *PPARB* and *CEBPB* genes. Our study supports the hypothesis that the WW strain of *L. plantarum* could be a potential probiotic to be used in functional foods to alter lipid metabolism and reduce cholesterol levels.

Key words: *Lactobacillus plantarum* WW, high-fat diet, lipid metabolism, hyperlipidemia

INTRODUCTION

The prevalence of hyperlipidemia around the world is related to daily dietary intake, especially the consumption of high-fat diets (HFD; Drouin-Chartier et al., 2016). A major feature of the disease is a rise in blood cholesterol levels and changes in body constitution. By 2016, the number of adults with hyperlipidemia reportedly exceeded 1.1 billion worldwide (Guan et al., 2017). In recent years, the number of hyperlipidemia patients in China has been increasing, and this problem has attracted a lot of attention. It is generally believed that

lipid metabolism disorder is the most important cause of hyperlipidemia. The pathogenesis includes increase of peripheral adipose tissue mobilization, leading to increase of free fatty acid in the liver and enhancement of triglyceride (TG) synthesis (Fan et al., 2003), and decrease of high-density lipoprotein cholesterol (HDL-C) synthesis and secretion, leading to increase of low-density lipoprotein cholesterol (LDL-C) synthesis and decrease of TG transport out of the liver (Zhao et al., 2008), which leads to the pathological evolution of lipid metabolism disorder. Although currently many people rely on drugs for treatment of hyperlipidemia, this cannot be viewed as an optimal long-term solution, because of the high prices and side effects of the commonly used drugs (Bełtowski, 2006; Bełtowski et al., 2009; Wiklund et al., 2013). Based on this situation, we see an urgent need to develop new, effective strategies and technologies to reduce serum cholesterol to prevent hyperlipidemia diseases.

Because the cholesterol-lowering effects of unfermented milk are not obvious, the main reason that fermented milk can reduce cholesterol is the selected probiotics during fermentation (Liu et al., 2016). Many studies have shown that soybean paste is an important resource for screening excellent strains (Wu et al., 2018; Xie et al., 2019; Zhang et al., 2019). Thus, it is necessary to screen the probiotics with high cholesterol-lowering effects. *Lactobacillus plantarum* is the most functional class of probiotics that lower cholesterol, regulate intestinal flora, and enhance immunity (Liu et al., 2017). This became the first experimental goal we would consider. The strain with the most obvious cholesterol-lowering effect can be obtained through in vitro experiments (Ji et al., 2010), and then the safety and some other characteristics of the strain can be evaluated (Choi and Chang, 2015), and finally the strain can be identified and applied to actual production. The *Lactobacillus plantarum* WW used in our study is based on this experimental process.

Few studies have examined the effects of fermented milk on specific genes associated with HFD. The *L. plantarum* WW we selected in this experiment has a cholesterol-lowering rate of 34.56% in vitro (Xin,

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2014). However, the in vivo lipid-lowering effect of the probiotic is not clear. In our experiment, we studied the effect of skim milk fermented with *L. plantarum* WW (FSM) on the expression of genes related to a high-fat diet, further confirming the lipid-reducing effect of the selected *L. plantarum* WW and the future industrial applications of the strain. We first confirmed the lipid-lowering effects of *L. plantarum* WW FSM via hematoxylin-eosin (HE) staining and detection of serum and liver-related biochemical indicators such as levels of total cholesterol (TC), TG, and HDL-C, and then used real-time quantitative PCR (RT-qPCR) and Western blot (WB) experiments to explore the lipid-lowering mechanism at the gene level to increase understanding of the role of *L. plantarum* WW FSM in lowering cholesterol.

MATERIALS AND METHODS

Experimental Scheme

The *L. plantarum* WW strain was isolated from natural fermented soybean paste in Northeast China and identified by the Institute of Microbiology, Chinese Academy of Sciences (Beijing). It was activated in aseptic de Man, Rogosa, and Sharpe medium for subsequent experiments. Fresh and odorless FSM was prepared according to the method of Hua et al. (2014) and divided into 2 portions. One portion was not processed and was stored at 4°C as a non-FSM, and the other portion was inoculated at a level of 8% (vol/vol) with an activated *L. plantarum* WW. After inoculation at 37°C, adjusting the bacterial concentration to 1×10^{10} cfu/mL, both portions were stored at $4 \pm 1^\circ\text{C}$.

Thirty-two male Sprague-Dawley rats from Liaoning Changsheng Biotechnology Co. Ltd. (Shenyang, China; average BW 112 g, 8 wk of age) were housed in cages with a density of 8 rats per cage. The cages were kept in a ventilated room with temperature of 28 to 30°C and 12-h light and dark cycle daily. Feed and water were provided ad libitum. All animals used in this study were cared for in accordance with the guidelines established by the National Institutes of Health (NIH, Bethesda, MD). All experimental procedures were approved by the Animal Protection Review Committee of Shenyang Agricultural University (No. 201106006; Shenyang, China).

After all rats were acclimatized, they were randomly assigned to 4 groups of 8 rats each, assigned to treatments as follows: (1) group A fed basal diet and sterile water; (2) group B fed HFD and sterile water; (3) group C fed HFD and sterile skim milk at 10 mL/kg of BW per day; and (4) group D fed HFD and *L. plantarum* WW FSM at 10 mL/kg of BW per day. Basic feed

consisted of 20% corn flour, 20% wheat flour, 20% bean material, 25% bran, 11% rice flour, 2% bone meal, and 2% fish meal (all mass fraction). The HFD consisted of 87.7% basic feed, 10% lard, 2% cholesterol, and 0.3% sodium cholate (all mass fraction). All groups were fed continuously for 12 wk. The body weight and food intake of rats in each group were recorded at the same time every week. Sterile stools from each group of rats were collected 3 d before the end of the experiment and preserved at -80°C for detection of fecal indicators. After 12 wk of feeding, each rat was weighed and fasted overnight, and blood was taken intravenously for detection of serum lipids. After blood collection, rats were immediately killed by cervical dislocation. The liver, spleen, kidney, and heart of each rat were excised immediately after dissection, washed with chilled saline water (0.85% NaCl, wt/vol), and blotted on filter paper for weighing, and the organ indexes of liver, spleen, kidney, and heart (weight of each organ/final weight) were calculated. Additionally, abdominal, perirenal, and epididymal fat were removed and weighed separately to calculate the fat coefficient (weight of each fat deposit/final weight) of each group of rats. Liver tissue blocks of the same size were cut out and fixed in a 10% formaldehyde solution for 24 h for HE staining. The rest of the liver was rapidly frozen with liquid nitrogen and stored in a refrigerator at -80°C for subsequent experiments.

Detection of Animal Sample Indicators

Determination of Serum, Fecal, and Liver Biochemical Markers. Liver samples of a certain weight were taken and physiological saline added according to a weight-to-volume ratio of 1:9 (g/mL). The mechanical homogenate was obtained under the conditions of ice water bath, and the homogenized sample was centrifuged at $2,500 \times g$ for 10 min and the supernatant removed. The determination of biochemical markers in feces was carried out in the same way. Blood samples were immediately centrifuged at $3,000 \times g$ for 15 min, and the serum was recovered for further analyses. Levels of serum and hepatic lipids, including total TC, HDL-C, LDL-C, TG, thiobarbituric acid (TBA), malondialdehyde, alanine aminotransferase (ALT), aspartate transaminase (AST), and levels of fecal lipids, including TC, TG, and TBA, were determined using assay kits (BeihuaKangtai Clinical Reagent Company, Beijing, China) according to the manufacturer's instructions.

HE Staining of Liver Tissue. Liver tissues of the same size in each group were fixed with 4% paraformaldehyde and embedded in paraffin for HE staining. The histopathology was performed as described in a previ-

ous report (Kang et al., 2015). Images were observed under a microscope and preserved.

Determination of Microbial Diversity in Feces. The Illumina MiSeq high-throughput sequencing method (Illumina, San Diego, CA) was used to analyze the microbial diversity of rat feces. The construction of the MiSeq library and MiSeq sequencing were commissioned by Shanghai Meiji Biotechnology Co. Ltd. (Shanghai, China). The experimental method was carried out with reference to Caporaso et al. (2012).

RT-qPCR Analyses of Gene Expression. Total RNA was extracted from the liver by the RNeasy Pure Tissue Kit (TianGenBiotech, Beijing, China). We obtained cDNA via reverse transcription using the FastKing RT Kit (TianGen Biotech) according to the manufacturer's instructions. Gene expression was detected by 7500 Fast Real-Time PCR System (College of Food, Shenyang Agricultural University, Shenyang, China). Data analysis was performed using the $2^{-\Delta\Delta CT}$ method and normalized with the house-keeping gene *ACTB*. The sequences of RT-qPCR primers were as follows (Meiji Biotechnology): peroxisome proliferator-activated receptor β (*PPARB*; Sanchez et al., 2017), F5'ACGCTATCCGCTT TGG R5'CGTTATGGCTGGACTTGC; cholesterol-hydroxylase (*CYP7A*; Zhaldak et al., 2017) F5'AGACGCACCTCGCTATCC R5'AGCCTCAG AGCCTCCTTG; cholesterol4 α -hydroxylase (*CEBPB*; Kita et al., 2018), F5'CTCCTCTTCCCACTCCCT R5'CCCTTTCGCTTATCCACT; sterol regulatory element binding protein-1c (*SREBP1c*; Choi and Chang, 2015), F5'AATGGTCCAGGCAAGTTCTG GGTC R5'TCCCTCTCAGCTGTGGTGGTGAA; fatty acid synthase (*FAS*; Peng et al., 2016), F5'AAAACAGGACTGGCAGAT R5'CAGGTTTCAC AGAGGAGGT.

WB Verification of Gene Expression. Western blot analysis (WanLei Biotech, Shenyang, China) was performed to verify the results obtained by RT-qPCR.

The specific experimental method was carried out with reference to Guo et al. (2019). The expression of *PPARB* and *CEBPB* genes were verified with *ACTB* as control.

Statistical Analysis

We used SPSS (version 17.0, SPSS Inc., Chicago, IL) for all statistical analyses. Significance testing with one-way ANOVA was used to compare the differences between the means of the experimental groups. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

During the 12 weeks of the experiment, the rats in each group had normal vital signs and none died. As can be seen in Table 1, there was no difference in the initial body weights of the 4 groups of rats. After the 12-wk feeding period, the final BW, weight gain, and total food intake of the B group were significantly greater than those of the A group ($P < 0.01$), which indicates that the HFD can cause increase of BW in rats (Lindström et al., 2006; Bazzano et al., 2011). This may be due to accumulation of lipid mass in body cells. Compared with the B group and the C group, the BW of the D group was reduced, but the difference was not significant ($P > 0.05$), and the total food intake was significantly lower ($P < 0.01$), indicating that *L. plantarum* WW FSM intake through diet can effectively inhibit increase of BW.

From Table 2, we can see that no significant differences occurred in the heart, kidney, and spleen indexes among the 4 groups ($P > 0.05$), which indicated that *L. plantarum* WW FSM had no side effects on the heart, kidney, or spleen of the rats ($P > 0.05$). The abdominal, perirenal, and epididymal fat coefficients of the B and C groups were significantly higher than those of the A group ($P < 0.01$; Bazzano et al., 2011). However, all 3

Table 1. Body weight gain and total food intake of rats in each experimental group¹

Group	Initial weight	Final weight	Weight gain	Total food intake
A	126.38 $\pm$ 5.85 ^{A,a}	387.00 $\pm$ 12.83 ^{B,b}	260.63 $\pm$ 13.72 ^{B,b}	18,444 $\pm$ 31.53 ^{B,b}
B	127.50 $\pm$ 5.68 ^{A,a}	447.13 $\pm$ 41.83 ^{A,a}	319.63 $\pm$ 41.50 ^{A,a}	21,689 $\pm$ 50.78 ^{A,a}
C	127.50 $\pm$ 5.40 ^{A,a}	445.63 $\pm$ 40.84 ^{A,a}	318.13 $\pm$ 37.89 ^{A,a}	22,153 $\pm$ 66.93 ^{A,a}
D	129.75 $\pm$ 6.41 ^{A,a}	432.13 $\pm$ 18.32 ^{A,a}	302.38 $\pm$ 17.44 ^{A,a}	19,419 $\pm$ 49.38 ^{B,b}

^{A,B}Different uppercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.01$).

^{a,b}Different lowercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.05$).

¹Measurements are all given in grams. Values are given as mean $\pm$ SD ($n = 8$). Group A was fed basal diet and sterile water; group B was fed a high-fat diet (HFD) and sterile water; group C was fed HFD and sterile skim milk at 10 mL/kg of BW per day; and group D was fed HFD and *Lactobacillus plantarum* WW fermented skim milk at 10 mL/kg of BW per day.

Table 2. Indexes of organ and fat coefficients of rats in each group¹ (%)

Group	Liver index	Cardia index	Renal index	Spleen index	Abdominal fat coefficient	Perirenal fat coefficient	Epididymal fat coefficient
A	3.01 ± 0.36 ^{A,b}	0.36 ± 0.04 ^{A,a}	0.92 ± 0.10 ^{A,a}	0.25 ± 0.03 ^{A,a}	0.32 ± 0.08 ^{C,c}	0.42 ± 0.06 ^{B,b}	0.49 ± 0.09 ^{B,b}
B	3.48 ± 0.24 ^{A,a}	0.36 ± 0.04 ^{A,a}	0.90 ± 0.08 ^{A,a}	0.24 ± 0.02 ^{A,a}	0.75 ± 0.16 ^{A,a}	0.68 ± 0.15 ^{A,a}	0.80 ± 0.11 ^{A,a}
C	3.31 ± 0.48 ^{A,a}	0.34 ± 0.06 ^{A,a}	0.91 ± 0.12 ^{A,a}	0.24 ± 0.06 ^{A,a}	0.71 ± 0.21 ^{A,a}	0.64 ± 0.08 ^{A,a}	0.83 ± 0.12 ^{A,a}
D	2.92 ± 0.50 ^{A,b}	0.32 ± 0.06 ^{A,a}	0.86 ± 0.09 ^{A,a}	0.22 ± 0.03 ^{A,a}	0.45 ± 0.09 ^{B,b}	0.44 ± 0.07 ^{B,b}	0.59 ± 0.08 ^{B,b}

^{A-C}Different uppercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.01$).

^{a-c}Different lowercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.05$).

¹Values are given as mean ± SD (n = 8). Group A was fed basal diet and sterile water; group B was fed a high-fat diet (HFD) and sterile water; group C was fed HFD and sterile skim milk at 10 mL/kg of BW per day; and group D was fed HFD and *Lactobacillus plantarum* WW fermented skim milk at 10 mL/kg of BW per day.

fat coefficients were significantly decreased ($P < 0.01$) after oral administration of *L. plantarum* WW FSM, which indicated that the HFD caused accumulation of fat in the body organs and that *L. plantarum* WW could effectively reduce the accumulation of fat in the organs and alleviate the damage from fat accumulation.

The contents of TC, TG, and LDL-C in the serum and livers of rats fed HFD were significantly higher than those of the A group (Table 3; $P < 0.01$), which indicated that the high-fat model was successfully established. Compared with the B and C groups, the contents of TC, TG, and LDL-C in the serum and livers of the D group were significantly decreased ($P < 0.01$). Compared with the B and C groups, the contents of TC, TG, and LDL-C in the serum and livers of the D group were significantly decreased ($P < 0.01$), and the contents of HDL-C in serum and liver increased ($P > 0.05$). The fecal indexes of each group were measured as shown in Table 4. The levels of TC and TG in the D group were higher than those of the B and C groups. The content of TBA in the feces of rats in group D was significantly higher than that of the A group ($P < 0.01$). The levels of malondialdehyde and TBA in the B and C groups were significantly higher than those of the A group (Figure 1 A and B; $P < 0.01$). The content

of malondialdehyde and TBA in serum of group D was significantly lower than those of groups B and C ($P < 0.01$), but it was significantly higher than that of group A ($P < 0.01$). Simultaneously, rats fed with *L. plantarum* WW FSM can significantly reduce ALT and AST in serum and liver (Figure 1; $P < 0.01$).

The effect of *L. plantarum* WW FSM on hepatic fat accumulation induced by HFD was further determined by HE staining of liver tissues of rats in each group. Observation of histological sections (Figure 2) showed that the hepatocytes of rats fed with normal diet were arranged in cords, the cytoplasm was red, and the nucleus was located in the centers of cells. The morphology of hepatocytes in group D was similar to that in group A. However, severe vacuolation and increased lipid deposition in the cytoplasm were observed in the livers of rats in the B and C groups, characterized by round cells, marginal nuclei, and fat vacuolation.

The results of microbial diversity analysis of feces from each rat via high-throughput sequencing are shown in Figure 3. From the analysis of community composition, we can see that the composition of microbial species in the feces of the 4 groups of rats is basically similar. The percentage of *Lactobacillus* and *Bacteroides* in the feces of group D was higher than that of group B, but the

Table 3. Effect of *Lactobacillus plantarum* WW fermented skim milk (FSM) on serum and liver lipid levels in rats¹ (mmol/L)

Group	TC		TG		LDL-C		HDL-C	
	Serum	Liver	Serum	Liver	Serum	Liver	Serum	Liver
A	2.40 ± 0.48 ^{C,c}	1.11 ± 0.23 ^{B,b}	1.06 ± 0.21 ^{B,c}	0.32 ± 0.04 ^{C,c}	0.32 ± 0.09 ^{C,c}	0.24 ± 0.05 ^{C,c}	0.84 ± 0.22 ^{A,a}	0.34 ± 0.10 ^{A,a}
B	5.67 ± 0.71 ^{A,a}	1.82 ± 0.09 ^{A,a}	2.67 ± 0.46 ^{A,a}	0.92 ± 0.18 ^{A,a}	0.69 ± 0.14 ^A	0.55 ± 0.12 ^{A,a}	0.33 ± 0.09 ^{C,c}	0.18 ± 0.05 ^{B,b}
C	5.50 ± 0.67 ^{A,a}	1.63 ± 0.20 ^{A,a}	2.63 ± 0.42 ^{A,a}	0.95 ± 0.17 ^{A,a}	0.67 ± 0.17 ^{A,a}	0.58 ± 0.09 ^{A,a}	0.42 ± 0.12 ^{bc}	0.20 ± 0.07 ^{B,b}
D	4.03 ± 0.83 ^{B,b}	1.28 ± 0.26 ^{B,b}	2.07 ± 0.59 ^{A,b}	0.69 ± 0.13 ^{B,b}	0.49 ± 0.09 ^{B,b}	0.40 ± 0.07 ^{B,b}	0.54 ± 0.07 ^{B,b}	0.25 ± 0.03 ^{AB}

^{A-C}Different uppercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.01$).

^{a-c}Different lowercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.05$).

¹Values are given as means ± SD (n = 8). TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol. Group A was fed basal diet and sterile water; group B was fed a high-fat diet (HFD) and sterile water; group C was fed HFD and sterile skim milk at 10 mL/kg of BW per day; and group D was fed HFD and *L. plantarum* WW FSM at 10 mL/kg of BW per day.

Table 4. Effect of *Lactobacillus plantarum* WW fermented skim milk (FSM) on total cholesterol (TC), triglycerides (TG), and thiobarbituric acid (TBA) levels of feces in rats¹

Group	TC (mmol/g)	TG (mmol/g)	TBA (μmol/g)
A	0.28 ± 0.05 ^{B,c}	0.24 ± 0.03 ^{B,c}	22.96 ± 3.44 ^{C,c}
B	0.47 ± 0.11 ^{AB,ab}	0.65 ± 0.07 ^{A,b}	43.97 ± 6.44 ^{B,b}
C	0.46 ± 0.04 ^{AB,b}	0.69 ± 0.09 ^{A,ab}	45.03 ± 3.44 ^{B,b}
D	0.59 ± 0.07 ^{A,a}	0.80 ± 0.03 ^{A,a}	58.65 ± 5.49 ^{A,a}

^{A-C}Different uppercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.01$).

^{a-c}Different lowercase superscript letters in the same column indicate that the difference between groups is significant ($P < 0.05$).

¹Values are given as means ± SD (n = 8). Group A was fed basal diet and sterile water; group B was fed a high-fat diet (HFD) and sterile water; group C was fed HFD and sterile skim milk at 10 mL/kg of BW per day; and group D was fed HFD and *L. plantarum* WW FSM at 10 mL/kg of BW per day.

proportion of *Streptococcus* was lower in group D than that of group B. The results show that *L. plantarum* WW FSM plays a regulatory role in the intestinal flora and has the potential to reduce the occurrence of cirrhosis.

Our study revealed a decrease in serum TC, TG, and LDL-C of group D, and enhancement of serum HDL-C, which may help reduce the risk of atherosclerosis (Pischon et al., 2009). The intake of HFD also increased the weight of some visceral organs (Ogunremi et al., 2015), the liver lipid levels, and the serum lipid profiles of rats. This result is consistent with previous reports (Gonçalves et al., 2015; Rocha-Rodrigues et al., 2016). This has been suggested to be due to the accumulation of lipid masses in liver cells (Kobayashi et al., 2012). It is worth mentioning that histologic analyses in the present study showed that feeding FSM had clear inhibitory effects on liver lipid deposition in rats. In addition, it is well known that ALT and AST are important factors in causing liver injury (Kim et al., 2018). In our experiment, the indexes of ALT and AST in the FSM group were lower than those of the normal diet group and the HFD group ($P < 0.01$). The results show that *L. plantarum* WW FSM plays an important role in the treatment of liver injury.

The role of the intestinal microbiota in host energy homeostasis is crucial and is greatly affected by high-fat diets (Bäckhed et al., 2004; Ramakrishna, 2013). The present study showed that the number of microbial communities in the feces of the 4 groups of rats differed significantly. The number of *Lactobacillus* and *Bacteroides* in the feces of the control group (A) was the highest, followed by the D group, and the B group was the lowest. It has been reported that *Streptococcus* may be a harmful strain causing cirrhosis (Hussain et al., 2019). In our experiment, the number of *Streptococcus*

decreased, which could regulate the intestinal flora and reduce the occurrence of liver cirrhosis.

Compared with previous research on the lipid-lowering effects of FSM (Kim et al., 2018), we verified the mechanism of lipid reduction of *L. plantarum* WW FSM for the first time via RT-qPCR and WB experiments. The levels of expression of hepatic mRNA related to fat metabolism, such as *FAS*, *CYP7A*, *PPARB*, *CEBPB*, and *SREBP1c* are shown in Figure 4. Compared with the normal diet group (A), the HFD resulted in significant upregulation of *PPARB*, *FAS*, and *CEBPB* mRNA expression in the B group ($P < 0.05$), and the expression level of *SREBP1c* in group D was lower than that of the normal diet group, whereas oral supplementation with *L. plantarum* WW FSM downregulated the mRNA expression of *PPARB* and *CEBPB*. Compared with the D group, the *FAS* mRNA expression levels were higher in groups A, B, and C, with the highest expression level in the B group.

The expression of *PPARB* and *CEBPB* protein in liver was measured via protein immunoblotting assay (Figure 5). As can be seen from the figure, expression of *PPARB* and *CEBPB* in each group was significantly different. The expression of *PPARB* and *CEBPB* in group B was significantly higher than in group A. The level of expression of *PPARB* and *CEBPB* in group D was lower than in groups B and C, and significant difference occurred compared with other groups ($P < 0.05$).

Among the *FAS*, *CYP7A*, *PPARB*, *CEBPB*, and *SREBP1c* genes, *PPARB* and *CEBPB* are the most important, with the most obvious changes, so we selected these 2 genes for WB verification. Although *FAS*, *CYP7A*, and *SREBP1c* were not verified by WB in our experiment, they are still closely related to lipid metabolism. An important downstream gene in the *PPAR* signaling pathway (Hollingshead et al., 2017), *CYP7A* is a microsomal cytochrome p450 enzyme that catalyzes the first and rate-limiting steps in the bile acid biosynthesis pathway, which is closely related to the overall rate of bile acid synthesis (Chiang, 2002). *Lactobacillus plantarum* WW FSM could increase the expression of the *CYP7A* gene in the liver of rats with hyperlipidemia, which would promote the digestion and absorption of fat and decrease TBA and other indicators in serum.

Sterol regulatory element binding protein (*SREBP*) plays an important role in animal fat metabolism and is closely related to lipid accumulation in non-adipose tissue such as the liver. *SREBP1c* is an isoform of *SREBP* with lipid-generating properties (Mengmei Hu, 2012). The expression of *SREBP1c* mRNA in the B group was higher than in the D group, which may be caused by

Figure 1. Effects of *Lactobacillus plantarum* WW fermented skim milk on liver and serum fat metabolism induced by high-fat diet in rats. Serum malondialdehyde (MDA, a), serum thiobarbituric acid (TBA, b), serum alanine aminotransferase (ALT, c), serum aspartate transaminase (AST, d), liver ALT (e), and liver AST (f) in rats treated with different diets for 12 wk. A = normal diet group, B = high-fat diet group, C = skim milk diet group, D = fermented skim milk diet group. Values with different letters are significantly different ($P < 0.05$). Results were expressed as mean $\pm$ SD ($n = 8$).

Figure 2. Effects of *Lactobacillus plantarum* WW fermented skim milk on pathological morphology of rat liver tissue. Liver tissue samples of the same size from each group were fixed with 4% paraformaldehyde and embedded in paraffin for hematoxylin-eosin staining. A = normal diet group, B = high-fat diet group, C = skim milk diet group, D = fermented skim milk diet group.

Figure 3. Effects of *Lactobacillus plantarum* WW fermented skim milk on intestinal microorganisms in rats. A = normal diet group, B = high-fat diet group, C = skim milk diet group, D = fermented skim milk diet group. (a) Community analysis; (b) one-way ANOVA at genus level for *Lactobacillus*; (c) one-way ANOVA at genus level for *Streptococcus*; (d) one-way ANOVA at genus level for *Bacteroides*.

the HFD. This is consistent with the results shown in Figure 2. *Lactobacillus plantarum* WW FSM lowers the deposition of fat in the liver.

A key enzyme of liver and a downstream target gene for *SREBP1c*, *FAS* is involved in the biosynthesis of long-chain fatty acids and thus is considered a target for treating obesity (Lauressergues et al., 2011; Bourrie et al., 2018). It can convert nutrients absorbed by the body into fatty acids, which raises the level of triglycerides. The expression of *FAS* in group B was significantly increased by HFD, which resulted in higher serum and liver TG levels in the B group than in the other groups.

The *PPAR* signaling pathway is one of the main pathways of adipogenesis and metabolism (Guan et al., 2017). One of the major genes of the *PPAR* signaling pathway and the ligand-dependent transcription factors of the nuclear receptor superfamily, *PPARB* is closely related to adipocyte differentiation. Peroxisome proliferator activated receptor beta is widely distributed in the body, mainly in the brain, adipose tissue, and skin (Barroso et al., 2011). The ligands of *PPARB* include natural ligands, such as linoleic acid, and synthetic ligands; *PPAR β* can play an important role in hyperlipidemia, atherosclerosis, and other diseases by regulating lipid metabolism and inhibiting inflammatory reactions (Pan et al., 2014). Our RT-qPCR assay showed that, compared with group B, the *CEBPB* gene in group D rats fed with *L. plantarum* WW FSM was

significantly downregulated, and this change was the most obvious when compared with the *FAS*, *CYP7A*, *PPARB*, and *SREBP1c* genes. We further verified via WB that the *CEBPB* gene was downregulated by *L. plantarum* WW FSM, and the level in group D was closer to that in group A.

The *CEBPB* gene is another one of the major genes of the *PPAR* signaling pathway. It is generally believed that *CEBPB* alone cannot induce differentiation of adipocytes (Rosen et al., 2002). By establishing an immortalized line of fibroblasts lacking *PPARG*, Rosen et al. demonstrated that *CEBPB* does not have the ability to promote lipogenesis without *PPARG*. Our RT-qPCR experiment showed that oral administration of *L. plantarum* WW FSM with HFD decreased expression of *PPARB* and *CEBPB*. The expression of *PPARB* and *CEBPB* proteins in the D group was further verified by WB analysis, which showed it to be lower than in the B group. We hypothesized that *L. plantarum* WW FSM could reduce lipids by inhibiting the expression of these 2 major genes.

Through RT-qPCR we found that the expression trends of *PPARB* and *CEBPB* in group D were basically the same as those in group A, whereas the expression trends of *PPARB* were more obvious. Serum and liver ALT, AST, TC, and LDL-C in B group are higher than in other groups, which we speculate may be related to the regulation of the *PPARB* and *CEBPB* genes. As

Figure 4. Hepatic mRNA expression levels in rats for the *FAS*, *CYP7A*, *PPARB*, *CEBPB*, and *SREBP1c* genes, as measured via real-time quantitative PCR (qRT-PCR). A = normal diet group, B = high-fat diet group, C = skim milk diet group, D = fermented skim milk diet group. Results were expressed as mean $\pm$ SD ($n = 8$).

Figure 5. Effects of *Lactobacillus plantarum* WW fermented skim milk on lipogenic proteins in hepatic tissues of rats. A = normal diet group, B = high-fat diet group, C = skim milk diet group, D = fermented skim milk diet group. Values with different letters are significantly different ($P < 0.05$). Results were expressed as mean $\pm$ SD ($n = 8$).

these are the 2 main genes of the PPAR pathway, the function of other genes such as *CYP7A* and *SREBP1c* may be affected by these 2 genes (Fernø et al., 2009). *PPARB* and *CEBPB* may be the most important genes involved in the regulation of lipid metabolism, and thus the potential targets of prevention for the treatment of cardiovascular diseases, but this conjecture requires further study.

CONCLUSIONS

In summary, the main findings of this study are that *L. plantarum* WW FSM has a positive effect on the treatment of hyperlipidemia induced by HFD in rats. In our investigation, *L. plantarum* WW FSM not only regulated lipid metabolism (as shown in levels of TC, TG, LDL-C, and HDL-C) and alleviate liver injury (demonstrated in levels of ALT and AST), but also played an active role in maintaining the stability of intestinal flora. *Lactobacillus plantarum* WW FSM is involved in regulating expression of the lipid metabolism-related genes *PPARB* and *CEBPB*, which may be one of the mechanisms of its lipid-lowering effect. It can be seen that the *L. plantarum* WW FSM has potential to treat hyperlipidemia.

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