

## Research Article

# Wheat Bran Protein Improves Lipid Metabolism by Downregulating the Amount of HMG-CoA Reductase in the Liver of High-Fat Diet-Fed Rats

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**Background.** The hypolipidemic effects of wheat bran have been widely documented; however, limited research has concurrently revealed the hypolipidemic and antioxidant properties of wheat bran protein (WBP). **Objective.** This study investigates the impact of WBP (5% daily dietary intake) on antioxidant activity, cholesterol metabolism, and serum/hepatic lipids in high-fat diet-fed rats. **Methodology.** In our animal experiment, we used 12 male Wistar rats that were 4 weeks old. Biochemical indicators of fecal, serum, intestinal, and liver were tested by kits or chemical techniques. The experiment on cholesterol micellar solubility was also carried out *in vitro*. **Results.** After 28 days of feeding, the results revealed that WBP significantly reduced the levels of total cholesterol ( $P < 0.05$ ) in serum and liver, the levels of triglycerides ( $P < 0.05$ ), the levels of MDA ( $P < 0.05$ ), and the levels of very low-density lipoprotein and low-density lipoprotein ( $P < 0.05$ ), and significantly increased the levels of high-density lipoprotein ( $P < 0.05$ ), the levels of glutathione peroxidase, and the level of total antioxidant capability. By downregulating the level of sterol regulatory element-binding protein-2 ( $P < 0.05$ ), WBP also reduced the content of 3-hydroxy-3-methylglutaryl-coenzyme A ( $P < 0.05$ ). **Conclusion.** *In vivo*, WBP demonstrated a substantial hypolipidemic and antioxidant impact ( $P < 0.05$ ), while *in vitro*, it significantly decreased the micellar solubility of cholesterol ( $P < 0.001$ ).

## 1. Introduction

Wheat (*Triticum aestivum* L.) is grown and harvested worldwide under the guidance of the Food and Agriculture Organization of the United Nations (FAO) and serves as the staple food for a significant proportion of the global population. According to FAO statistics, the globe produced 800 million tons of wheat in 2019, with China accounting for nearly 20% of that total [1]. One of the main by-products produced when milling wheat grain is wheat bran (WB). Pericarp, aleurone, and testa tissue are all referred to as bran [2]. WB is typically utilized as an excellent source of fiber, vitamins, and minerals in animal feed, accounting for roughly 85% of all use [3]. WB is also increasingly incorporated into

human diets due to its inexpensive cost. On a dry matter basis, WB contains 6 to 19 percent starch, 14 to 18 percent protein, 5 to 8 percent ash, and 3 to 6 percent lipids [4].

Numerous studies have utilized *in vitro*, rat, and human experiments to provide credible evidence that ingesting WB or the modified WB does have certain specific health effects. By promoting fecal evacuation, decreasing transit time, softening stools, and minimizing constipation, WB may improve the digestive system [5]. The fiber in WB, which has a strong bulking impact due to its capacity to hold water, provides the basis for the aforementioned effect [6]. Additionally, WB's bulking properties can reduce the amount of poisonous and potentially harmful compounds in the colon, thereby diminishing the risk of colorectal cancer [7].

The aleurone layer of the wheat grain contains a high concentration of antioxidant phytochemicals, with ferulic acid being the predominant compound [8]. Ferulic acid can be absorbed in the small intestine and express its antioxidant activity in the liver by conjugating with glucuronide and sulfate [9].

Consequently, WB serves as a valuable source of antioxidants, mitigating oxidative stress in cells and serum, and protecting against oxidative damage induced by free radicals [10, 11]. Antioxidants reduce the possibility of acquiring chronic illnesses such as cardiovascular disease (CVD) [11]. Since the 1950s, many scientists have examined the impact of WB on blood lipid profiles and cholesterol levels. A study conducted in 1979, instructed healthy male participants to consume 0.5 g of WB per kg of body weight daily for four weeks, resulting in a 10% reduction in total serum cholesterol levels and a 24% decrease in total serum triglycerides [12]. *In vitro* experiments conducted between 1982 and 1989 demonstrated that WB inhibits pancreatic lipase function, preventing triglyceride hydrolysis and absorption [13]. Moreover, research in the same period indicated that WB inhibits pancreatic lipase activity *in vitro*, hindering triglyceride hydrolysis and absorption [14–16]. A 2005 study found that WB extracts mitigate tissue damage by scavenging oxygen radicals generated during the lipid peroxidation of LDL cholesterol [17]. However, early research reported that adding WB to the diets of rats, healthy individuals, and individuals with hypercholesterolemia, obesity, and type 2 diabetes could not reduce blood lipid levels [18, 19]. Nevertheless, a recent study demonstrated that wheat bran hydrolysate possesses *in vitro* antioxidant properties and lowers blood pressure in hypertensive rats [20].

To delve deeper into the hypolipidemic and antioxidant properties of wheat bran, we conducted *in vitro* and *in vivo* research, focusing on wheat bran protein (WBP) as a key bioactive component. We evaluated the levels of total cholesterol (TC), triglycerides (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein cholesterol (HDL) which are indications of hyperlipidemia to determine the hypolipidemic effect of WBP. Additionally, we investigated the levels of sterol regulatory element-binding protein-2 (SREBP2), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), ATP-binding cassette transporter A1, cholesterol 7  $\alpha$ -hydroxylase (CYP7A1), Niemann–Pick type C1-like 1 (NPC1L1), and acyl-CoA:cholesterol acyltransferase-2 (ACAT2), all of which are mediators and enzymes involved in cholesterol metabolism, as well as components of the low-density lipoprotein receptor (LDL-R) acyl-coenzyme A [21]. Atherosclerosis initiation and progression are linked to the synthesis of lipid hydroperoxides and the transformation of LDL cholesterol into the atherogenic form of oxidized-LDL, both influenced by the high concentration of free radicals in arteries [22, 23]. Our work demonstrates that WBP has the potential to enhance the antioxidant system's functionality, as indicated by levels of the antioxidant markers catalase (CAT), malondialdehyde

(MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant capability (T-AOC). In conclusion, our findings suggest that WBP contributes to the hypolipidemic and antioxidant effects of WB and holds promise as a potential therapeutic intervention for reducing atherosclerotic CVD risk.

## 2. Materials and Methods

**2.1. Preparation of Wheat Bran Protein.** Wheat bran was procured from the Bayannaer Academy of Agricultural and Animal Sciences. WBP was isolated from the wheat bran by using alkali and acid separation methods, followed by grinding into a powder. A 10% wheat bran solution was prepared by dissolving it in distilled water in a 2 L beaker, and the mixture was agitated for two hours at 350 r/min at 50°C by using a constant temperature magnetic stirrer (Scilogex, Hartford, CT, USA). After adjusting the pH to 9.5 with 0.1 mol/L of NaOH, the solution was centrifuged at 3000  $\times$  g for 20 min to obtain the supernatant. Subsequently, the supernatant was incubated at pH 4.5 with 0.1 mol/L HCl for two hours, followed by another centrifugation at 3000  $\times$  g for 20 minutes to collect the precipitate. After rinsing the precipitate with distilled water and adjusting the pH to 7.0, the residue was dried by using a vacuum freeze dryer (Beijing Boyikang Experimental Instrument Co., Ltd., Beijing, China). The purity of WBP was determined to be 88 percent.

**2.2. Animal Experiment.** Male Wistar rats, aged four weeks weighing 70–80 g, were procured from Sibefu Beijing Biotechnology Co. Ltd. The rats were individually housed with free access to food and water, maintained on a 12-hour light/dark cycle, and housed in temperature-controlled settings at 22  $\pm$  2°C. The rats were separated into two groups and fed a commercial, unpurified diet for one week. The Hetao College's Ethics Committee for Animal Research allowed the use of animals in research (SCXK 2019-0010, 13 October 2021).

The high-fat control (HC) group and the WBP group were the final two experimental groups, each consisting of six rats ( $n=6$ ). During the trial period, various high-cholesterol diets were available to the HC and WBP groups. The HC group's diet included casein (20%), lard (5%), maize oil (1%), AIN-93G-MX mineral mix (3.5%), AIN-93-VX vitamin mix (1%), choline chloride (0.2%), cellulose (5%), cholesterol (1%), sodium cholate (0.5%), sugar (20.2%), and cornstarch (42.6%). In contrast, the WBP group's diet replaced 20% casein with 15% casein and 5% WBP. Food intake and body weight were regularly monitored during the study. To identify fecal steroids, rats' feces were collected between days 26 and 28. The rats were fasted for 18 hours on the final day. The rats were then euthanized using ether anesthesia. Polypropylene tubes were used to collect the blood, which was subsequently centrifuged at 3000  $\times$  g for 15 min at 4°C and kept at –80°C. For additional research, the liver of each rat was removed, weighed, and refrigerated at –80°C.

**2.3. Analysis of Liver Biochemical Indicators.** Biochemical indicators, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), ATP-binding cassette transporter A1 (ABCA1), sterol regulatory element-binding protein-2 (SREBP2), acyl-CoA: cholesterol acyltransferase-2 (ACAT2), cholesterol 7  $\alpha$ -hydroxylase (CYP7A1), and low-density lipoprotein receptor (LDL-R), were measured in the liver using Rat ELISA Kits (SBJ-M0611, SBJ-H1086, SBJ-R1128-96T, SBJ-M0610, SBJ-R0777, and SBJ-H1101. Sen Shellfish Gamma Biotechnology Co., Ltd., Nanjing, China). Total lipids from the liver were extracted by chloroform/methanol solution (2:1, vol/vol), as described by Folch et al. [24]. The concentrations of total cholesterol (TC) and triglycerides (TG) in the liver were determined using assay kits (A111-1-1 and A110-1-1; Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd., Nanjing, China).

**2.4. Analysis of Intestine Biochemical Indicators.** Rat ELISA Kits were used to determine the amount of ATP-binding cassette transporter A1 (ABCA1), acyl-CoA: cholesterol acyltransferase-2 (ACAT2), and Niemann-Pick type C1-like 1 (NPC1L1) in the colon (SBJ-H1086, SBJ-M0610, and SBJ-G074; Sen Shellfish Gamma Biotechnology Co., Ltd., Nanjing, China).

**2.5. Antioxidant Activity Assays of Serum and Liver.** The levels of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and total antioxidant capability (T-AoC) in the liver and serum were determined using assay kits (A003-2-2, A007-2-1, A005-1-1, A001-1-1, and A015-1-1; Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd., Nanjing, China).

**2.6. Serum and Fecal Lipid Assays.** By using commercial test kits, the serum concentrations of TC, TG, and high-density lipoprotein cholesterol (HDL) were determined enzymatically (A111-1-1, A110-1-1, and A112-2-1; Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd., Nanjing, China). The calculation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) serum concentrations was achieved through the subtraction of HDL from TC. With the aid of commercial assay kits, TC extracted from the fecal matter was quantified (A111-1-1; Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd., Nanjing, China). Bile acid isolation from feces followed methods detailed by Bruusgaard et al. [25] and Malchow-Moller et al. [26]. The measurement of bile acid commenced with the combination of 0.5 mL of supernatant with 3 mL of 40 percent  $H_2SO_4$  and 1 mL of 0.3 percent furfural. Subsequently, the mixture was subjected to a water bath maintained at 65°C, and after a duration of 30 minutes, the absorbance at 620 nm was measured when the pink hue was formed with the greatest intensity.

**2.7. In Vitro Micelle Experiment.** The cholesterol micelle method described by Kirana et al. [27] was modified by us. Lipids were dissolved in chloroform and dried with nitrogen

(0.1 mmol/L cholesterol, 0.1 mmol/L oleic acid, 0.05 mmol/L monoallelic acid, and 0.06 mmol/L L- $\alpha$ -phosphatidylcholine). The taurocholic acid solution, which was dissolved in 15 mmol/L phosphate buffer/NaCl (pH = 7.4), was then incorporated into the lipid mixture. An ultrasonicator was used to sonicate the micelle suspension for 3 min, followed by incubation at 37°C in an incubating shaker for the entire next day. Subsequently, 10 mg of WBP was added to one tube containing 1 mL of the aforementioned mixture, and nothing else was added to the other tube, just the 1 mL of the mixture. Following a 3-minute ultrasonication, two tubes were incubated for 2 h at 37°C for 2 hours. A 0.22-micron microporous membrane was employed to separate the solution, and 50  $\mu$ L of the filtrate was utilized to measure the cholesterol by using a total cholesterol test (Nanjing Jiancheng Biological Engineering Research Institute Co., Ltd., Nanjing, China). The absorbance at 510 nm was measured using a UV-visible spectrophotometer (Hitachi, Ltd., Chiyoda-ku, Tokyo, 100-8280 Japan). Based on the cholesterol calibration standard's standard curve, the concentrations were determined.

$$\text{Inhibition ability (\%)} = \left[ \frac{(C_0 - C_1)}{C_0} \right] \times 100, \quad (1)$$

where  $C_1$  is the cholesterol concentration of the micelles with sample fractions and  $C_0$  is the cholesterol concentration of the original micelles.

**2.8. Statistical Analysis.** All experimental data are expressed as mean  $\pm$  standard error of the mean (SEM). Data analysis was conducted using SPSS software version 21 (IBM software, New York, NY, USA). One-way analysis of variance (ANOVA) was used to assess the data, followed by the LSD (L) test, and a significant difference was defined as  $P < 0.05$ .

### 3. Results

**3.1. Effect of WBP Treatment on Growth Biomarkers.** Table 1 shows no significant differences between the HC and WBP groups for initial body weight or liver relative weight. However, there were significant differences between the groups for final body weight ( $P < 0.01$ ) and food intake ( $P < 0.01$ ). Therefore, the high-fat diet-fed rats' body growth is affected by feeding the WBP which suggests that the rats in the WBP group would consume more cholesterol than the rats in the HC group.

**3.2. Effect of WBP Treatment on Serum Lipid Profiles.** Figure 1 illustrates the impact of WBP on serum lipid profiles in high-fat diet-fed rats. WBP considerably lowers the level of TC in serum compared to the HC group ( $P < 0.05$ ). However, the level of TG in serum cannot be appreciably altered by endowing WBP. Furthermore, the HDL content in the serum of the WBP group was considerably lower than that of the HC group ( $P < 0.05$ ) and the LDL + VLDL content was significantly lower in the WBP group than in the HC group ( $P < 0.05$ ).

TABLE 1: Effect of wheat bran protein on body weight and food intake in high-fat diet-fed rats.

Parameters	Experimental groups	
	HC	WBP
Initial body weight (g)	105.1 ± 4.9	107.1 ± 1.6
Final body weight (g)	254.2 ± 3.6	296.2 ± 1.5**
Liver relative weight (g)	5.6 ± 0.1	5.5 ± 0.1
Food intake (g/d)	14.3 ± 0.2	16.9 ± 0.3**

Liver relative weight (g) = (liver weight/final body weight) × 100 g. Values are presented as mean ± SEM ( $n = 6$ ). \* $p < 0.05$ ; \*\* $p < 0.01$  vs the HC group.

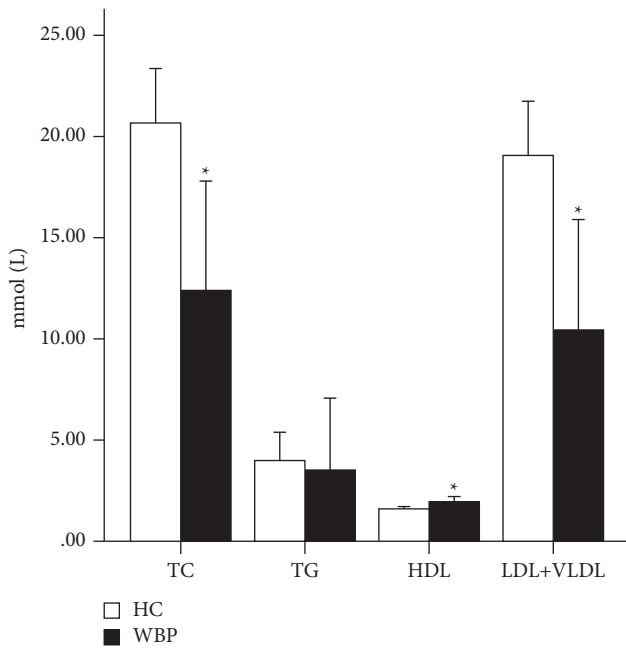


FIGURE 1: Wheat bran protein intake ameliorated the serum lipid profile in high-fat diet-fed rats. TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein. Values are presented as mean ± SEM ( $n = 6$ ). \* $P < 0.05$  vs the HC group.

3.3. *Effect of WBP Treatment on Liver Lipid Profiles.* In comparison to the HC group, WBP significantly decreases the levels of TC and TG in the liver, as depicted in Figure 2 ( $P < 0.05$ ). Additionally, the total lipid content in the liver of the WBP group is comparable to that of the HC group.

3.4. *Effect of WBP Treatment on Enzymatic Activity and Mediated Proteins Related to Cholesterol Metabolism.* Figure 3 displays the impact of WBP administration on enzymatic activity and mediated proteins related to cholesterol metabolism. WBP has no discernible effect on the enzymatic activity of CYP7A1 or ACAT2 in the liver, but it significantly decreases HMG-CoA activity compared to the HC group ( $P < 0.05$ ). Additionally, the WBP group significantly had lower levels of the mediated proteins ABCA1 and SREBP2 in the liver than the HC group ( $P < 0.05$ ). Furthermore, the liver LDL-R concentration in the WBP group is comparable to that in the HC group. Figure 4 shows that the levels of the proteins NPC1L1 and ABCA1 mediated by

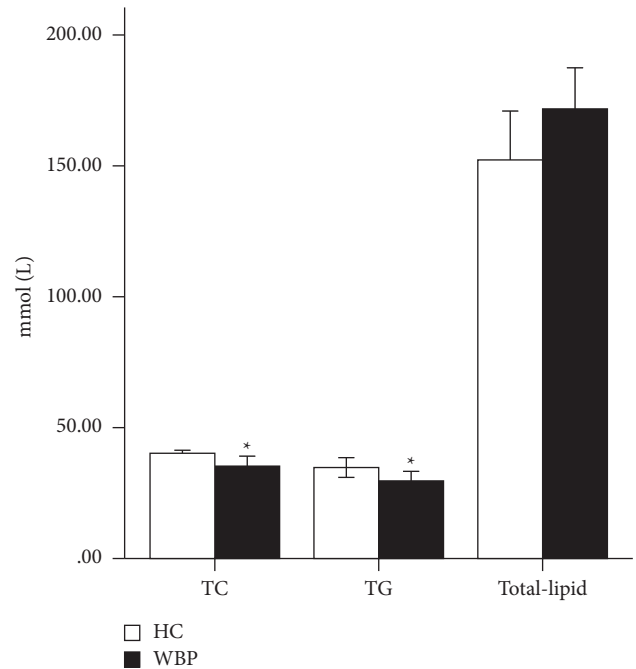


FIGURE 2: The effects of wheat bran protein on the liver lipid profile of high-fat diet-fed rats. TC: total cholesterol; TG: triglycerides. Values are presented as mean ± SEM ( $n = 6$ ). \* $P < 0.05$  vs the HC group.

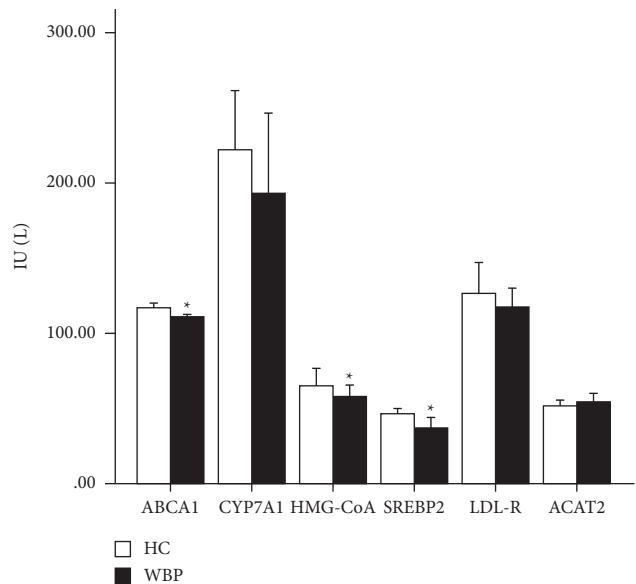


FIGURE 3: Wheat bran protein modulated the concentrations of enzymes CYP7A1, ACAT2, and HMG-CoA and the mediated proteins' content of ABCA1, SREBP2, and LDL-R in the liver of high-fat diet-fed rats. Values are presented as mean ± SEM ( $n = 6$ ). \* $P < 0.05$  vs the HC group.

the enzyme ACAT2 in the gut of the WBP group are similar to those in the HC group.

3.5. *Effects of WBP on the Indicators Related to Antioxidant Ability.* Figure 5 illustrates the impact of WBP treatment on serum antioxidant capacity indicators. The administration of WBP significantly increased the concentration of the

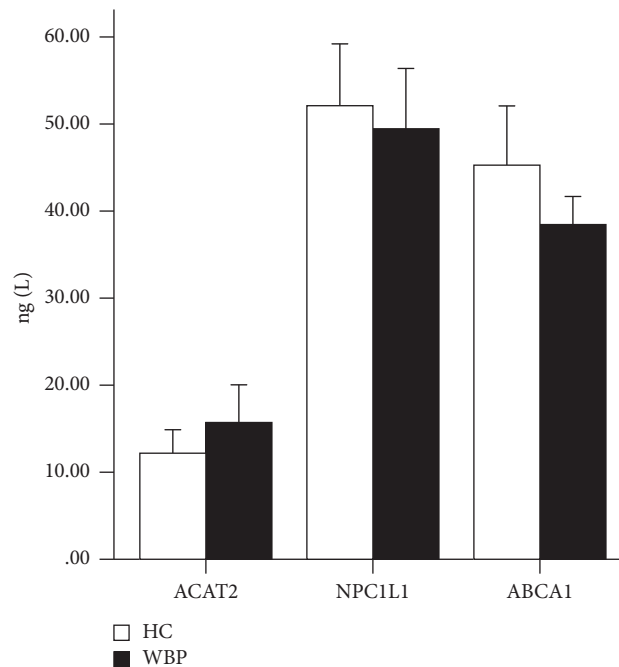


FIGURE 4: Wheat bran protein modulated the concentrations of enzyme ACAT2 and the levels of mediated proteins ABCA1 and NPC1L1 in the intestine of high-fat diet-fed rats. Values are presented as mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.05$  vs the HC group.

enzyme GSH-Px (Figure 5(a),  $P < 0.05$ ) and enhanced the amount of T-AoC (Figure 5(b),  $P < 0.05$ ). Moreover, it significantly decreased the content of MDA (Figure 5(b),  $P < 0.05$ ) in the serum. While the concentration of the enzyme SOD showed a slight improvement, the concentration of the enzyme CAT showed a slight decrease.

Notably, the levels of the enzymes GSH-Px and SOD in the WBP group were slightly lower than in the HC group. WBP treatment significantly decreased the concentration of CAT (Figure 6(a),  $P < 0.05$ ) and significantly reduced the amount of MDA (Figure 6(b),  $P < 0.05$ ) but did not significantly increase the amount of T-AoC.

**3.6. Effect of WBP Treatment on Fecal Matter.** Figure 7(a) demonstrates that the WBP group exhibited significantly higher fecal excretion than the HC group. However, as depicted in Figure 7(b), the fecal bile acid level in the WBP group was marginally higher than that of the HC group. There was no discernible difference between the WBP group and the HC group in terms of fecal cholesterol (Figure 7(c)).

**3.7. Effect of WBP on Micellar Cholesterol Solubility In Vitro.** In an *in vitro* experiment, WBP significantly reduced the solubility of cholesterol micelles compared to the untreated cholesterol micelles (Figure 8,  $P < 0.001$ ). This proves that the WBP effectively prevents the production of cholesterol micellar.

#### 4. Discussion

Cholesterol homeostasis is a crucial aspect in maintaining the health of living organisms. Disturbances in cholesterol homeostasis are associated with various disorders, including

cancer, neurological diseases, and cardiovascular disease. Four fundamental processes are involved in maintaining the dynamic balance of cholesterol: production, absorption, export, and esterification [28]. Our results establish that WBP could significantly reduce the levels of lipid profiles by two distinct mechanisms. SREBP2 is an endoplasmic reticulum-anchored precursor that precisely regulates the transcription of HMG-CoA, a rate-limiting enzyme that governs the production of cholesterol [29]. Our findings provide a scientifically sound explanation for the correlation between the levels of SREBP2, HMG-CoA, TC, and TG. WBP first downregulates the content of SREBP2, which subsequently inhibits HMG-CoA production (Figure 3,  $P < 0.05$ ). The limited HMG-CoA availability then restricts the synthesis of TC and TG (Figures 1 and 2,  $P < 0.05$ ). This discovery aligns with previous studies that demonstrated that whole wheat bran (WB) could reduce TC and TG levels in the blood [12]. Our study goes further by confirming that WBP, a component of WB, contributes significantly to lowering lipid profiles and provides a mechanistic explanation.

VLDL, LDL, and HDL are crucial lipoproteins for transporting cholesterol in the body. Endogenously produced cholesterol can be transported from the liver to peripheral tissues by VLDL and LDL in the blood, while exogenously acquired cholesterol can also be transported by VLDL and LDL from the intestine to peripheral tissues [30]. HDL plays a pivotal role in the recycling and removal of cholesterol from peripheral tissues [31]. Our data show that feeding WBP efficiently increases HDL levels and decreases VLDL and LDL levels in serum (Figure 1,  $P < 0.05$ ). These findings can theoretically explain the lower TC and TG levels in the HC group. WBP appears to enhance the function of recycling and removing cholesterol from peripheral tissues, thereby

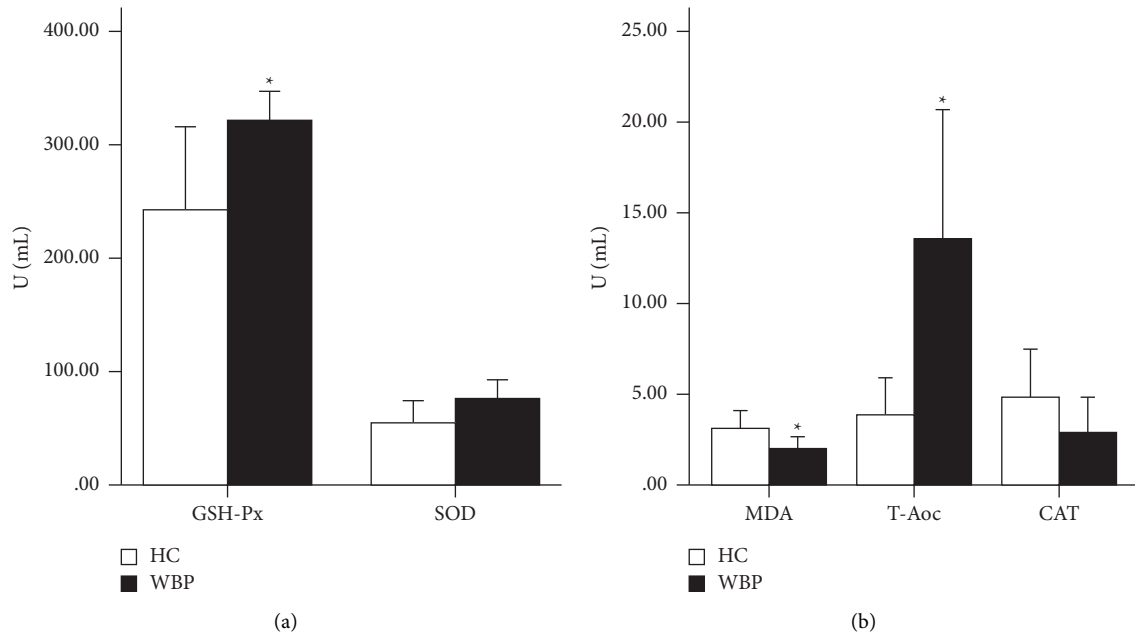


FIGURE 5: Treatment of wheat bran protein on serum antioxidant parameters, (a) GSH-Px, SOD, (b) MDA, T-Aoc, and CAT, in the high-fat diet-fed rats. Values are presented as mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.05$  vs the HC group.

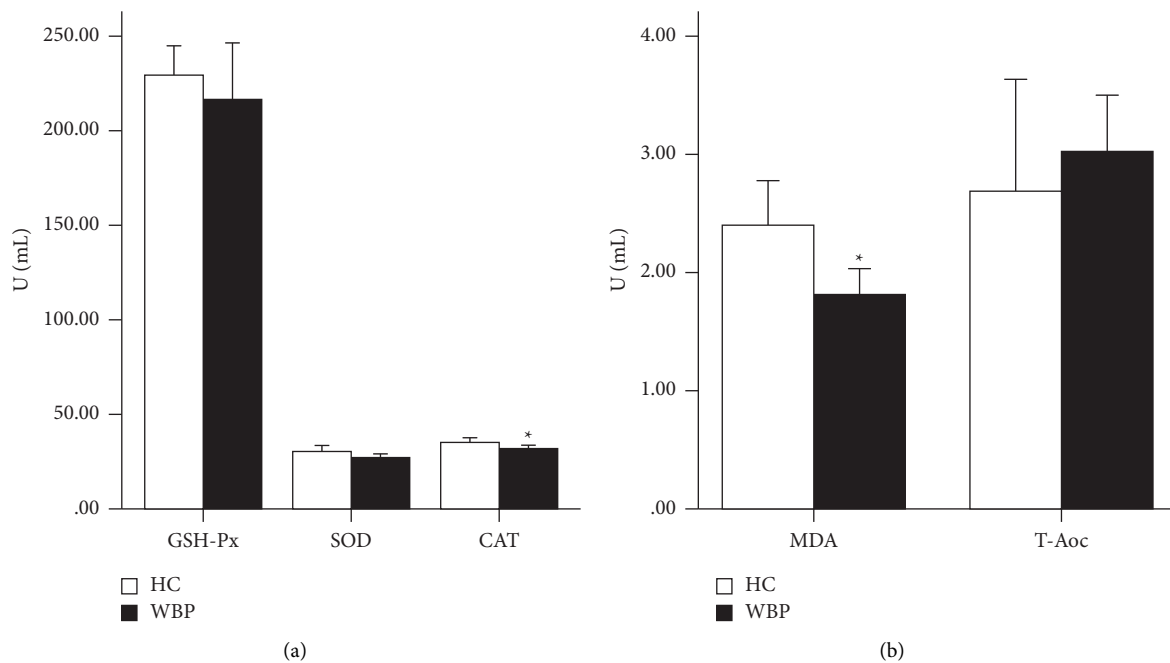


FIGURE 6: Treatment of wheat bran protein on liver antioxidant parameters, (a) GSH-Px, SOD, CAT, (b) MDA, and T-Aoc, in the high-fat diet-fed rats. Values are presented as mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.05$  vs the HC group.

constraining the distribution of cholesterol in the serum. Although the levels of ABCA1, a protein responsible for HDL production [32], in the liver and gut were not significantly affected by WBP in our study, additional research is needed to elucidate the mechanism underlying the interaction between WBP and VLDL, LDL, and HDL. In conclusion, our findings demonstrate that WBP, as a natural plant protein, offers a novel strategy for reducing the risk of atherosclerosis.

The liver's cholesterol catabolic pathway, which converts cholesterol into bile acids, is initiated by the rate-limiting enzyme CYP7A1 [33]. This study observed that the level of CYP7A1 in the WBP group did not significantly change when compared to the HC group. This result is consistent with the conclusion drawn from Figure 7(b), which indicates that WBP ingestion has no significant impact on fecal bile acid excretion. NPC1L1 is a crucial mediator protein that

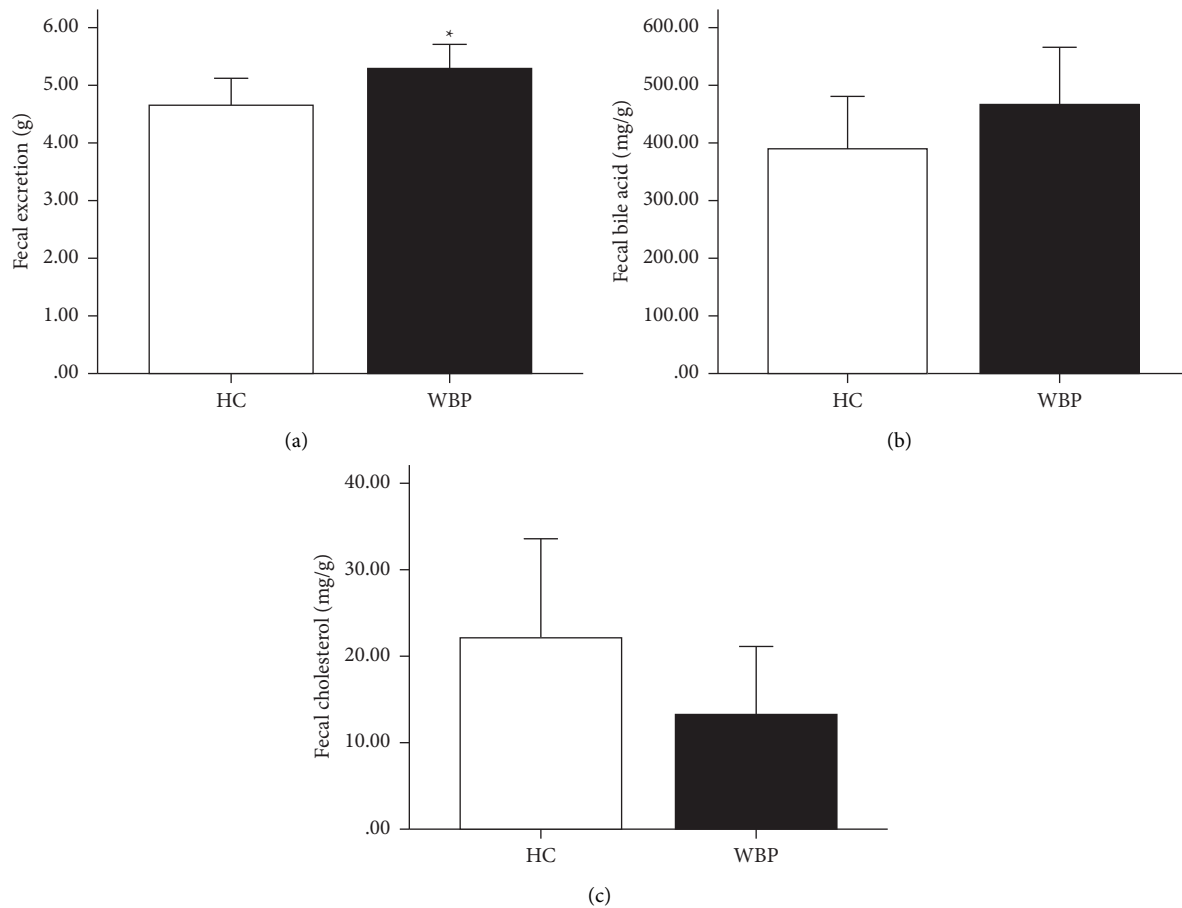


FIGURE 7: Analysis of fecal excretion (a), fecal bile acid (b), and fecal cholesterol (c) in high-fat diet-fed rats after 4 weeks. Values are presented as mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.05$  vs the HC group.

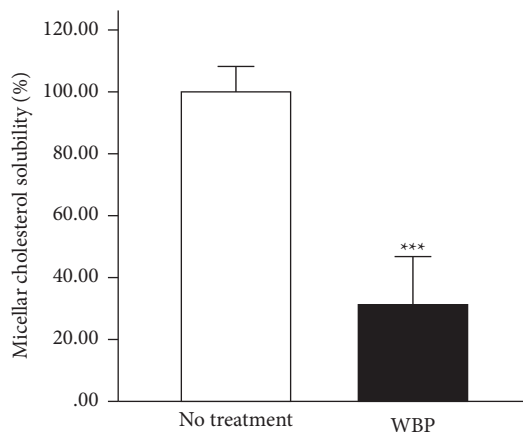


FIGURE 8: Effect of wheat bran protein (10 mg/mL) on the micellar solubility of cholesterol *in vitro*. Values are presented as mean  $\pm$  SEM ( $n=4$ ). \*\*\* $P < 0.001$  vs no treatment.

regulates cholesterol absorption at the intestinal lumen and hepatic canalicular space through clathrin-mediated endocytosis [34]. This investigation revealed that WBP administration did not significantly affect the level of NPC1L1 in the gut, suggesting that WBP does not significantly alter the amount of cholesterol consumed by rats. This observation is

in line with findings in Figure 7(c), which indicate that despite WBP's strong *in vitro* ability to disrupt cholesterol micellar structures, fecal cholesterol excretion in the WBP group is not substantially higher than in the HC group. Based on these data, it appears that the hypolipidemic effect of WBP is not manifested through a reduction in cholesterol uptake or an increase in cholesterol export. Another crucial mechanism for preventing the accumulation of free cholesterol in cells involves esterification. ACAT2, a membrane protein mainly expressed in enterocytes and to a lesser extent in hepatocytes, catalyzes the esterification of cholesterol, facilitating its intestinal absorption [35]. In our study, WBP therapy had no discernible effect on the levels of ACAT2 in the liver and gut. Therefore, the hypolipidemic effect of WBP does not appear to be achieved through cholesterol esterification.

Oxidative stress is a term used to describe an imbalance between the production of oxidants and the body's antioxidant defenses [36]. Oxidative stress can lead to lipid peroxidation which is responsible for the accumulation of plaque in the intimal layer of arteries and the development of atherosclerosis. Three major antioxidant enzymes SOD, GSH-Px, and CAT play important roles in antioxidant defense. SOD is the sole antioxidant enzyme capable of neutralizing superoxide in mammalian cells and is essential

for resisting oxidative stress [37]. CAT is an enzyme that breaks down hydrogen peroxide into water and oxygen through the action of a protein complex [38]. GSH-Px as a selenium-containing antioxidant enzyme can efficiently convert lipid peroxides and hydrogen peroxides to water and lipid alcohols [39]. In our investigation, WBP treatment significantly increased the concentration of GSH-Px in serum (Figure 5(a),  $P < 0.05$ ) but had no discernible effect on the level of GSH-Px in the liver. The level of SOD in both serum and the liver were not significantly affected by WBP. Furthermore, WBP did not significantly alter the serum level of CAT but significantly reduced the hepatic level of CAT (Figure 6(a),  $P < 0.05$ ). These findings indicate that WBP exhibits strong antioxidant capacity by increasing GSH-Px levels. Thus, WBP may serve as a potent natural antioxidant.

T-AoC assesses the overall antioxidant status of a biological sample by evaluating the antioxidant capacity of all antioxidants, including both enzymatic and nonenzymatic antioxidants [40]. Our findings suggest that WBP significantly enhances T-AoC levels in serum (Figure 5(b),  $P < 0.05$ ). These data are consistent with previous research studies that states that WB could absorb oxygen radicals. Thus, our findings confirmed that WBP is an important antioxidant component in WB. MDA is typically used as a sign of oxidative stress and the level of antioxidant protection because it is the principal end product of polyunsaturated fatty acid peroxidation [41]. In our investigation, WBP showed a high ability of eliminating MDA in the liver (Figure 6(b),  $P < 0.05$ ) and serum (Figure 5(b),  $P < 0.05$ ). This implies that WBP efficiently mitigates the oxidative stress and may contribute to preventing the development of atherosclerosis.

## 5. Conclusions

In conclusion, our study establishes that WBP, a natural plant protein, efficiently reduces lipid profile levels and enhances antioxidant capacity. Furthermore, our results elucidate the fundamental mechanism by which WBP lowers TG levels in the liver through the regulation of SREBP2 (Figure 3,  $P < 0.05$ ) and its downstream target HMG-CoA. Additionally, WBP demonstrates potent antioxidant properties by increasing GSH-Px (Figure 5(a),  $P < 0.05$ ) levels, enhancing T-AoC levels (Figure 5(b),  $P < 0.05$ ), and reducing MDA levels (Figure 5(b),  $P < 0.05$ ). Thus, WBP may represent a novel therapeutic strategy for lowering the risk of atherosclerotic cardiovascular disease.

## Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

## Ethical Approval

The animal study protocol was approved by the Institutional Review Board of Hetao College (SCXK 2019-0010, 13 October 2021).

## Disclosure

This study did not involve humans.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Y.S., C.L., and L.Y. conceptualised the study. Y.S., C.W., and X.B. developed the methodology. Y.S. and R.B. performed software analysis. Y.S. and Y.Z. validated the study. Y.S., C.L., and L.Y. performed the formal analysis. Y.S. and R.B. investigated the data. C.L. and L.Y. collected the resources. Y.S., C.L., and Y.Z. curated the data. Y.S. wrote and prepared the original draft. Y.S. and J.W. reviewed and edited the data. Y.S. and J.W. visualised and supervised the study and administered the project. C.L. and J.W. acquired the fundings. All authors have read and agreed to the published version of the manuscript. Yi Sun and Cong Liu contributed equally to this work.

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