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Effects of dietary wheat bran arabinoxylans on cholesterol metabolism of hypercholesterolemic hamsters

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ABSTRACT

The aim of the present study is to investigate the effects of dietary wheat bran arabinoxylans (AXs) on cholesterol metabolism in hypercholesterolemic hamsters. The hamsters were divided into 3 groups and fed the experimental diets containing AXs or oat β-glucan at a dose of 5 g/kg for 30 days. As the results, the AXs lowered plasma total cholesterol and LDL-cholesterol concentrations, and increased excretions of total lipids, cholesterol and bile acids, as well as oat β-glucan. The AXs reduced the activity of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase, and increased the activity of cholesterol 7-α hydroxylase (CYP7A1) in liver. Moreover, the AXs increased propionate and the total short-chain fatty acids (SCFAs) concentrations. These results indicated that dietary AXs reduced the plasma total cholesterol and LDL-cholesterol concentrations by promoting the excretion of fecal lipids, regulating the activities of HMG-CoA reductase and CYP7A1, and increasing colonic SCFAs in hamsters.

1. Introduction

Wheat bran arabinoxylans (AXs) extracted from by-product of wheat milling are the hemicelluloses. AXs one of the main components of wheat grain cell walls consist of backbone chains of β-(1-4)-linked D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains (Fincher & Stone, 1986). Annual output of nearly 200,000 tons of wheat bran in China provides a wealth of raw materials (Zhou et al., 2010) for AX extraction and utilization. It is well known that dietary AXs prevent lifestyle-related diseases, such as cardiovascular diseases (CVD) and type II diabetes (Broekaert et al., 2011; Garcia et al., 2006), reduce the risk of gastrointestinal cancer (Schatzkin et al., 2007), and increase immunopotentiating activity in mice (Ogawa, Takeuchi, & Nakamura, 2005).

A previous study in our laboratory showed that AXs extracted from wheat bran by alkaline or enzyme-aided procedures all could be explored as the good source of natural immunomodulator (Zhou et al., 2010). In addition, it was discovered that the AXs inhibited significantly the growth of transplanted tumors in mice (Cao et al., 2011). The soluble corn bran AXs have been reported to lower cholesterol in rats by reducing uptake of cholesterol from a cholesterol-rice diet (Lopez et al., 1999). Hu, Wang, and Xu (2008) demonstrated that the corn bran AXs enhanced the catabolism of lipids by down-regulated transcription of intestine-bile acid binding protein, and up-regulated ileum farnesoid X receptor, liver peroxisome proliferator-activated receptors, lipoprotein lipase and transcription of hepatic cholesterol 7 alphahydroxylase.

The replacement of refined wheat flours with whole flour in various starchy foods improves the development of CVD by reducing the plasma cholesterol levels (Adam et al., 2001), and the whole wheat flours can significantly increase cecal SCFAs which are effective plasma cholesterol-lowering agents. However, it is still not clear whether AXs are directly responsible for the observed effects. Although previous studies demonstrated that the wheat bran AXs promoted fecal output in rats and humans (Lu, Gibson, Muir, Fielding, & O'Dea, 2000; Lu, Walker, Muir, & O'Dea, 2004), little is known of about the beneficial effects of wheat bran AXs in cholesterol metabolism.

In the present study, AXs were isolated from wheat bran by alkaline extraction according to our previous methods (Zhou et al., 2010). Oat β-glucan one of the world recognized hypolipidemic functional food was used as positive control to investigate the effects of AXs on cholesterol metabolism in hamsters fed a

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hypercholesterolemic diet. AXs was fed to hamsters so as to elevate the serum cholesterol concentrations (Nath, Wiener, Harper, & Eicvehjem, 1959).

2. Materials and methods

2.1. Materials

Fresh wheat bran was provided by Beijing Guchuan Foodstuffs Co., Ltd (Beijing, China) and stored at 4 °C. Oat (*Avena sativa* L.) seeds were provided by Zhangjiakou City Academy of Agriculture Sciences (Zhangjiakou, China).

2.2. Pre-treatment of wheat bran and alkaline extraction of AXs

Pre-treatment of wheat bran and alkaline extraction of polysaccharides were carried out according to our previous methods (Zhou et al., 2010). Briefly, the wheat bran was soaked with de-ionized water at 4 °C for 60 min and passed through 200 mesh, and then the moist material was washed with 5–6 volume [w/v] of de-ionized water three times to remove starch. Water was squeezed from the suspension of washed bran which was heated at 50 °C for 12 h (the content of water in washed bran decreased to about 10%). The dried wheat bran was ground to powder by a hammer mill and passed through 60 mesh. The de-starched bran was stored at 4 °C.

As for extraction of AXs, 100 g dried powder of de-starched wheat bran was mixed with 1.5 L of 0.15 mol/L NaOH including 0.5% H_2O_2 [v/v] for 90 min at 80 °C, followed by cooling to room temperature and centrifugation at 5000 × *g* for 30 min. The pH of supernatant was adjusted with 0.2 mol/L HCl to pH 4.5, centrifuged at 5000 × *g* for 30 min, concentrated to the range from 1/4 to 1/5 volume of the original solution under reduced pressure, precipitated by ethanol (final concentration was 65%), and then centrifuged at 5000 × *g* for 5 min at 4 °C. The sediments were dissolved in water and centrifuged again at 5000 × *g* for 30 min at 4 °C. The supernatant was precipitated by ethanol as mentioned above and freeze-dried. The obtained fraction was AXs.

2.3. Extraction of oat β-glucan

Oat β-glucan was prepared according to the method of Papageorgioua, Lakhdarab, Lazaridouc, Biliaderisd, and Izydorczyk (2005) with some modifications. The milled oat sample (1 kg) was defatted by shaking 3 times with 3 L petroleum ether at 4 °C for 24 h. The washed residue was filtered followed by freeze-drying to obtain defatted oat flour. The oat flour was extracted with 16 times [w/v] NaOH solution with pH 12.5 by shaking at 40 °C for 3 h. The mixture was cooled to room temperature and centrifuged at 5000 × *g* for 30 min. The pH of supernatant was adjusted with 0.2 mol/L HCl to pH 4.5, and then centrifuged at 5000 × *g* for 30 min to remove proteins. The supernatant concentrated to the range from 1/4 to 1/5 volume of the original solution under reduced pressure. After the pH level was adjusted to 7.0, the obtained concentrate was precipitated with ethanol (final concentration was 55%) at 4 °C for 24 h, and then centrifuged at 5000 × *g* at 4 °C for 30 min. The residue was dehydrated by 95% ethanol 3 times, followed by filtering and freeze-drying. The obtained extractant was oat β-glucan.

2.4. Determination of polysaccharide

The polysaccharide content of AXs was measured by Orcinol–HCl method (Shogren, Hashimoto, & Pomeranz, 1987). The purity of AXs was 70.3%. The determination of oat β-glucan content was performed according to the AOAC Official Method 995.16. The purity of oat β-glucan was 70.1%.

2.5. Determination of viscosity

The viscosity of AXs and oat β-glucan was measured by an Ubbelohde viscometer with 4% polysaccharide.

2.6. Determination of average molecular weight

The average molecular weights and molecular weight distributions of AXs and oat β-glucan were determined according to our previous methods (Zhou et al., 2010). Briefly, the average molecular weights were measured by high-performance size-exclusion chromatography equipped with online multi-angle laser light scattering (DAWN-EOS, Wyatt Technology Inc., USA) detector using a K5 cell and a He–Ne laser (λ = 690 nm), and differential refractive index detector (OPTILAB DSP). The column (TSK-gel G 5000, TOSOH, Japan) was eluted with 0.1 M phosphate buffer (pH 7.2) at a flow rate of 0.5 mL/min. The data were analyzed by use of Astra software (Version 4.90.04, Wyatt). The concentration of each eluted fraction was determined by differential refractive index (ERC 7515 A) according to the known value of dN/dC = 0.15 (Zhou et al., 2010).

2.7. Animals and diets

Male golden hamsters (4 weeks old, $n = 24$) were obtained from Vital River Lab Animal Technology Co., Ltd. (Beijing, China). All hamsters housed individually in stainless steel cages were acclimatized to the laboratory conditions (22 ± 2 °C, 55 ± 5% humidity, and 12 h light/dark cycle) for 3 days prior to the experiment. Hamsters were divided into 3 groups (Control, AXs, and Oat β-glucan) so that the average body weight of each group was same. The hamsters were given de-ionized water, and fed 3 experimental diets for 30 days. Experimental diets were prepared according to the American Institute of Nutrition (AIN)-93G formula with some modifications. The composition of the experimental diets is shown in Table 3.

The plasma samples were taken from the eye-veniplex on the 0th, 10th and 20th day of experiment, respectively. Rat feces were collected for 1 week before the hamsters were sacrificed. The hamsters were fasted for 16 h and then sacrificed by the removal of blood from the eye-veniplex. Livers frozen by liquid nitrogen were kept at −20 °C.

All experiments were carried out according to the PR China legislation regarding the use and care of laboratory animals and were approved by the Bioethics Committee of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

2.8. Analysis of metabolic parameters in hamsters

The concentrations of plasma lipids were measured using Automatic Chemistry Analyzer (Hitachi, Tokyo, Japan). The concentrations of total cholesterol (tissue total cholesterol assay kit, E1015, Applygen Technologies Co., Ltd, Beijing, China), free cholesterol (tissue free cholesterol assay kit, E1016, Applygen Technologies Co., Ltd, Beijing, China), triglyceride (tissue triglyceride assay kit E1003-2, Applygen Technologies Co., Ltd, Beijing, China) in liver were measured with commercially available kits, as well as the activities of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase (Rat HMGR ELISA Kit, Nanjing Sen Shellfish Gamma Biotechnology Co., Ltd, Nanjing, China) and cholesterol 7-α hydroxylase (CYP7A1) (Rat CYP7A1 ELISA Kit, Nanjing Sen Shellfish Gamma Biotechnology Co., Ltd, Nanjing, China).

Table 1
Molecular weight distribution of AXs and oat β-glucan.

	AXs			Oat β-glucan		
	Mw (Da)	Polydispersity (Mw/Mn)	Mass fraction (%)	Mw (Da)	Polydispersity (Mw/Mn)	Mass fraction (%)
Peak 1	3.47×10^6	1.36	6.15	3.77×10^6	1.49	8.09
Peak 2	3.10×10^5	1.26	59.21	2.70×10^5	2.27	24.6
Peak 3	1.75×10^5	1.05	15.40	1.44×10^4	4.62	66.1
Peak 4	6.32×10^4	1.03	13.04			
Peak 5	1.06×10^5	1.16	6.20			

Means were determined in triplicate for each cultivar.

Table 2
Viscosities of AXs and oat β-glucan.

	AXs	Oat β-glucan
Flow time (s)	265 ± 2a	238 ± 3b
η_r	22.5 ± 0.2a	20.2 ± 0.1b
η_{sp}	21.5 ± 0.2a	19.2 ± 0.2a
[η] app (mL/g)	151 ± 1a	142 ± 2b

Means expressed with standard deviation were determined in triplicate for each cultivar. Different letters indicated significant differences at $P < 0.05$ (t-test).

The fecal total lipids were measured by Soxhlet method. The fecal cholesterol content was measured using tissue total cholesterol assay kit E1015 (Applygen Technologies Inc, Beijing, China). The fecal bile acid content was measured using rat bile acid ELISA kit (Nanjing Sen Shellfish Gamma Biotechnology Co., Ltd, Nanjing, China). Fecal SCFAs were measured by ion chromatography (DIONEX ICS-3000, USA) equipped with a high-performance capillary column (IonPac AS11-HC, 4 mm × 250 mm), a guard column (IonPac AG11-HC, 4 mm × 50 mm), and a suppressors (ASRS 300 4-mm). The column temperature was 30 °C. The gradient elution was performed by 0.8 mM KOH for 0–12 min, 0.8–34 mM KOH for 12–40 min, and 34 mM KOH for 40–50 min. The lactate, acetate, propionate, and butyrate (Sigma, USA) were used as standard.

2.9. Statistical analysis

The data were expressed as means with standard errors (SE) and analyzed by Tukey–Kramer's multiple comparison post hoc test. The analysis was carried out with SPSS (Version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. AXs molecular weight and viscosity

The molecular weight distribution of AXs and oat β-glucan were measured based on our previous studies (Table 1). The molecular weight of AXs was larger than that of oat β-glucan ($P < 0.05$). The viscosity of AXs and oat β-glucan was measured with an Ubbelohde viscometer (Table 2).

3.2. Growth and metabolism parameters of hamsters

Growth and metabolism parameters of hamsters are shown in Table 4. The plasma total cholesterol and LDL-cholesterol concentrations were reduced significantly by both AXs and oat β-glucan, compared with the control group ($P < 0.05$). There was no difference in growth and other metabolism parameters among the 3 groups (Table 4).

Table 3
Diet composition (g/kg diet).

	Control	AXs	Oat β-glucan
Casein	200	200	200
Corn Starch	397	397	397
Soybean oil	70	70	70
Cellulose	50	45	45
AXs		5	
Oat β-glucan			5
Sucrose	38.486	38.486	38.486
t-Butylhydroquinone	0.014	0.014	0.014
Choline bitartrate	2.5	2.5	2.5
L-Cystine	3	3	3
Maltodextrin 10	132	132	132
Mineral mix	35	35	35
Vitamin mix	10	10	10
Cholesterol	10	10	10
Bile salt	2	2	2
Lard	50	50	50

The diets were prepared according to the AIN-93G formula with some modifications.

3.3. Activities of HMG-CoA reductase and CYP7A1 in liver

HMG-CoA reductase and CYP7A1 activities in liver were analyzed and shown in Fig. 1. The HMG-CoA reductase activity in liver of AXs group was significantly lower than that of the control groups (Fig. 1A), while the oat β-glucan group was not significantly different from the control group. The CYP7A1 activities in liver of the AXs and oat β-glucan groups were significantly increased compared with the control group (Fig. 1B).

3.4. Fecal lipids

Fecal excretion and lipids concentrations are shown in Table 5. The fecal weight, fecal total lipids excretion, cholesterol and bile acids excretion are increased in AXs and oat β-glucan groups compared with the control group ($P < 0.05$). In addition, the bile acids excretion in the AXs group was higher than that in the oat β-glucan group ($P < 0.05$).

3.5. Fecal SCFAs concentrations

As shown in Table 5. There was no difference in fecal lactate and acetate concentrations among 3 groups. The propionate concentration in AXs group was higher than that in the control group ($P < 0.05$), but that was slightly lower than that of oat β-glucan ($P < 0.05$). Compared with the control group, the total SCFAs concentrations were increased in the AXs and oat β-glucan groups ($P < 0.05$). In addition, the butyrate was detected only in the oats group.

4. Discussion

It has been widely reported that oat β-glucan as cereal dietary fiber effectively lowers the plasma total cholesterol, plasma LDL-cholesterol cholesterol, without affecting HDL cholesterol or

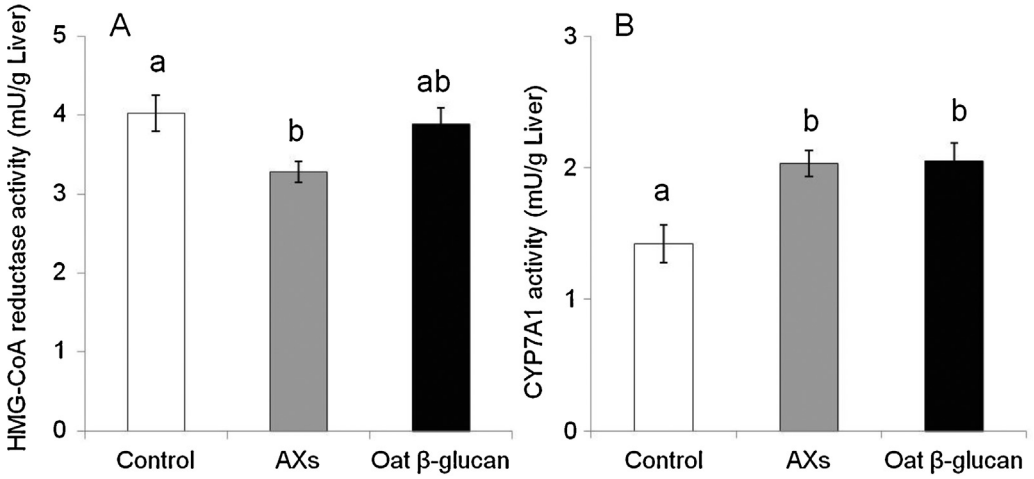

Fig. 1. Liver HMG-CoA reductase activity (A) and CYP7A1 acitivity (B) of the experimental hamsters. Means and SE were determined from 8 hamsters per group. □, Control group, ■, AXs, ■, Oat β-glucan. Different superscript letters indicate significant differences at $P < 0.05$.

Table 4
Effects of dietary AXs and oat β-glucan on the metabolic parameters of hamster fed a hypercholesterolemic diet.[a]

	Control	AXs	Oat β-glucan
Initial body weight (g)	112.9 ± 3.0	113.0 ± 2.5	113.6 ± 1.8
Final body weight (g)	126.8 ± 2.9	123.9 ± 3.3	127.8 ± 3.7
Body weight gain (g)	13.9 ± 1.7	10.9 ± 1.7	13.0 ± 2.5
Food intake (g/day)	8.15 ± 0.23	8.15 ± 0.19	8.15 ± 0.21
Liver weight (g)	6.13 ± 0.33	5.92 ± 0.16	5.85 ± 0.25
Plasma lipids (mmol/L)			
Cholesterol			
Total	7.38 ± 0.25a	6.69 ± 0.20b	6.52 ± 0.32b
LDL	2.03 ± 0.12a	1.56 ± 0.15b	1.60 ± 0.14b
HDL	0.35 ± 0.01	0.36 ± 0.01	0.34 ± 0.02
Triglycerides	3.40 ± 0.64	2.57 ± 0.47	3.11 ± 0.72
Liver lipids (μmol/g)			
Cholesterol			
Total	70.9 ± 3.6	72.6 ± 4.2	66.5 ± 7.3
Free	21.8 ± 2.0	20.0 ± 1.3	17.4 ± 1.4
Ester	49.1 ± 5.6	52.6 ± 3.8	49.1 ± 6.5
Triglycerides	22.9 ± 1.4	23.5 ± 0.9	25.3 ± 2.4

[a] Means expressed with SE were determined from 8 hamsters per group. Different letters indicated significant differences at $P < 0.05$ (Tukey–Kramer's multiple comparison post hoc test).

Table 5
Effects of dietary AXs and oat β-glucan on the fecal parameters of hamster fed a hypercholesterolemic diet.[a]

	Control	AXs	Oat β-glucan
Fecal weight (g/day)	5.58 ± 0.20b	6.93 ± 0.15a	6.25 ± 0.11ab
Total lipids (mg/day)	35.2 ± 2.2b	42.7 ± 3.2a	43.6 ± 1.5a
Cholesterol (mg/day)	33.6 ± 3.7a	52.3 ± 3.0b	44.1 ± 3.9b
Bile acid (mg/day)	2.43 ± 0.07c	2.93 ± 0.09a	2.60 ± 0.02b
SCFAs (μg/day)			
Lactate	228 ± 8	176 ± 15	195 ± 3
Acetate	2382 ± 85	2740 ± 140	2510 ± 44
Propionate	74 ± 3c	135 ± 12b	205 ± 4a
Butyrate	–	–	39 ± 1
Total[b]	2684 ± 27b	3051 ± 75a	2911 ± 10a

[a] Means expressed with SE were determined from 8 hamsters per group.
[b] The data of fecal SCFAs were the sum of lactate, acetate, propionate, and butyrate. Different letters indicated significant differences at $P < 0.05$ (Tukey–Kramer's multiple comparison post hoc test). SCFAs, short-chain fatty acids; –, unidentified.

triglycerides concentrations, and reduces the risk of developing CVD in animal models and hypercholesterolemic subjects (Braaten et al., 1994; Kerckhoffs, Hornstra, & Mensink, 2003; Queenan et al., 2007). In this study, we demonstrated that the dietary AXs, as the same as oat β-glucan, reduced significantly plasma total and LDL-cholesterol concentrations in hypercholesterolemic hamsters fed with a hypercholesterolemic diet containing 0.5% AXs (Table 4). This result indicated that increased intake of AXs was one possible way to prevent the development of CVD.

Lopez et al. (1999) reported that soluble corn bran AXs reduced serum and liver cholesterol levels in rats by reducing uptake of cholesterol from a cholesterol-rice diet, but not by decreasing triglycerides levels. Moreover, the regulated effects of corn bran dietary fiber in rats on the expression of several genes involved in lipid metabolism were studied (Hu et al., 2008). It was demonstrated that the corn bran AXs enhanced the catabolism of lipids by down-regulated transcription of intestine-bile acid binding protein, up-regulated ileum farnesoid X receptor, liver peroxisome proliferator-activated receptors, lipoprotein lipase and transcription of hepatic cholesterol 7 alphahydroxylase. Although the cholesterol-lowering mechanisms of corn bran AXs were now well documented, little is known of the beneficial effects of wheat bran AXs. In this study, we determined the effect of AXs on fecal weight, cholesterol excretion, the concentrations of total lipids and bile acids excretion. As a result, the fecal weight in AXs group was significantly increased compared with control group. This finding is consistent with the previous studies regarding the wheat bran AXs promote fecal output in rats and humans (Lu et al., 2000, 2004). The total lipids, cholesterol and bile acids concentrations in the AXs and oat β-glucan groups were significantly increased (Table 5), indicating that the AXs contribute to reduce plasma total and LDL cholesterol concentrations by inhibiting cholesterol absorption in the intestine and promoting the excretion of bile acids.

The liver is an important organ of cholesterol synthesis and excretion, which is regulated by HMG-CoA reductase and CYP7A1 as rate-limiting enzymes, respectively (Sato, Ohuchi, Sook, Toyomizu, & Akiba, 2003). In order to clarify the mechanism(s) of the hypocholesterolemic effect of AXs, its effects on cholesterol metabolism in liver were determined. As a result, AXs reduced cholesterol synthesis significantly, and increased its decomposition into bile acids through regulating HMG-CoA reductase and CYP7A1 in liver. These findings supported that, in addition to the inhibition of cholesterol absorption in intestine, AXs promoted the bile acids pump through the regulation of HMG-CoA reductase and CYP7A1 in liver. In addition, the oat β-glucan can only promote cholesterol decomposition, not inhibit cholesterol synthesis. This may be one

of the reasons that AXs showed stronger capacity of promoting bile acids excretion than oat β-glucan.

Studies have shown that the highly water-soluble β-glucan, with moderate and/or high molecular weight, plays the better role in lowering cholesterole than β-glucan with the low water-solubility and low molecular weight (Theuwissen & Mensink, 2008). It has been postulated that highly water-soluble β-glucan with moderate and/or high molecular weight have higher intestinal viscosity, it may preserve the bile acids and reduce their reabsorption, leading to the bile acid synthesis from cholesterol, which finally lower plasma cholesterol concentrations. In this study, the molecular weight of AXs was larger than that of oat β-glucan (Table 1). In addition, the intrinsic viscosity of AXs was higher than that of oat β-glucan (Table 2). The difference in molecular weight and intrinsic viscosity explained the result that AXs increase bile acids excretion levels better than oat β-glucan (Table 5).

Adam et al. (2001) demonstrated that the replacement of refined wheat flours with whole flour in various starchy foods can exert a beneficial effect of fibers on developing CVD through the decrease of plasma cholesterol. Moreover, they observed that the whole wheat flours can significantly increase cecal SCFAs which are effective plasma cholesterol-lowering agents. The cellulose is usually incompletely fermented or not fermented, but the AXs and oat β-glucan are fermented partly or completely by intestinal microflora (Drzikova, Dongowski, Gebhardt, & Habel, 2005). Major end products of fermentation are the SCFAs, such as, lactate, acetate, propionate, and butyrate, which are very important for plasma cholesterol-lowering effects. In this study, the propionate and total SCFAs concentrations in AXs and oat β-glucan groups were higher than that in the control group. These findings indicated that the hypocholesterolemic effects of AXs were related to the increased colonic SCFAs, besides the promoting fecal and fecal lipids output. Furthermore, the fecal butyrate concentration was detected only in the oats group, and the propionate concentration in AXs group was slightly lower than that of oat β-glucan group (Table 5). Given that the degree of dietary fibers fermentation depended on their structural and physicochemical parameters (Bourquin, Titgemeyer, & Fahey, 1996; Drzikova et al., 2005; Wood, Arrigoni, Miller, & Amadò, 2002), it was speculated that the difference in fecal butyrate and propionate concentrations between AXs and oat β-glucan groups could be partially attributed to their disparity of the molecular structural and solubility. However, further study is required to explain the causes of this discrepancy.

In conclusion, the present study clearly showed that dietary wheat bran arabinoxylans reduces the plasma total cholesterol and LDL-cholesterol concentrations by promoting the excretion of fecal lipids, regulating activity of HMG-CoA reductase and CYP7A1, and increasing colonic short-chain fatty acids in hypercholesterolemic hamsters.

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