



Lipid-Lowering Effect of Red *Glutinous Sorghum* in High-Fat Diet-Induced Obese Mice

Yanlin Qin¹, Ren Zou¹, Qiming Yu^{1,2*}

¹The School of Public Health, Guilin Medical University, Guilin, China

²Guangxi Key Laboratory of Environmental Exposomics and Entire Lifecycle Health, Guilin Medical University, Guilin, China

Email: 3318188036@qq.com, *qm-yu19@glmc.edu.cn

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Abstract

This study aimed to investigate the lipid-lowering effect of Red *Glutinous Sorghum* (RGS) in high-fat diet-induced obese mice. Mice were randomly divided into a normal control group (NC, n = 6, normal diet) and a high-fat diet group (HFD, n = 12, high-fat diet) and fed for 9 weeks to establish an obesity model. After successful model establishment, the HFD-fed mice were randomly re-assigned to either the HFD group (n = 6, high-fat diet) or the RGS group (n = 6, RGS-diet) for a 7-week intervention period. Body weight, liver weight, and fat weight were recorded throughout the experiment. Hepatic pathological changes were observed using hematoxylin-eosin (HE) staining. Serum and hepatic levels of TG, TC, LDL-C, and HDL-C were measured. Western blot analysis was employed to assess protein expression levels of IL-6 and FASN in liver tissue. The results showed that, compared with the HFD group, RGS treatment significantly decreased body weight, liver index, fat index, and serum/hepatic levels of TG, TC, LDL-C ($P < 0.05$), while significantly increasing HDL-C levels ($P < 0.05$). Hepatic pathological examination revealed that the RGS group exhibited markedly improved hepatocyte structure and a significant reduction in lipid vacuoles. Furthermore, the protein expression levels of IL-6 and FASN in liver tissue were significantly downregulated in the RGS group ($P < 0.05$). In summary, RGS effectively inhibited high-fat diet-induced weight gain, improved lipid metabolism disorders, and reduced hepatic lipid accumulation. The underlying mechanism may be related to the downregulation of IL-6 and FASN protein expression, thereby alleviating inflammatory responses and suppressing fatty acid synthesis.

Subject Areas

Food Science & Technology

Keywords

Red *Glutinous Sorghum*, High-Fat Diet, Obesity, Lipid-Lowering Effect

1. Introduction

Since the beginning of the 21st century, rapid advances in science and technology have progressively improved living standards worldwide. However, changes in dietary patterns and the widespread adoption of sedentary lifestyles have led to increased consumption of high-fat diets and chronic energy surplus, thereby promoting the development of obesity [1]. In recent years, the global prevalence of obesity has continued to rise, significantly increasing the risk of metabolic diseases such as hyperlipidemia and cardiovascular disease [2]. This trend has also substantially elevated the risk of premature mortality and imposed a considerable socioeconomic burden [3]. As a result, obesity has emerged as one of the most critical public health challenges of this century [4].

Current treatment strategies for obesity primarily include dietary intervention, pharmacotherapy, and bariatric surgery [5]. However, long-term use of weight-loss medications may be associated with adverse effects such as nausea, vomiting, and abdominal pain [6]. Similarly, bariatric surgery carries risks including reduced bone mineral density, increased fracture risk, and micronutrient deficiencies [7]. In contrast, dietary intervention is regarded as a more promising approach for obesity management owing to its high safety profile and strong sustainability [8]. In recent years, natural products derived from plants and herbs have demonstrated promising effects in alleviating obesity with favorable safety profiles, offering new insights for its prevention and management [9].

Sorghum ranks as the fifth most widely consumed cereal grain globally. It is rich in various bioactive compounds, including phenolic compounds, carotenoids, and vitamin E, and exhibits anti-obesity, antioxidant, and anti-inflammatory bioactivities [10]. Owing to its unique phenolic composition, *sorghum* shows considerable potential as a functional food [11]. Studies have demonstrated that *sorghum* seed extract can significantly reduce malondialdehyde and hydrogen peroxide levels while markedly increasing the activities of superoxide dismutase and glutathione peroxidase [12]. Furthermore, phenolic extracts from *sorghum* have been reported to exhibit significant antibacterial activity against liver abscess-causing pathogens [13].

Sorghum exhibits strong tolerance to drought and high temperatures, requiring minimal inputs while yielding high productivity. Beyond its widespread use as food, feed, and fodder, *sorghum* has been further developed for the production of syrup, biofuels, and various bio-based products [14]. Regular consumption of whole-grain *sorghum* foods can exert positive effects on human health. However, the limited variety of *sorghum*-based food products currently available on the global market restricts consumer choices; therefore, collaborative innovation is

urgently needed to promote new product development [15].

The present study selected the novel *sorghum* variety “red *glutinous sorghum*”, officially recognized by the Department of Agriculture and Rural Affairs of Guangxi Zhuang Autonomous Region as the research object. This variety possesses several notable advantages: it demonstrates extremely strong wind resistance and root anchorage, making it well-suited for cultivation on dry land; it has a soft and glutinous texture with high starch content, making it a viable alternative to certain conventional grains. Moreover, *sorghum* is one of the traditional crops widely cultivated in China. Promoting the cultivation of red glutinous *sorghum* in the Guangxi region and deeply developing its functional attributes is expected to generate significant economic benefits. Therefore, this study aimed to investigate the lipid-lowering effects of red *glutinous sorghum* in a high-fat diet-induced obese mouse model, with the goal of providing a theoretical basis for the comprehensive utilization of red *glutinous sorghum* resources and the development of related functional foods.

2. Materials and Method

2.1. Experimental Animals and Materials

A total of eighteen male SPF ICR mice aged 5 - 6 weeks were used in this study. The *sorghum* grains were processed and milled into flour prior to the experiment. The experimental diets were formulated according to the AIN-93G guidelines (Table 1).

Table 1. Composition of experimental diets (g/kg).

Name	High-fat diet (g)	Normal diet (g)	RGS-diet (g)
Casein	20	20	20
Soybean protein	90	90	90
Fish meal	50	50	50
Soybean meal	150	150	150
L-cysteine	3	3	3
Corn starch	147.5	317.5	0
Lard	170	0	170
Pregelatinized corn starch	132	132	0
Sucrose	100	100	100
Wheat bran	20	20	20
Soybean oil	70	70	70
Mineral mix	35	35	35
Vitamin mix	10	10	10
Choline bitartrate	2.5	2.5	2.5
BHT	0.014	0.014	0.014
Red <i>glutinous sorghum</i> flour	0	0	450
Energy (kcal)	4000	3601	4099

Assay kits for total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Reagents and antibodies, including RIPA lysis buffer, BCA protein assay kit, ECL substrate, and specific primary (IL-6, FASN, GAPDH) and secondary (Goat Anti-Rabbit IgG) antibodies, were obtained from Beyotime Biotechnology (Shanghai, China). The instruments used included an electronic balance (Sartorius Scientific Instrument (Beijing) Co., Ltd.), an Alpha chemiluminescence gel imaging system (Purseen Biotechnology (Shanghai) Co., Ltd.), an optical microscope and RM2125 rotary microtome (Leica, Germany), and a multifunctional microplate reader (Thermo Scientific, USA).

2.2. Method

2.2.1. Experimental Grouping and Animal Feeding

All the experimental animals were housed in SPF animal facility maintained at (22 ± 2) °C with a 12 h light/dark cycle, with ad libitum access to food and water. Following a 1-week acclimatization period, the mice were randomly divided into two groups using a random number table: the normal control (NC) group (n = 6), fed a normal diet, and the High-fat diet (HFD) group (n = 12), fed a High-fat diet. After successful model establishment (defined as the HFD group exhibiting a mean body weight ≥ 20% greater than the NC group), the HFD-fed mice were randomly reassigned to two groups (n = 6 per group): the RGS group, which received the RGS-based diet, and the HFD group, which continued on the high-fat diet. The intervention period lasted 7 weeks. All animal experiments received approval from the Experimental Animal Ethics Committee of Guilin Medical College (approval number: GLMC202005013).

2.2.2. Sample Collection and Preparation

At the end of the experiment, mice were fasted for 12 - 16 h. Body weight was measured, and whole blood (1 - 2 mL) was collected via orbital venous plexus puncture and centrifuged to separate serum. The supernatant was stored at -80 °C until analysis. Following euthanasia, the liver was rapidly excised and weighed. Approximately 0.5 g of liver tissue was fixed in 10% neutral formalin for histopathological analysis. The remaining liver tissue, bilateral abdominal adipose tissue, and epididymal adipose tissue were snap-frozen and stored at -80 °C for subsequent biochemical and molecular analysis.

2.2.3. Histopathological Analysis of Liver Tissue

Liver tissue samples were fixed in 10% neutral formalin, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections were cut at 4 μm thickness, stained with hematoxylin and eosin (HE), and mounted with coverslips. The morphological structure of hepatocytes was examined under a light microscope.

2.2.4. Determination of Liver Index and Fat Index

The liver was gently blotted dry with filter paper and immediately weighed. The

liver index was calculated according to Equation (1):

$$\text{Liver index (\%)} = \frac{\text{Liver Weight}}{\text{Body Weight}} \times 100 \quad (1)$$

Abdominal and epididymal adipose tissues were carefully dissected, with adhering blood vessels and non-adipose tissues removed. The tissues were then blotted dry with filter paper and weighed. The fat index was calculated using Equation (2):

$$\text{Fat index (\%)} = \frac{\text{Fat Weight}}{\text{Body Weight}} \times 100 \quad (2)$$

2.2.5. Determination of Serum and Hepatic Biochemical Indices

The levels of TC, TG, LDL-C, and HDL-C in mouse serum and liver tissue were measured using commercially available assay kits. All procedures were performed strictly according to the manufacturer's instructions.

2.2.6. Western Blot Analysis of IL-6 and FASN in Liver Tissue

Liver tissues were homogenized on ice in RIPA lysis buffer supplemented with protease inhibitors. The homogenate was centrifuged at $3000 \times g$ for 17 min at 4°C . The supernatant was collected for subsequent analysis. Protein concentration was determined using a BCA protein assay kit. Equal amounts of protein were separated by SDS-PAGE and subsequently transferred onto a PVDF membrane using the wet transfer method. After transfer, the membrane was blocked with 5% skimmed milk for 2 h at room temperature. Following three washes with TBST, the membranes were incubated overnight at 4°C with primary antibodies against FASN (1:5000) and IL-6 (1:7500). After washing with TBST, the membranes were incubated with the secondary antibody (1:10,000) for 1 h at room temperature. GAPDH was used as the internal loading control. Protein bands were visualized using an ECL chemiluminescent substrate, and grayscale analysis was performed to quantify the relative expression levels of the target proteins.

2.3. Statistical Analysis

Data were processed and analyzed using SPSS version 25.0 (IBM, USA). The data met the assumptions of normality and homogeneity of variance. Results are expressed as mean \pm SD. Comparisons among multiple groups were performed using one-way ANOVA, followed by pairwise comparisons using the least significant difference (LSD) test. A P -value < 0.05 was considered statistically significant. Graphs were prepared using GraphPad Prism version 9.5.

3. Results

3.1. Effect of RGS on Body Weight in Mice

The changes in body weight throughout the experiment are shown in **Figure 1**. Food intake was not recorded during the experimental period. During the 9 weeks modeling period, the body weight of mice in the NC group showed a gradual phys-

iological increase and subsequently stabilized, whereas the body weight of mice in the HFD group increased progressively. By the end of the modeling period, the mean body weight of the HFD group was 8.70 g higher than that of the NC group, confirming the successful establishment of the obesity model. Before the dietary intervention, there was no statistically significant difference in body weight between the HFD (37.93 g) and RGS (38.38 g) groups ($P > 0.05$). Therefore, the grouping did not affect the results of subsequent experiments. Following the 7-week dietary intervention, the body weight of mice in the RGS group was significantly lower than that of the HFD group, with a reduction of 2.86 g ($P < 0.01$). The results indicate that RGS significantly suppressed high-fat diet-induced body weight gain in mice, thereby effectively controlling body weight and preventing further obesity progression.

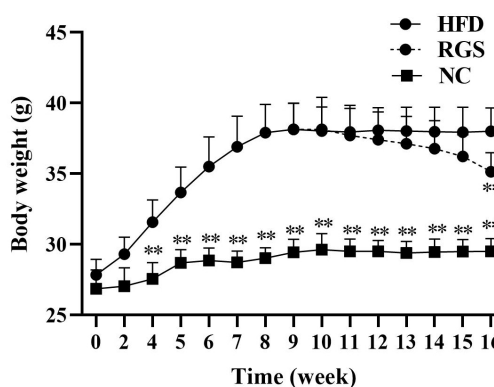


Figure 1. Body weight of mice throughout the experimental period. HFD: High-fat diet group; RGS: Red *glutinous sorghum* group; NC: Normal control group. Compared with the High-fat diet group, * $P < 0.05$; ** $P < 0.01$.

3.2. Effects of RGS on Liver and Fat Accumulation in Mice

The effects of RGS on liver weight, liver index, fat weight, and fat index are shown in **Figure 2**. Compared with the NC group, both liver weight and liver index were significantly increased in the HFD group ($P < 0.05$), with liver weight increasing by 0.40 g (**Figure 2(A)** and **Figure 2(B)**). Following RGS intervention, liver weight and liver index were significantly reduced compared to the HFD group ($P < 0.05$), with liver weight decreasing by 0.18 g. These results suggest that long-term high-energy intake impairs lipid metabolism, promotes hepatic lipid deposition, aggravates metabolic burden, and induces liver damage, all of which can be effectively alleviated by RGS intervention.

The fat weight and fat index of the mice are shown in **Figure 2(C)** and **Figure 2(D)**. Both fat weight and fat index were significantly higher in the HFD group than in the NC group ($P < 0.05$), with fat weight increasing by 0.97 g. Compared with the HFD group, fat weight and fat index were significantly reduced in the RGS group ($P < 0.05$), with fat weight decreasing by 0.46 g. These results indicate that a high-fat diet can promote fat accumulation in mice, leading to an increase in body weight and body fat percentage, whereas RGS intervention can effectively

inhibit fat accumulation and reduce body fat levels.

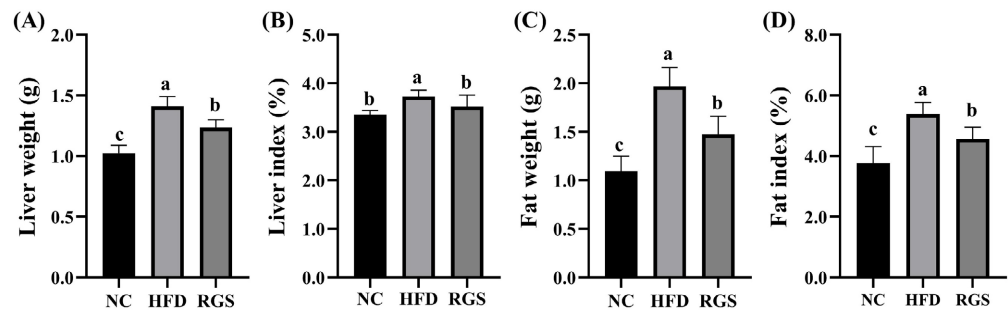


Figure 2. Effects of RGS on liver weight, liver index, fat weight, and fat index in mice. (A) Liver weight; (B) Liver index; (C) Fat weight; (D) Fat index. HFD: High-fat diet group; RGS: Red *glutinous sorghum* group; NC: Normal control group. Different letters indicate significant differences ($P < 0.05$).

3.3. Effect of RGS on Liver Histomorphology in Mice

The results of liver histopathological examination are shown in **Figure 3**. In the NC group, hepatocytes were tightly and regularly arranged with intact morphology, and no obvious lipid vacuoles or inflammatory infiltration were observed. In the HFD group, hepatocytes were disorganized, some cells exhibited irregular morphology and prominent lipid vacuoles were clearly visible. In contrast, hepatocytes in the RGS group were orderly arranged with relatively tight intercellular junctions and essentially intact morphology; no obvious inflammatory response was observed, and only a few lipid vacuoles were present. These results indicate that RGS intervention can effectively alleviate high-fat diet-induced hepatic lipid accumulation and structural damage.

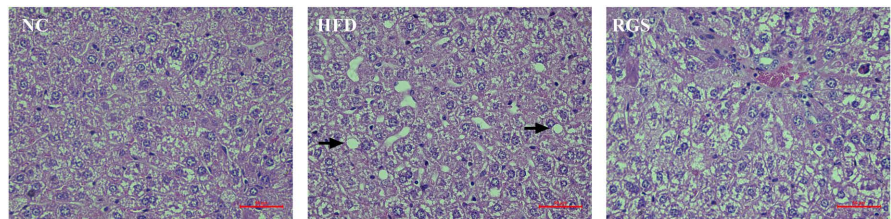


Figure 3. Liver HE staining sections of mice (400 \times magnification). HFD: High-fat diet group; RGS: Red *glutinous sorghum* group; NC: Normal control group. Arrows indicate lipid vacuoles.

3.4. Effect of RGS on Serum and Hepatic Biochemical Parameters in Mice

The serum and hepatic biochemical parameters are shown in **Figure 4**. Compared with the NC group, the serum and hepatic levels of TC, TG, and LDL-C in the HFD group were significantly increased ($P < 0.05$), while the HDL-C level was significantly decreased ($P < 0.05$), indicating that long-term high-fat diet feeding induces dyslipidemia and hepatic lipid deposition, ultimately leading to lipid metabolism disorders. Compared with the HFD group, the serum and hepatic levels of TC, TG, and LDL-C in the RGS group were significantly decreased ($P < 0.05$),

while HDL-C level was significantly increased ($P < 0.05$). These results demonstrate that RGS effectively suppresses high-fat diet-induced elevation of lipid levels and ameliorates abnormal lipid metabolism.

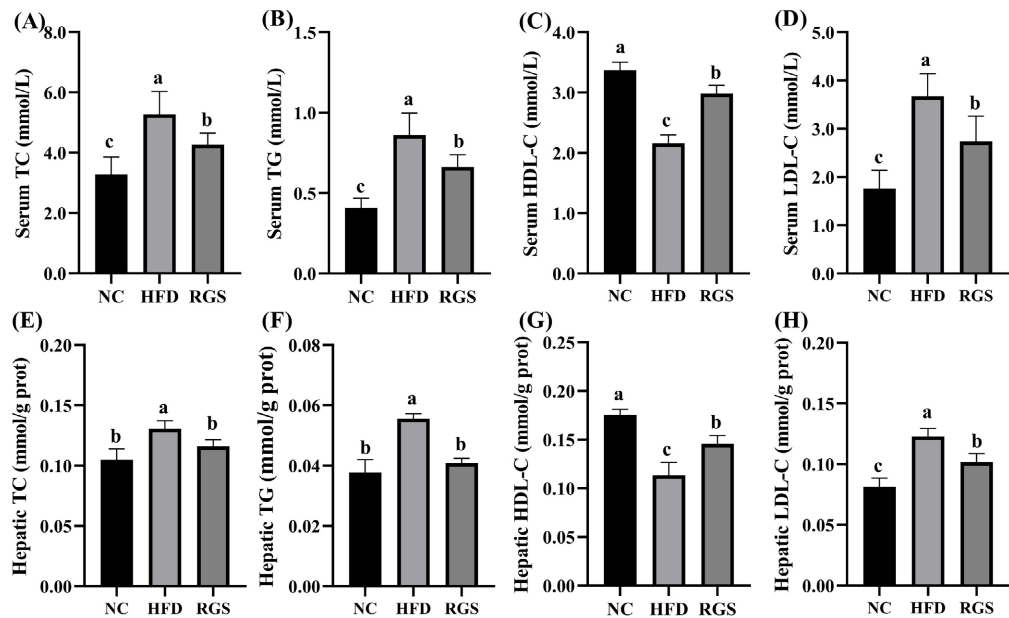


Figure 4. Effects of RGS on serum and hepatic biochemical parameters in mice. (A-D) Serum levels of TC, TG, HDL-C, and LDL-C; (E-H) Hepatic levels of TC, TG, HDL-C, and LDL-C. HFD: High-fat diet group; RGS: Red *glutinous sorghum* group; NC: Normal control group. Different letters indicate significant differences ($P < 0.05$).

3.5. Effects of RGS on the Protein Expression of IL-6 and FASN

The protein expression levels of IL-6 and FASN in liver tissue are shown in **Figure 5**. Compared with the NC group, the protein expression levels of both IL-6 and FASN were significantly upregulated in the HFD group ($P < 0.05$). Following RGS intervention, the expression of both IL-6 and FASN proteins was significantly reduced compared with the HFD group ($P < 0.05$). These results suggest that RGS

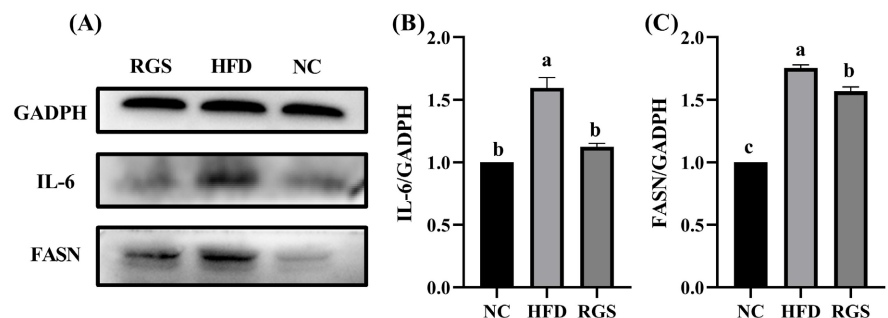


Figure 5. Effects of RGS on IL-6 and FASN protein expression in liver tissue. A: Representative protein bands of IL-6 and FASN; B: Relative expression level of IL-6 protein; C: Relative expression level of FASN protein. HFD: High-fat diet group; RGS: Red *glutinous sorghum* group; NC: Normal control group. Different letters indicate significant differences ($P < 0.05$).

may exert its lipid-lowering effects partly through the suppression of hepatic inflammatory signaling and *de novo* lipogenesis.

4. Discussion

Obesity has become a significant global public health burden owing to its role in promoting the development of various chronic diseases, including hyperlipidemia, type 2 diabetes, and hypertension [1]. Among various intervention strategies, dietary intervention has demonstrated favorable therapeutic efficacy and a superior safety profile [8]. *Sorghum*, as a grain rich in polyphenols and dietary fiber, has been shown to possess the potential to improve lipid metabolism and attenuate inflammation [16]. Therefore, this present study established a high-fat diet-induced mouse model of obesity to systematically investigate the lipid-lowering effects and potential mechanisms of RGS.

The results of this study showed that the body weight of mice in the RGS group was reduced by 8.14% compared with the HFD group. This reduction may be primarily attributed to the bioactive components in *sorghum*, which can influence both appetite and energy metabolism. Specifically, the starch in *sorghum* is digested more slowly than that in conventional cereals, leading to prolonged satiety and subsequently reduced food intake [17]. Furthermore, *sorghum* has been reported to stimulate the release of appetite-regulating hormones, thereby inhibiting appetite and regulating body weight by delaying gastric emptying [16].

Obesity is frequently accompanied by dysfunction of white adipose tissue, leading to ectopic lipid deposition in organs such as the liver [18]. Consequently, increases in liver and adipose tissue weights are considered important indicators of obesity-related metabolic dysfunction [19]. In the present study, high-fat diet feeding increased liver weight and adipose tissue weight in mice by 39.22% and 94.17%, respectively, and prominent lipid vacuoles were observed in the liver tissue. These findings further confirm the occurrence of obesity-related ectopic lipid deposition. Studies have shown that *sorghum* bran extract can inhibit adipocyte differentiation and lipid accumulation [20]. Additionally, extruded *sorghum* flour has been reported to effectively reduce obesity, adipose tissue weight (epididymal and total), and the Lee index in mice [21]. Furthermore, resistant starch derived from *sorghum* has been found to effectively reduce the number of steatotic cells in the hepatic lobules, alleviate the severity of steatosis, and ameliorate cellular vacuolation [22]. After RGS intervention, liver weight and fat weight were reduced by 12.68% and 23.00%, respectively. Moreover, pronounced improvements in hepatic histopathology were observed. These findings further suggest the potential application of *sorghum* in ameliorating obesity and liver injury.

Obesity can promote the transformation of HDL particles from large, cholesterol ester-rich particles to small, triglyceride-rich particles, resulting in decreased HDL-C levels; simultaneously, LDL-C, TC, and TG levels are elevated, collectively leading to lipid metabolism disorders [23] [24]. Following RGS intervention, mice in the RGS group exhibited significantly increased HDL-C levels and significantly

decreased LDL-C, TC, and TG levels in both serum and liver tissue. These results indicate that RGS effectively ameliorates lipid metabolism disorders and attenuates abnormal lipid deposition in the blood and liver.

To further elucidate the underlying mechanism, Western blot analysis was employed to detect the protein expression levels of IL-6 and FASN in liver tissue. FASN serves as a key regulatory enzyme in *de novo* fatty acid synthesis [25], and its upregulated expression is closely associated with lipid accumulation. IL-6, as a pleiotropic cytokine, can induce severe systemic inflammatory responses when overexpressed [26]. In the present study, the expression levels of IL-6 and FASN in the RGS group were reduced by 29.52% and 10.56%, respectively, compared with the HFD group. These findings suggest that RGS may ameliorate lipid metabolism disorders through a dual pathway: on one hand, by inhibiting FASN expression to reduce *de novo* fatty acid synthesis and decrease lipid production; on the other hand, by downregulating IL-6 expression to alleviate obesity-associated chronic low-grade inflammation, thereby improving insulin sensitivity and restoring metabolic homeostasis.

5. Conclusion

The present study demonstrates that RGS intervention effectively alleviates high-fat diet-induced body weight gain and fat accumulation in mice. RGS ameliorates lipid metabolism disorders by reducing TC, TG, and LDL-C levels while increasing HDL-C levels. Furthermore, RGS attenuated hepatic steatosis and pathological damage by downregulating the expression of the inflammatory cytokine IL-6 and the lipogenic enzyme FASN in the liver. These findings suggest that RGS may mitigate abnormal lipid deposition through the synergistic inhibition of inflammation and lipogenesis, thereby restoring metabolic homeostasis. It is important to acknowledge that this study measured only two hepatic protein markers (IL-6 and FASN). Therefore, future studies will investigate additional molecular targets to further clarify the underlying mechanisms. This study not only provides a theoretical basis for the further development of *sorghum* as a functional food but also offers new insights for the innovative utilization of *sorghum* resources and their industrial development.

Conflicts of Interest

The authors declare no conflicts of interest.

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