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Tartary Buckwheat Sprout Powder Lowers Plasma Cholesterol Level in Rats

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Summary We examined the effects of different types of buckwheat sprouts on the plasma cholesterol concentration, fecal steroid excretion and hepatic mRNA expression related to cholesterol metabolism in rats. Rats were fed a cholesterol-free diet with 5 g of Kitawasesoba common buckwheat sprout powder (KS)/100 g, 5 g of Hokkai T no. 8 tartary buckwheat sprout powder (HS-8)/100 g or 5 g of Hokkai T no. 9 tartary buckwheat sprout powder (HS-9)/100 g of diet for 4 wk. Control rats were fed a diet with α-cornstarch instead of sprout powder for 4 wk. There were no significant differences in food intake, body weight, liver weight or cecal contents among the groups. Plasma total cholesterol concentrations in the HS-8 and HS-9 groups were significantly lower than in the control group, whereas there was no significant difference between the KS and control groups. Fecal bile acid excretion and cecal short-chain fatty acid concentrations in the KS, HS-8 and HS-9 groups were significantly greater than in the control group. Furthermore, fecal matter excretion in the KS, HS-8 and HS-9 groups tended to be increased compared to the control group, with that in the HS-8 group being significantly higher than in the control group. Hepatic cholesterol 7α-hydroxylase mRNA expression in the KS, HS-8 and HS-9 groups and hepatic HMG-CoA reductase mRNA expression in the HS-9 group were significantly higher than in the control group. The results suggest that tartary buckwheat sprout powder has a serum cholesterol-lowering function by enhancing fecal bile acid excretion through increased fecal matter excretion or the upregulation of hepatic cholesterol 7α-hydroxylase mRNA expression in rats.

Key Words bile acid, buckwheat sprout powder, hepatic mRNAs, serum cholesterol, short-chain fatty acid

Tartary buckwheat has relatively more functional compounds than common buckwheat (*1*, *2*), but the production regions of tartary buckwheat are not as widespread as those of common buckwheat. Usually, buckwheat seed is used for making noodles or herb tea, and the leaves are consumed as a salad vegetable. On the other hand, the buckwheat sprout has recently been introduced as a new vegetable (*3–5*), and dietary consumption of buckwheat sprouts has attracted much interest because the sprout is considered an ideal food that follows the nutritional principle of whole-food nutrition (*3*).

Buckwheat seed is well known to be a high protein source (*6*) that has a hypocholesterolemic effect mediated by high fecal steroid excretion and low digestibility in rats (*7*). Furthermore, rutin is one of the major compounds in buckwheat, and its hypolipidemic effect and effect on cardiac function have also been reported in several experimental animal models (*8–10*). In fact, the buckwheat sprout has greater nutritional value (more amino acids, minerals, protein, polyunsaturated fatty acids and crude fibers) than the seeds or seed products (*3*, *5*, *11*). Several researchers have reported that the consumption of sprouts from various vegetables has beneficial health effects in experimental animals (*12–15*). For example, Taniguchi et al. (*12*) have suggested that the Japanese radish sprout has hypoglycemic activity and improves lipid metabolism in rats. However, little information about the beneficial health effects of the buckwheat sprout is available, although the sprout has been recognized as a functional food (*16*).

Recently, new varieties of tartary buckwheat (Hokkai T no. 8 and Hokkai T no. 9) that are richer in rutin than common buckwheat varieties have been developed by the National Agricultural Research Center for the Hokkaido Region in Japan. Thus, in this study, we examined

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the comparative effects of common buckwheat and tartary buckwheat sprouts on the plasma cholesterol concentration, fecal steroid excretion and hepatic mRNA expression related to cholesterol metabolism in rats.

MATERIALS AND METHODS

Plant materials. One common buckwheat cultivar (Kitawasesoba) and two tartary buckwheat cultivars (Hokkai T no. 8 and Hokkai T no. 9) were harvested in Hokkaido. Buckwheat seeds were germinated at 22°C for 4 d in the dark. In the next step, the germinated seeds were grown at 22°C for 5 d in the light. At 9 d after germination of Kitawasesoba and 10 d after germination of Hokkai T no. 8 and Hokkai T no. 9, the stems and leaves of the sprouts were weighed and freeze-dried. Finally they were ground into powders.

Micronutrient content. Dietary fiber, protein, lipid, carbohydrate, moisture and ash concentrations in sprout powders were determined by the AOAC procedure (*17*). The rutin concentration in sprout powders was determined according to the method of Suzuki et al. (*18*). In brief, 1 g of sprout powder was homogenized with 10 mL of 0.01% phosphoric acid in methanol and extracted at 80°C for 1 h. After centrifugation, the supernatant was filtered and then subjected to HPLC.

Animals and diets. Male F344/DuCrj rats (8 wk old) were purchased from Charles River (Yokohama, Japan). Rats were housed individually in stainless steel cages with ad libitum access to food and water. The animal facility was maintained on a 12:12 h light:dark cycle at a temperature of 22±1°C with 60±5% relative humidity. Animals were randomly divided into four groups (n=5). There were no significant differences in the body weight or serum total cholesterol concentration at the start of the experiment. The composition of each diet is shown in Table 1. All diets were based on the AIN-93G purified rodent diet (*19*). Rats were fed a cholesterol-free diet with 5 g of common buckwheat (Kitawasesoba) sprout powder (KS)/100 g diet, and 5 g of tartary buckwheat (Hokkai T no. 8) sprout powder (HS-8)/100 g diet or 5 g of tartary buckwheat (Hokkai no. 9) sprout powder (HS-9)/100 g diet for 4 wk. Control rats were fed a diet with α-cornstarch instead of sprout powders for 4 wk. Body weight and food consumption were recorded daily. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. All animals were cared for and handled in line with the standard principles in the Guide for the Care and Use of Laboratory Animals (*20*).

Table 1. Compositions of the experimental diets.

	Control	KS	HS-8	HS-9
	(g/kg diet)			
Casein	200	200	200	200
α-Cornstarch	528.5	478.5	478.5	478.5
Sucrose	100	100	100	100
Soy bean oil	70	70	70	70
Cellulose	50	50	50	50
Mineral mix[1]	35	35	35	35
Vitamin mix[1]	10	10	10	10
L-Cystine	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5
TBHQ[2]	1	1	1	1
Kitawasesoba (KS)	—	50	—	—
Hokkai T no. 8 (HS-8)	—	—	50	—
Hokkai T no. 9 (HS-9)	—	—	—	50

[1] These diets were based on the AIN-93G diet composition.

[2] *Tert*-butyl hydroquinone.

Analytical procedures. At the end of the experimental period of 4 wk, all fecal excretion during 3 d was collected. Blood samples (1 mL) were also collected from the caudal veins of fasting rats at the end of the experimental period of 4 wk. The blood was taken into tubes with an anticoagulant. After the samples stood at room temperature for 30 min, plasma was prepared by centrifugation at 1,500 ×g for 20 min. Soon after, the rats were fed the diets again. Twenty-four hours after blood was collected, rats were anesthetized with diethyl-ether inhalation and killed. Then the liver and cecum were quickly removed, and the liver was washed with cold saline (9 g NaCl/L), blotted dry on filter paper and weighed before freezing for storage at −80°C.

Chemical analysis. Plasma total cholesterol, HDL cholesterol and triglyceride concentrations were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX, USA). The non-HDL cholesterol concentration was calculated as follows: non-HDL-cholesterol=total cholesterol−HDL cholesterol. Total lipids were extracted from the liver and feces by a mixture of chloroform-methanol (2 : 1, v/v) (*21*). The neutral sterol in each total lipid obtained by saponification was acetylated (*22*), and measured by gas liquid chromatography (GLC) using a Shimadzu 14A chromatograph (Shimazu, Kyoto, Japan) with a DB17 capillary column (0.25 mm×30 m; J&W Scientific, Folsom, CA, USA) with nitrogen as a carrier gas. Acidic sterols in the feces were measured by GLC following the method of Grundy et al. (*23*). Each short-chain fatty acid (SCFA) in the cecum was measured by GLC using a chromatograph (Shimadzu) with a glass column (2,000×3 mm) packed with 80–100 mesh Chromosorb W-AW DMCS with H_3PO_4 (100 mL/L) as the liquid phase after adding H_3PO_4 by the procedure of Hara et al. (*24*).

RNA isolation, RT-PCR and Southern blot analysis. Hepatic total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan) (*25*). mRNAs encoding 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, used as an invariant control) were analyzed by semi-quantitative RT-PCR and subsequent Southern hybridization of PCR products with each inner oligonucleotide as previously described (*26*). The expected sizes of DNA fragments amplified with these primers were 245 bp for HMG-CoA reductase, 306 bp for cholesterol 7α-hydroxylase and

702 bp for GAPDH. The relative quantity of mRNA was estimated by densitometric scanning with X-ray film.

Statistical analysis. Data are expressed means±SD. For comparison between groups, the Turkey-Kramer test (StatView, SAS Institute, Cary, NC) was carried out. $p<0.05$ was regarded as significant.

RESULTS

Micronutrient contents

Table 2 shows the micronutrient contents in Kitawasesoba, Hokkai T no. 8 and Hokkai T no. 9 sprout powders. The total fiber contents in Kitawasesoba, Hokkai T no. 8 and Hokkai T no. 9 sprout powders were 30.1, 37.0 and 34.4 g/100 g powder, respectively. The rutin contents in Kitawasesoba, Hokkai no. 8 and Hokkai no. 9 sprout powders were 0.6, 5.9 and 8.0 g/100 g powder, respectively. The total fiber and rutin contents in tartary buckwheat sprout powders were higher than those in common buckwheat sprout powder.

Feed intake, body weight, tissue weight and fecal matter excretion

Table 3 shows feed intake, body weight, tissue weight and fecal matter excretion in rats. Body weight gain and food intake were unaffected by dietary treatment. The liver weight and cecum weight in the KS, HS-8 and HS-9 groups were not significantly different from those in the control group. Fecal matter excretion in the KS and HS-8 groups was significantly ($p<0.05$) higher than in the control group. However, there was no significant difference in fecal matter excretion between the control and the HS-9 groups.

Tissue lipid concentration

Table 4 shows plasma total cholesterol, HDL cholesterol, non-HDL cholesterol and triglyceride concentrations in rats at 4 wk. Plasma total cholesterol levels in the HS-8 and HS-9 groups were significantly ($p<0.05$) lower than in the control group, whereas there was no significant difference between the KS and control groups. There were no significant differences in plasma HDL cholesterol, non-HDL cholesterol or triglyceride concentrations among the groups.

Table 2. Properties of various buckwheat sprout powders.

	Kitawasesoba sprout powder	Hokkai T no. 8 sprout powder	Hokkai T no. 9 sprout powder
	(g/100 g powder)		
Moisture	7.8	5.4	5.6
Protein	29.1	30.3	27.5
Lipid	6.5	6.3	6.2
Ash	9.6	9.3	10.3
Carbohydrate	46.4	42.8	42.4
Total dietary fiber	30.1	37.0	34.4
Soluble dietary fiber	5.6	5.9	6.5
Insoluble dietary fiber	24.5	31.1	27.9
Rutin	0.6	5.9	8.0

Table 3. Body weight gain, feed intake, and liver weight in rats fed various experimental diets for 4 wk.

	Dietary group			
	Control	KS	HS-8	HS-9
Initial body weight (g)	173±3	174±4	174±4	174±4
Final body weight (g)	255±6	256±6	255±15	256±15
Body weight gain (g/4 wk)	68±6	67±6	69±15	68±15
Feed intake (g/4 wk)	400±14	401±21	389±19	382±26
Liver weight (wet g/100 g bw)	3.2±0.2	3.2±0.2	3.3±0.1	3.4±0.1
Cecum weight (wet g/100 g bw)	1.5±0.2	1.6±0.1	1.5±0.2	1.5±0.3
Fecal matter excretion (dry g/3 d)	2.1 ± 0.7^{b}	3.1 ± 0.4^{a}	3.3 ± 0.4^{a}	2.8 ± 0.7^{ab}

Values are means±SD of five rats in each group. Values not sharing a row superscript are significantly different at $p<0.05$.

Table 4. Plasma total cholesterol, HDL-cholesterol, and non-HDL cholesterol concentrations in rats fed various experimental diets at 4 wk.

	Dietary group			
	Control	KS	HS-8	HS-9
Total cholesterol (mmol/L)	2.30 ± 0.25^{a}	2.20 ± 0.10^{ab}	1.87 ± 0.32^{c}	1.93 ± 0.16^{bc}
HDL cholesterol (mmol/L)	1.22±0.39	1.09±0.17	0.99±0.55	0.96±0.09
Non-HDL cholesterol (mmol/L)	1.08±0.32	1.11±0.16	0.88±0.35	0.97±0.21
Triglyceride (mmol/L)	1.42±0.39	1.52±0.12	1.36±0.46	1.27±0.36

Values are means±SD of five rats in each group.
Values not sharing a row superscript are significantly different at $p<0.05$.
Non-HDL cholesterol=Total cholesterol−HDL cholesterol.

Table 5. Liver cholesterol and fecal steroid concentrations in rats fed various experimental diets for 4 wk.

	Dietary group			
	Control	KS	HS-8	HS-9
Liver cholesterol (μmol/g)	8.13±1.57	6.72±2.44	5.62±2.21	6.97±1.70
Fecal cholesterol (μmol/3 d)	3.14±2.29	4.58±2.00	5.68±2.70	6.21±1.50
Fecal coprostanol (μmol/3 d)	12.36±8.17	25.53±12.19	19.82±10.60	25.35±12.99
Fecal total neutral steroids[1] (μmol/3 d)	15.49±10.14	30.12±13.56	25.50±12.94	31.56±13.50
Fecal cholic acid (μmol/3 d)	0.11±0.08^{b}	0.21±0.17^{ab}	0.32±0.08^{a}	0.16±0.08^{ab}
Fecal deoxycholic acid (μmol/3 d)	0.08±0.02^{b}	0.19±0.11^{ab}	0.21±0.05^{a}	0.19±0.14^{ab}
Fecal chenodeoxycholic acid (μmol/3 d)	0.03±0.01^{b}	0.12±0.04^{a}	0.16±0.04^{a}	0.11±0.07^{a}
Fecal lithocholic acid (μmol/3 d)	0.29±0.07^{b}	0.58±0.22^{a}	0.50±0.15^{ab}	0.51±0.26^{ab}
Fecal total bile acid[2] (μmol/3 d)	0.51±0.13^{b}	1.10±0.42^{a}	1.19±0.19^{a}	0.97±0.46^{a}

Values are means±SD of five rats in each group.
Values not sharing a row superscript are significantly different at $p<0.05$.
[1]Total neutral steroids=cholesterol+coprostanol.
[2]Total bile acid=cholic acid+deoxycholic acid+chenodeoxycholic acid+lithocholic acid.

Table 6. Cecal short chain fatty acid (SCFA) concentrations and pH in rats fed various experimental diets for 4 wk.

	Dietary group			
	Control	KS	HS-8	HS-9
Acetate (μmol/g wet contents)	12.76±4.85^{b}	23.59±8.79^{a}	22.73±12.82^{a}	27.51±8.12^{a}
Propionate (μmol/g wet contents)	3.99±0.93^{b}	7.36±2.91^{a}	5.88±3.06^{ab}	6.38±1.87^{ab}
butyrate (μmol/g wet contents)	1.93±0.62^{b}	9.19±3.77^{a}	5.98±1.76^{a}	9.15±3.59^{a}
Total SCFA (μmol/g wet contents)	18.68±5.60^{b}	40.14±12.49^{a}	34.59±17.11^{a}	43.04±12.68^{a}
pH	8.10±0.53	7.89±0.25	7.87±0.24	7.75±0.24

Values are means±SD of five rats in each group.
Values not sharing a row superscript are significantly different at $p<0.05$.
Total SCFA=acetate+propionate+butyrate.

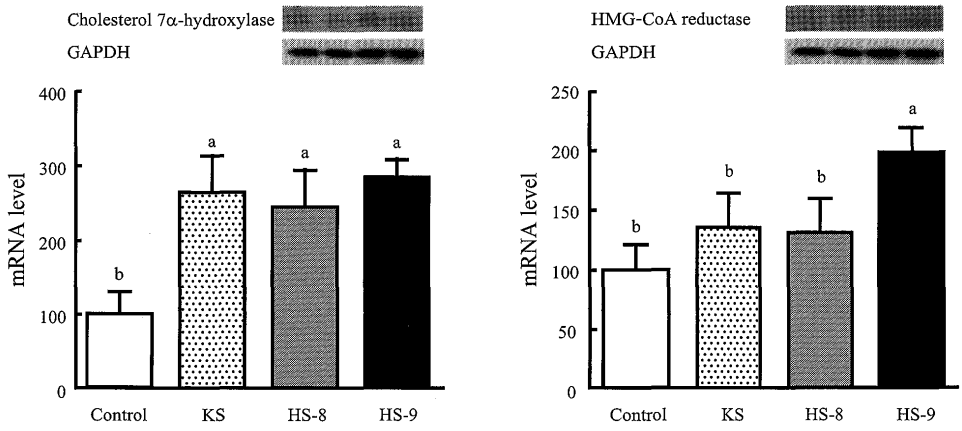

Fig. 1. Hepatic cholesterol 7α-hydroxylase and HMG-CoA reductase mRNA levels in rats fed various buckwheat sprout powders for 4 wk. Values are means for five rats with standard deviations indicated by bars.

Table 5 shows the hepatic cholesterol concentrations and fecal steroid excretions of the rats. There were no significant differences in hepatic cholesterol, fecal cholesterol or fecal coprostanol concentrations among the groups. The fecal total bile acid excretions in the KS, HS-8 and HS-9 groups were significantly ($p<0.05$) greater than in the control group.

Cecal SCFA concentration

Table 6 shows the cecal SCFA concentrations of the rats. Cecal acetate and *n*-butyrate concentrations in the KS, HS-8 and HS-9 groups were significantly ($p<0.05$) higher than those in the control group. The cecal propionate concentration in the KS group was significantly higher than in the control group. Cecal total SCFA con-

centrations in the KS, HS-8 and HS-9 groups were significantly ($p<0.05$) higher than in the control group.

Hepatic mRNA expression

Figure 1 shows the hepatic cholesterol 7α-hydroxylase mRNA and HMG-CoA reductase mRNA levels of rats. Hepatic cholesterol 7α-hydroxylase mRNA levels in the KS, HS-8 and HS-9 groups were significantly ($p<0.05$) higher than in the control group. The hepatic HMG-CoA reductase mRNA level in the HS-9 group was significantly ($p<0.05$) higher than in the control, KS and HS-8 groups.

DISCUSSION

In the present study, we found that the consumption of tartary buckwheat sprout powders reduced the plasma total cholesterol concentration. Although little information on the hypocholesterolemic effects of vegetable sprouts is available, Taniguchi et al. (*12*) recently reported that the radish sprout had a hypocholesterolemic effect in rats. They suggested that the effect might be due to the dietary fiber content in sprout powder (*12*), since dietary fiber is well known to decrease blood cholesterol in rats (*27*, *28*). The hypocholesterolemic effect of dietary fiber is mainly explained by the greater fecal steroid excretion (*26*, *28*, *29*). For example, Buhman et al. (*29*) reported that feeding psyllium to rats enhanced the fecal excretion of bile acid and total steroids. Drzikova et al. (*28*) also reported that higher excretion of bile acids was connected with a lowering of the blood cholesterol level in rats fed dietary fiber-rich oat-based product diets. In this study, compared to the 5% fiber content (as cellulose) in the control diet, approximately 6.5–6.8% total dietary fiber (cellulose+each buckwheat sprout fiber) was present in each buckwheat sprout powder diet. Furthermore, the consumption of buckwheat sprout powders significantly upregulated hepatic cholesterol 7α-hydroxylase mRNA expression, which is similar to the report (*30*) that soluble dietary fiber such as barley β-glucan increased the cholesterol 7α-hydroxylase activity and mRNA level in cholesterol-fed rats. However, Kubo and Nanba (*31*) also reported that the antihyperlipemic activities of maitake containing abundant insoluble dietary fiber were due to acceleration of cholesterol and bile acid excretion, and of cholesterol conversion into bile acid. The upregulated hepatic cholesterol 7α-hydroxylase mRNA expression may be due to the quality of the KS, HS-8, HS-9 dietary fibers containing about 80–84% insoluble dietary fiber. In fact, hepatic cholesterol 7α-hydroxylase mRNA expression had a negative correlation to the plasma total cholesterol concentration of week 4 ($r=-0.516$, $p<0.05$) and a positive correlation to the fecal total bile acid excretion of week 4 ($r=0.564$, $p<0.05$) in this experiment. Therefore, the results suggest that the stimulation of hepatic bile acid synthesis from cholesterol might be one mechanism by which buckwheat sprout powders decrease the plasma cholesterol concentration in rats. However, there was not a high positive correlation between the fecal total bile acid excretion and plasma total cholesterol concentration ($r=-0.405$, $p<0.1$) in this experiment.

Several dietary fibers are well known to increase cecal fermentation due to microfloral activity in the hindgut (*28*, *32*). In this study, cecal fermentation processes were more intensive in the case of the consumption of buckwheat sprout powders relative to the control diet, as cecal SCFA (acetate, propionate and *n*-butyrate) concentrations in the KS, HS-8 and HS-9 groups were greater than in the control group. It has been reported that cecal SCFA might be responsible for a plasma cholesterol-lowering effect (*27*), because the digestion through the colon is stimulated by a high proportion of *n*-butyrate, thereby promoting gastrointestinal transit time as well as fecal bulking (*33*). In this study, there was a negative correlation between the cecal total SCFA level and plasma total cholesterol concentration ($r=-0.560$, $p<0.05$). Therefore, it might be possible that the cecal fermentation processes increased the fecal matter excretion, resulting in greater steroid excretion in the KS, HS-8 and HS-9 groups than in the control group.

Some workers have reported that rutin is one of the major compounds in buckwheat, and that tartary buckwheat has a higher rutin content than common buckwheat (*1*, *2*). We also found that the level of the compound in tartary buckwheat sprout powders was approximately 9-fold higher than in common buckwheat sprout powders. Recently, rutin was revealed to have hypolipidemic and cardiac protective effects in several experimental animal models (*8–10*). In this study, tartary buckwheat sprout powders more efficiently decreased plasma total cholesterol than the common buckwheat sprout in rats. Santos et al. (*8*) have reported that a dose of rutin of 5 mg/kg body weight presents the largest percentile reduction of cholesterol. In this experiment, rats in the KS, HS-8 and HS-9 groups were fed 17, 160 and 213 mg/kg body weight/d, respectively. On the other hand, Stanely and Kannan (*10*) have reported that oral administration of rutin to streptozotocin-induced diabetic rats significantly decreased cholesterol and triglyceride concentrations in plasma and tissues, while rutin administration to normal rats did not exhibit any significant changes in any of the parameters. These beneficial effects of rutin on lipids could be due to its antioxidant property. The differences found in the hypocholesterolemic effect of the KS and HS groups might be due to the difference in the antioxidant property in normal rats caused by different rutin contents in these sprout powders. However, the mechanism of the cholesterol-lowering function of rutin in this experiment could not be clarified.

Furthermore, we found that though hepatic HMG-CoA reductase mRNA expression was higher than in the control group only for the HS-9 group, there were also slight increments in the KS and HS-8 groups compared with the control group. The increments of hepatic HMG-CoA reductase mRNA expression in the KS, HS-8 and HS-9 groups might have been due to an increment of cholesterol 7α-hydroxylase mRNA expression because the HMG-CoA reductase mRNA expression had

a high positive correlation to the cholesterol 7α-hydroxylase mRNA expression (r=0.720, p<0.001). Furthermore, although there was no significant difference in the hepatic cholesterol concentrations among the groups, the tendency toward lower hepatic cholesterol levels might have been related to the plasma cholesterol concentration. However, there was no correlation between the HMG-CoA reductase mRNA expression and plasma total cholesterol concentration (r=−0.39, p<0.1), and the precise mechanism is unknown at present.

In conclusion, the hypocholesterolemic effect of tartary buckwheat sprout powders was evident compared to the control diet in rats. It appears that the cholesterol-lowering effect of buckwheat sprout powder is dependent on fecal steroids, especially bile acid, excretion, and the cholesterol 7α-hydroxylase mRNA level. These results demonstrate that buckwheat sprout powder positively influences plasma and liver lipid metabolism.

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