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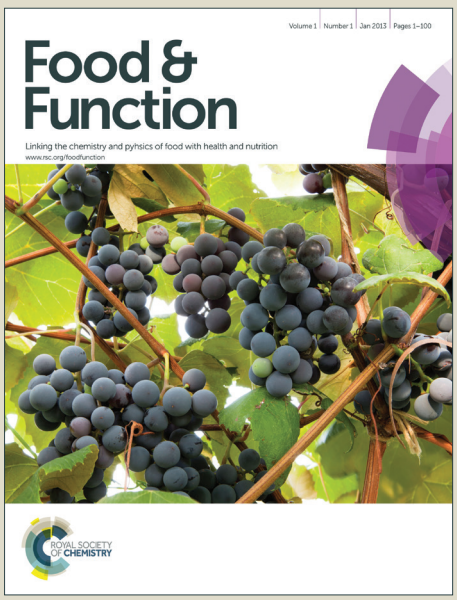

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Effect of Particle Size of Cellulose from Sweet Potato Residues on Lipid Metabolism and Cecal Conditions in Ovariectomized Rats

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ABSTRACT

This study aims to examine the effect of the particle size of cellulose from sweet potato residues on lipid metabolism and cecal conditions in ovariectomized rats. Forty mature female Wistar rats were divided into five groups. The sham-operated group was used as the sham control. The other four groups were double-ovariectomized and assigned to the model, ordinary cellulose (100g/kg diet), microcrystalline cellulose (100g/kg diet), and cellulose nanocrystal (100g/kg diet) groups. As the cellulose particle size decreased, the body weight gain and food intake were decreased. The plasma lipids and hepatic lipids were decreased. In addition, the mRNA levels of cholesterol 7 α-hydroxylase, farnesoid X receptor, and 3-hydroxy-3-methylglutaryl coenzyme A reductase were decreased, whereas those of ileal apical sodium-dependent bile acid transporter, and intestinal bile acid binding protein were increased. The cecum weight, cecum content, and short-chain fatty acid concentration and the amount of total bile acids in the small intestinal content, as well as the bile acids and neutral steroids in fecal excretion were increased. These results indicate that as the particle size decreased, cellulose was more effective in preventing ovarian hormone deficiency-induced hyperlipidemia and in improving intestinal health.

Keywords: *Cellulose, Ovariectomized rats, lipid metabolism, Gene expression, Cecal condition*

1 Introduction

Estrogens are important regulators of lipid homeostasis. [1] Obesity, which results from declining estrogen levels after menopause, increases the risk of heart disease, diabetes, and hypertension. Previous studies have shown prevalence for a more atherogenic lipid profile in postmenopausal women, who tend to have higher triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels, as well as lower high-density lipoprotein cholesterol (HDL-C) levels, than pre-menopausal women.[2,3] Progressive withdrawal of estrogen during pre-menopause is also associated with many features of the metabolic syndrome profile. [4,5] Menopause itself is a risk factor for the metabolic syndrome and is independent of the effects of estrogen deficiency on central obesity in nondiabetic women. [6]

Fiber is a vital component of healthy diets and has well-documented health benefits. [7-10] Increased consumption of fiber is associated with reduced risks for cardiovascular disease and perhaps certain cancers.[11,12]An effective method of increasing cellulose intake is by eating food supplemented with rich cellulose.[13,14] However, formulating this type of food is difficult because the addition of a large amount of cellulose-rich ingredients affect the appearance, texture, and taste of food. [15.16] The importance of cellulose has led to the development of a large and potential market for fiber-rich products and ingredients, and encourages food scientists to search for better sources of food fibers. Previous reports demonstrated that the physicochemical properties of cellulose determine its physiological and metabolic effects upon their consumption. [17] In vitro fermentation experiments showed that

wheat bran of small particle size increases short-chain fatty acid (SCFA) production than wheat bran of large particle size.[18] These results showed that particle size reduction effectively enhances the cholesterol-lowering activities of insoluble carrot fiber and cellulose. Other reports demonstrated that the high water-holding capacity and swelling capacity of insoluble fibers can increase digesta viscosity by reducing the free water content in digesta, thus decreasing nutrient absorption. [19]

However, given the nutritional relevance of cellulose, efforts have been made to modify the physicochemical properties of various types of cellulose using techniques such as microcrystalline cellulose (MCC) and cellulose nanocrystals (CNCs). Microtechnology and nanotechnology are emerging fields with high potential in nutraceuticals and functional foods for human health improvement. [20] The larger surface area per unit mass of tiny particles than that of larger-sized particles of the same chemistry results in higher biological activity.[21] The application of micronization or nanotechnology in food research and development has gained considerable attention. The reduction in the cellulose particle size to micro- or nanosizes by micronization or nanotechnology, respectively, can affect the surface areas, structures, and functional properties of cellulose and lead to new applications in food development.[22,23] Preliminary studies revealed that the reduction in the cellulose particle size to micro- or nanoscale enhances some of the physicochemical properties of cellulose.[24] A comprehensive understanding of the effects of micronization or nanotechnology on the characteristics and different physicochemical properties of cellulose will be useful to improve its functionality and potential applications.

Sweet potato is a root crop largely grown in different countries, including China. The industrial exploitation of sweet potato starch involves the elimination of soluble sugars and the separation of most fibers, resulting in a purified starch and a solid residue called sweet potato residue (SPR). SPR mainly consists of residual starch and cellulose fibers.[25] After residual starch is removed, the cellulose fiber content is approximately 75 wt%. We have studied the preparation and characteristics of ordinary cellulose (OC), MCC, and CNCs from SPRs.[26] These results indicated that particle size reduction effectively enhances the physicochemical properties. The successful enhancement of the functions of cellulose from SPR will increase its use, and subsequently benefit the starch industry and the environment as well as contribute to the development of fiber-rich food.

Recent findings revealed that the reduction in cellulose particle size to microsized fiber powder by different micronizing technologies effectively enhances the physicochemical properties and in vitro hypoglycemic potential of cellulose.[27,] However, the effects of the particle size of cellulose from SPR on serum lipids, hepatic lipids, and intestinal health in estrogen insufficiency-induced obesity are of significant interest. However, plasma cholesterol can be lowered by several mechanisms, including decreasing hepatic cholesterol biosynthesis, increasing plasma cholesterol removal, decreasing dietary cholesterol uptake, accelerating hepatic cholesterol degradation into bile acids, and disrupting bile acid reabsorption. Therefore, whether cellulose can affect these mechanisms by regulating genes involved in cholesterol homeostasis needs to be investigated.

Estrogen insufficiency in humans can be modeled using ovariectomized (OVX) rats. This model is characterized by mild obesity and is useful in studying the effects of hypoestrogenism on adiposity. This study was performed to evaluate and compare the effects of the particle size of cellulose from SPRs on serum lipids, hepatic lipids, gene expression and intestinal health in OVX rats. The understanding of the effects of cellulose particle size on physiological functions could broaden the potential applications of micronization and nanotechnology in the fiber-rich food industry.

2. Materials and methoes

2.1 Materials

SPR was acquired from Sichuan Guangyou Shuye Co., Ltd. (Sichuan, China). OC (83.57% α-cellulose), MCC (85.02% α-cellulose), and CNCs (94.85% α-cellulose) from SPRs were prepared using the method of Lu et al.[26]

2.2 Animals and diets

Eight-week-old female Wistar rats were obtained from Chongqing Tengxin Inc. (Chongqing, China). The rats were housed in individual plastic cages in a temperature- and humidity-controlled room (23 ± 1 °C and 60 ± 5% relative humidity) with a 12 h light/dark cycle (lights, 08:00 to 20:00). The rats were given free access to a commercial solid diet and distilled water. After a 7 d adaptation period, 40 rats were randomly divided into the sham group ($n = 8$) and the OVX groups ($n = 32$). The sham group was subjected to sham operation, whereas the OVX groups were

subjected to bilateral ovariectomy performed via a dorsal midline incision under ether anesthesia. The rats were fed a commercial solid diet during the 7 day recovery period. Upon recovery from anesthesia, the rats were assigned to the OVX-CON, OVX-OC, OVX-MCC, and OVX-CNC groups, with eight animals per group per experiment. The rats were given free access to one of the experimental diets for 28 d. The compositions of each diet are shown in **Table 1**. Body weight and food intake were recorded daily in the morning before replacing the food. All experimental procedures were performed in accordance with the protocols approved by Institutional Animal Care and Research Advisory Committee.

2.3 Sampling and analytical procedures

Before the rats were killed, fresh fecal excretion samples were collected from each rat on the last 3 d of the experimental period. The feces were freeze-dried, weighed, and milled. On the last day of the experiment, the rats were fasting 12 h and then anesthetized with anhydrous ether and sacrificed by decapitation. Blood samples were collected from the neck of each rat at night into a blood collection tube (Vacutainer; Liuyang City Medical Instrument Factory, Hunan, China) containing heparin as an anticoagulant. Plasma was separated by centrifugation at 1400 g at 4 °C for 15 min and stored at –80 °C until analysis. After blood collection, the liver was immediately perfused with cold saline (9 g/L NaCl), removed, washed with cold saline, blotted dry on filter paper, weighed, and stored at –20 °C until analysis. Then, the small intestine and cecum were removed. The contents of the small intestine were transferred into a pre-weighed tube, freeze-dried, and weighed. The cecum was also weighed. Then,

0.4 g of the cecal contents was transferred into a tube, and 2 mL of 10 mmol/L sodium hydroxide was immediately added. The mixture was freeze-dried and used for short-chain fatty acid (SCFA) analysis. An aqueous solution containing 0.5 g/L crotonic acid was used as an internal standard. The pH of the cecal contents was measured with a compact pH meter using a sampling sheet (PHS-3, calibrated at 20 °C; Shanghai, China) immediately after removal. The water content of the cecal contents was determined as the difference between the wet mass and the dry mass of the cecal contents after freeze-drying. The cecal ammonia level was determined spectrophotometrically in the deproteinized [4 mL of 0.25 mol sulfuric acid and 50 g of sodium tungsten dihydrate per liter, 1:1 (v/v), for 50 mg contents] supernate (1500 × g, 10 min) of the cecal content (Okuda & Fujii, 1966). The cecal wall was flushed with ice-cold saline (9 g/L NaCl, 4 °C), blotted onto filter paper, and then weighed.

2.4 Bichemical analysis

2.4.1 Plasma lipid

The total cholesterol (TC), TG, LDL-C, and HDL-C concentrations in the plasma were determined enzymatically with a HITACHI 7020 automatic biochemistry analyzer (Hitachi High Technologies Corp., Tokyo, Japan) using commercial diagnostic kits (Beihai Biotechnology, Shanghai, China). The non-HDL-C concentration was calculated by subtracting the HDL-C concentration from the TC concentration. The atherosclerosis index (AI) was calculated using the formula as follows:

$$\text{Atherogenic index} = \frac{\text{total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$$

2.4.2 Liver lipids

The level of liver total lipids was determined gravimetrically after extraction using the method of Folch et al.[28] The liver TG and cholesterol concentrations were determined enzymatically as described elsewhere.[29] The liver index was calculated using the formula, liver index = liver weight/body weight.

2.4.3 Neutral sterols and total bile acid (TBA)

Steroids were extracted from the feces and digestive tract contents (small intestine) with a chloroform/methanol mixture (1:1, vol/vol) at 70 °C for 60 h.[30] The TBA concentrations in the small intestinal contents and feces were determined enzymatically by the 3α-dehydrogenase assay method of Sheltaway and Losowsky [31] using taurocholic acid as standard. The cholesterol and coprostanol concentrations in feces were analyzed by capillary gas chromatography (GC-2010; Shimadzu Corporation, Kyoto, Japan). 5a-Cholestane (Nacalai Tesque Inc., Kyoto, Japan) was used as the initial standard for neutral sterol analysis.

2.4.4 Total lipid and cellulose contents of fecal excretion

The total lipids of fecal excretion were determined gravimetrically after extraction by the method of Folch et al.[28] The cellulose content of fecal excretion was determined according to the method described in TAPPI T19m-54.

2.4.5 The surface area of cecum

The fully spread cecum was fixed to the white plastic with a standard calibration

(cm), and printing the picture after take a photo. Then, cut the picture along the cecum profile, and measure the length of 1 cm scale line in the picture (L). Weighing outline drawings (W_1) and peace square centimeter of paper (W_2) by the accuracy of 0.0001 g of electricity. Surface area (cm^2) = (W_1(g)/ W_2(g))*L^2(cm^2)

2.4.6 RNA extraction from liver and real time-polymerase chain reaction (RT-PCR) analysis of gene expression.

Total RNA was extracted from the frozen livers according to the method described by Chomczynski and Sacchi.[3] RNA integrity was veried by agarosegel electrophoresis using Oligotex-dT30 (Takara Bio, Shiga, Japan). A total of 1mg puried mRNA was used for cDNA synthesis using reverse transcriptase (TaKaRa Biotechnology Co., Ltd., Dalian, China) in accordance with the manufacturer's instructions. The messenger RNA (mRNA) expressions for cholesterol 7 α-hydroxylase (CYP7A1), farnesoid X receptor, (FXR), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), apical sodium-dependent bile acid transporter (ASBT), and ileum bile acid binding protein (IBABP) were determined by RT-PCR using a Light Cycler Instrument (Roche Diagnostics, Mannheim, Germany). 2 μL of cDNA was amplified in a total volume of 20 μL using the SYBR Premix Ex *Taq*II (TaKaRa Biotechnology Co., Ltd., Dalian, China) and specic primers of 0.4 μM each. The reaction mixture was incubated for initial denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 20 s. Table 2 lists the sequences of the gene-specific primers (Sangon Biological Engineering, Shanghai, China) used in the study. Relative gene expression was calculated using the crossing point of each target gene; the

β-actin gene was used as the reference.

2.5 Statistical analyses

Data are expressed as means and standard deviations ($n = 8$). The data were subjected to one-way analysis of variance using Statistical Product and Service Solutions software (version 16.0). The differences among groups were examined by Tukey's multiple-range test. $p < 0.05$ was considered significant.

3 Result

3.1 Body weight gain and food intake

The OVX rats had significantly higher ($p < 0.05$) body weight gain and food intake than those in the sham group rats (**Figure 1**), indicating that these rats were estrogen-deficient. Among the OVX rats, the body weight gain and food intake were significantly higher ($p < 0.05$) in the OVX-OC rats than those in the OVX-CON rats, whereas no differences were observed among the OVX-OC, OVX-MCC, and OVX-CNC groups. However, as the cellulose particle size decreased, the body weight gain and food intake of the cellulose-fed OVX rats decreased.

3.2 Plasma lipids and liver lipids

The plasma TC, TG, LDL-C, non-HDL-C, and AI were significantly higher ($p < 0.05$) in the OVX rats than those in the sham rats (**Table 3**). In the OVX rats, the plasma HDL-C concentration and AI were unaffected by cellulose addition. However, plasma TG significantly decreased ($p < 0.05$) in the cellulose-fed OVX rats than that in the OVX-CON group. In the OVX-CNC group, plasma TC, LDL-C, and non-HDL-C significantly decreased ($p < 0.05$) than those of the OVX-CON group. As the cellulose particle size decreased, TC, TG, LDL-C, and non-HDL-C also decreased.

The liver weights did not differ among the groups. The lipid, TC, and TG concentrations in the liver significantly increased ($p < 0.05$) in the OVX rats than those in the sham rats (**Table 4**). Compared with the OVX-CON group, the lipid and TG concentrations in the liver significantly decreased ($p < 0.05$) in the OVX-OC, OVX-MCC, and OVX-CNC groups. However, the level of liver total lipids was not correlated with the cellulose particle size. Among the cellulose-fed OVX rats, the liver TG logarithmically decreased as the cellulose particle size decreased. However, the difference was not significant.

3.3 TBAs in the small intestinal contents and fecal excretion

The TBA levels in the small intestinal contents and fecal excretion were lower in the OVX rats than those in the sham rats (**Table 5**). The dry weight of the small intestine significantly decreased in the OVX-CON group than that in the sham group. In the cellulose-fed OVX rats, the dry weight of the small intestine increased with decreasing particle size, whereas those of bile acid levels in the fecal excretion and small intestinal contents were significantly increased ($p < 0.05$). Among the cellulose-fed OVX rats, the TBA levels of the fecal excretion and small intestinal contents generally increased as the cellulose particle size decreased. In the OVX-CNC group, the TBA levels in the fecal excretion were significantly higher ($p < 0.05$) than those in the OVX-MCC and OVX-OC groups. However, the TBA levels in the small intestinal contents were significantly ($p < 0.05$) higher in the OVX-MCC and OVX-CNC groups than those in the OVX-OC group.

3.4 Effects of cellulose particle size on the dry weight, cellulose content, lipids,

and neutral sterol of the fecal excretion of OVX rats

The dry weights of fecal excretion of the sham and OVX–CON groups showed no difference. The cellulose content and lipids of the fecal excretion of the OVX–CON rats were higher than those of the sham rats (Table 5). However, the cholesterol, coprostanol, and total neutral sterols significantly decreased ($p < 0.05$) in the OVX-CON group than those in the sham group. By contrast, among the OVX groups, the dry weight, cellulose contents, lipids, and neutral sterols of fecal excretion of the cellulose-fed OVX rats were significantly higher ($p < 0.05$) than those of the OVX-CON rats. As the cellulose particle size decreased, the fecal excretion dry weight, cellulose contents, lipids, and neutral sterols exponentially increased. The lipid, cholesterol, coprostanol, and total neutral sterol levels of fecal excretion were significantly higher ($p < 0.05$) in the OVX-CNC groups than those in the OVX-OC and OVX-MCC groups.

3.5 Effects of cellulose particle size on the cecum and cecal content of OVX rats

The cecal tissue weight and contents in the OVX-CON rats significantly decreased ($p < 0.05$) than those in the sham group (Table 5). However, ammonia in the cecal content significantly increased ($p < 0.05$). The cecal tissue weight, cecal contents, and cecal content moisture were significantly higher ($p < 0.05$) in the cellulose-fed OVX rats than those in the OVX-CON rats. In the cellulose-fed OVX rats, the cecal tissue weight and moisture increased as the cellulose particle size decreased. The cecal surface area in the OVX-CNC rats was increased than those in the OVX-OC and OVX-MCC, but no significant differences were found among the

groups. As the particle size decreased, the ammonia concentrations and pH increased. However, no significant differences were found among the groups.

3.6 Effects of cellulose particle size on the cecum SCFA content in OVX rats

The acetic acid, propionic acid, isobutyric acid, and total SCFA concentrations in the cecal contents significantly decreased ($p < 0.05$) in the OVX-CON groups than those in the sham group. In the OVX groups, the acetic acid, propionic acid, isobutyric acid, n-butyric acid, and total SCFA amounts in the cecal contents increased after feeding with cellulose (**Table 6**). For the propionic acid and total acid concentrations, the values for the cellulose-fed OVX groups was significantly higher ($p < 0.05$) than those for the OVX-CON group, and no significant difference was found among the cellulose-fed OVX groups. The acetic acid, propionic acid, and total SCFA amounts were higher in OVX-MCC than those in OVX-OC and OVX-CNC. No significant difference was found among these groups. The n-butyric acid and isobutyric acid concentrations were higher in OVX-MCC and OVX-CNC than those in OVX-OC.

3.7 Effects of cellulose particle size on the hepatic and ileac genes expression

To understand the mechanism of the hypocholesterolemic effect of cellulose, the expression of hepatic and ileac genes involved in cholesterol homeostasis in the rats were measured (**Figure 2**). The mRNA levels of CYP7A1, FXR and HMG-CoA reductase in liver, and ASBT and IBABP in ileac tissues were significantly up-regulated in the OVX-CON group compared with the sham group. In the OVX rats (**Figure 2A**), the mRNA levels of CYP7A1, FXR and HMG-CoA reductase in liver

were down-regulated linearly as the cellulose particle size decreased, whereas those of ASBT and IBABP were significantly up-regulated (**Figure 2B**). The mRNA levels of CYP7A1, FXR and HMG-CoA reductase in liver were significantly decreased ($p < 0.05$) in the OVX-CNC groups than those in the OVX-OC and OVX-MCC groups, but those of ASBT and IBABP were significantly increased ($p < 0.05$).

4 Discussion

In this study, we investigated the effects of cellulose particle size on the plasma lipids, liver lipids, and cecal condition of rats. With decreasing cellulose particle size, the plasma lipid decreased without building up liver lipid. The amount of bile acids in the small intestinal contents, as well as the excretions of bile acids and neutral steroids in the feces, increased with decreasing cellulose particle size. These results imply that cellulose effectively enhanced cholesterol-lowering activities, and as the cellulose particle size decreased, the more enhanced the effect.

The hypocholesterolemic effect of fiber may be due to the fiber-induced changes in the lipoprotein metabolism, bile acid metabolism, intestinal absorption, or fermentation by-products and their effects on hepatic cholesterol synthesis.[32,33] Possible explanations for the action of cellulose are as follows: (a) cellulose restricts dietary energy, which causes a reduction in the hepatic synthesis of cholesterol; (b) cellulose increases the fecal excretion and intestinal pool of bile acids; (c) cellulose increases the synthesis of fermentation products such as propionic acid, which possibly reduce cholesterol synthesis in the liver.

In this study, the body weight and food intake increased in rats fed with cellulose

than those in the OVX-CON group. However, no significant difference was found in the rats fed with different particle sizes of cellulose. This result indicates that the cellulose particle size did not affect the energy intake of rats. The liver is the main site for endogenous cholesterol synthesis, in which HMG-CoA reductase is the rate-limiting enzyme. Our data indicated that the mRNA level of hepatic HMG-CoA reductase was significantly increased by ovariectomy, but decreased as the cellulose particle size decreased. Therefore, cellulose can inhibit the biosynthesis of cholesterol in the liver. The mRNA levels of HMG-CoA reductase in liver was down-regulated linearly as the cellulose particle size decreased. These suggest that inhibition of the mRNA expression of hepatic HMG-CoA reductase and reduction of the hepatic cholesterol biosynthesis may be a functional mechanism operated by cellulose in OVX rats.

Previous studies indicated that the hypocholesterolemic effects of some bioactive components are mainly induced by reducing the reabsorption of bile acids at the terminal ileum for enterohepatic recycling.[28] Intestinal absorption of bile salts is mediated significantly by the ASBT in brush-border membranes and IBABP in epithelial cells of the intestinal villus. In the present study, the mRNA levels of ASBT and IBABP were up-regulated by cellulose. As a result, the bile acids, which return to the liver via the enterohepatic circulation, were increased. Bile acids in the feces were also significantly increased in the groups fed with cellulose comparedwith the OVX-CON group. Therefore, the promotion of bile acid reabsorption in enterohepatic circulation may be attributed to the decreased amounts of cholesterol biosynthesis and

the increased surface of the small intestine. TBA excretion in the lumen increased with the cellulose particle size decreased (Table 5), while mRNA levels of hepatic CYP7A1 and FXR decreased. Because the absorption of cellulose was increased with the cellulose particle size decreased, the bile acids were absorption by the cellulose in the small intestine. Thus, an increased fecal excretion of TBAs through bile acids bound by cellulose can cause an increase in hepatic synthesis of bile acid, resulting in decreased plasma lipids. In this study, the dry weight, cellulose contents, lipids, and neutral sterols of the fecal excretion of the cellulose-fed OVX rats were significantly higher ($p < 0.05$) than those of the OVX-CON rats. As the particle size decreased, the crystallinity, water-holding capacity, swelling capacity and absorption of cellulose was increased. Then, the dry weight, cellulose contents, lipids, and neutral sterols in the feces exponentially increased as the cellulose particle size decreased, whereas the plasma lipid levels generally decreased. The previous study also reported that particle size reduction effectively enhances the cholesterol-lowering activities of insoluble carrot fiber and cellulose.[17]

The physicochemical properties of cellulose determine its physiological and metabolic effects upon consumption. As the particle size decreased, cellulose became more viscous, highly crystalline, and strongly absorbent.[34] In our previous studies, the specific area, water-holding capacity, swelling capacity, and oil-holding capacity of sweet potato cellulose increased with decreasing particle size.[26] The high water-holding capacity and swelling capacity of micro- and nanocellulose can increase digesta viscosity by decreasing the free water content in digesta, thus

decreasing nutrient absorption. The viscosity of fibers slows the digestion of lipid nutrients by preventing the bulk diffusion of food across the intestinal lumen. The ability of cellulose to absorb fat or oil is vital in nutrition, in which the ability to absorb or bind bile acids and increase their excretion is associated with plasma cholesterol reduction.[35] CYP7A1 is the first rate-limiting enzyme responsible for converting cholesterol to bile acids in the liver.[27] FXR, a bile-acid-activated nuclear receptor that plays a major role in the regulation of bile acid metabolism, and has been proposed to play a central role in the feedback repression of the CYP7A1 gene.[36,37] FXR is activated by bile acids.[38] Bile acid secretion induces biliary lipid secretion. On the other hand, the cellulose can bind bile acids. In our OVX rat model, the level of mRNA for CYP7A1 and FXR were significantly decreased in the groups fed with cellulose compared with the OVX-CON group. However, the level of mRNA for ASBT and IBABP were significantly up-regulated. Therefore, the increased amounts of bile acids and cholesterol in the small intestinal contents might due to increased biliary secretion and decreased reabsorption. The high absorption and viscosity of CNC resulted in increased binding with bile acids, which hindered the digestion of lipids in the intestine, impeded the reabsorption of bile acid in the ileum, and induced the excretion of more bile acids in the feces. In this study, the TBA levels in the fecal excretion and small intestine of rats fed with sweet potato cellulose diets were higher than those of the OVX-CON groups, and increased as the cellulose particle size decreased. In the OVX-CNC group, the TBA concentrations in the fecal excretion and small intestinal contents were significantly higher ($p < 0.05$) than those in the

OVX-MCC and OVX-OC groups. Moreover, the cellulose, lipid, bile acid, and neutral sterol contents in the fecal extract increased with decreasing cellulose particle size. These results indicate that the resistance to digestion and absorption of cellulose increased as the cellulose size decreased. Moreover, the specific surface area and absorption capacity of cellulose increased with decreasing particle size.

Ammonia produced from the amino acid degradation in the body is converted to urea in the mammalian liver, and hydrolyzed into ammonia by microbial urease . Urease-producing bacteria are important in both their nutritional and pathological aspects because they are involved in nitrogen recycling, and the resulting product, ammonia, can be harmful to animal health. A decreased level of ammonia formed in caecum or along the intestinal tract was beneficial for the improvement of intestinal health[39]. One study on cecal ammonia with fiber supplementation found, as we did, that fiber lowers ammonia concentrations in the cecum.[40] As the particle size decreased, the ammonia concentrations increased. However, no significant differences were found among the groups. In rats, the cecum is a site of vigorous microbial activity, wherein dietary fibers undergo fermentation to yield SCFAs. SCFAs, the end products of microbial fermentation, are important for a healthy colonic mucosa and can promote mineral absorption.[41] The beneficial effects of SCFAs on colon health have been well-documented. Specific SCFAs may reduce the risk of developing gastrointestinal disorders, cancer, and cardiovascular diseases.[42,43] Acetate is the principal SCFA in the colon. After absorption, acetate has been shown to promote cholesterol synthesis. However, propionate has been shown to inhibit cholesterol

synthesis. The rate and amount of SCFA production depend on the species and amounts of microflora present in the colon, as well as on the substrate source and gut transit time. In vitro fermentation experiments showed that wheat bran with small particle sizes increase SCFA production than wheat bran with large particle sizes.[18] In the present study, the cellulose-fed OVX rats exhibited increased SCFA concentrations. In the OVX-MCC group, the total SCFA, acetic acid, propionic acid, isobutyric acid, and n-butyric acid concentrations in the cecal content were higher than those in the OVX-OC and OVX-CNC groups. Micro- and nanotechnology can effectively break the fiber matrix and pores, thus explaining the ready fermentation of MCC compared with that of OC. However, given the higher crystallinity of CNC, it is not fermented in the small intestine. Thus, the SCFA concentration in the CNC-fed rats decreased than that in the MCC-fed rats. These results indicate that all cecal constituents, including cecal bacteria, may be involved in the improvement of cholesterol metabolism. However, cecal bacteria were not included in this study. Thus, the relationship between cecal constituents and cholesterol metabolism must be further elucidated in the future.

Sweet potato cellulose exhibited cholesterol-lowering effects and improved the cecal condition in OVX rats. This hypolipidemic effect of cellulose became more obvious as its particle size decreased. The mRNA levels of CYP7A1, FXR, which are the genes related to bile acid metabolism, and HMG-CoA reductase in liver were decreased as the cellulose particle size decreased, but those of ASBT and IBABP were increased. The amount of bile acids in the small intestinal contents, as well as the

excretion of bile acids and neutral steroids in the feces, increased with decreasing cellulose size. These results indicate that smaller cellulose particle sizes were more effective in preventing ovarian hormone deficiency-induced hyperlipidemia and in improving intestinal health.

Abbreviations

SPR	sweet potato residue
CON,	control
OC	ordinary cellulose
MCC	microcrystalline cellulose
CNC	cellulose nanocrystal
OVX	total cholesterol
TC	total cholesterol
TG	triglyceride
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
TBA	total bile acid
SCFA	short-chain fatty acid
CYP7A1	cholesterol 7 α-hydroxylase
FXR	farnesoid X receptor
HMG-CoAR	3-hydroxy-3-methylglutaryl coenzyme A reductase
ASBT	apical sodium-dependent bile acid transporter
IBABP	ileum bile acid binding protein

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Table 1

Composition of experimental diets (g kg^{-1} diet)

Ingredient	Sham	OVX			
		Control	OC	MCC	CNC
Corn starch	549.5	549.5	449.5	449.5	449.5
Casein	200	200	200	200	200
Soy bean oil	100	100	100	100	100
Sucrose	100	100	100	100	100
Mineral mixture[a]	35	35	35	35	35
Vitamin mixture[b]	10	10	10	10	10
L-Cystine	3	3	3	3	3
Choline chloride	2.5	2.5	2.5	2.5	2.5
OC	-	-	100		
MCC	-	-		100	
CNC	-	-			100

[a] AIN-76 mineral mixture (% mixture).

[b] AIN-76 vitamin mixture (% mixture).

Table 2

Primer sequence and product size

Gene	Primer sequence		Product size(bp)
	Sense	Antisense	
HMG-CoAR	GACCAACCTTCTACCTCAGCAAG	ACAACTCACCAGCCATCACAGT	117
FXR	GCTAAGGAAGTGCAGAGAGATGG	ATAGCTTGGTCGTGGAGGTCACT	167
CYP7A1	GAGGGATTGAAGCACAAGAACC	ATGCCCAGAGAATAGCGAGGT	139
ASBT	GTGACATGGACCTCAGTGTTAGC	GTAGGGGATCACAATCGTTCCT	125
IBABP	CAGACTTCCCCAACTATCACCAG	TCAAGCCACCCTCTTGCTTAC	110

Table 3

Effects of cellulose particle size on serum lipids in ovariectomized rats

	Sham	OVX			
		OVX-CON	OVX-OC	OVX-MCC	OVX-CNC
TG (mmol/L)	0.29±0.02*	0.40±0.07^{a}	0.34±0.03^{b}	0.32±0.02^{bc}	0.31±0.01^{bc}
TC (mmol/L))	1.30±0.15*	1.73±0.14^{a}	1.75±0.09^{a}	1.66±0.09^{ab}	1.56±0.21^{b}
HDL-C (mmol/L)	0.42±0.06	0.51±0.06	0.50±0.05	0.48±0.06	0.48±0.08
LDL-C (mmol/L)	0.13±0.01*	0.17±0.03^{a}	0.16±0.02^{a}	0.15±0.02^{ab}	0.14±0.01^{b}
non-HDL-C(mmol/L)	0.88±0.10*	1.22±0.11^{a}	1.24±0.07^{a}	1.19±0.09^{ab}	1.08±0.16^{b}
AI	2.10±0.19*	2.40±0.04	2.49±0.31	2.54±0.43	2.27±0.32

All data are expressed as means ± SD, n=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. $^{a, b, c}$ Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

Table 4

Effects of cellulose particle size on liver lipids in ovariectomized rats

	Sham	OVX			
		OVX-CON	OVX-OC	OVX-MCC	OVX-CNC
Liver weight (g)	6.12±0.57	6.24±1.10	6.21±0.81	6.36±0.92	6.16±0.79
Liver total lipids (mg/g liver)	51.91±4.94*	66.74±18.93[a]	50.07±7.28[b]	54.56±9.39[b]	52.14±5.40[b]
TC (mmol/g liver)	0.14±0.07*	0.23±0.09	0.21±0.08	0.20±0.07	0.20±0.07
TG (mmol/g liver)	0.48±0.12*	0.67±0.14[a]	0.51±0.13[b]	0.50±0.12[b]	0.49±0.12[b]

All data are expressed as means ± SD, n=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. [a, b] Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

Table 5

Effects of cellulose particle size on fecal excretion, small intestine contents, cecum and cecum contents in ovariectomized rats

	Sham	OVX			
		OVX-CON	OVX -OC	OVX-MCC	OVX-CNC
Fecal excretion					
Dry weight (g/4d)	1.26±0.76	1.26±0.43^{a}	4.23±0.79^{b}	4.83±1.00^{bc}	5.72±1.70^{c}
Cellulose content (g/100g)	9.01±0.75	10.38±1.07^{a}	37.89±1.58^{b}	43.58±0.97^{bc}	52.61±1.69^{c}
Total lipids (mg/d)	20.38±8.13	30.74±8.28^{a}	59.82±7.54^{b}	64.67±17.41^{b}	81.31±11.62^{c}
Total bile acids(μmol/d)	40.99±24.91	23.70±5.41^{a}	89.76±25.45^{b}	97.23±55.88^{b}	160.27±69.04^{c}
Neutral sterols (μmol /d)	92.86±10.50*	70.33±10.21^{a}	93.00±10.42^{b}	105.16±6.20^{c}	123.59±8.80^{d}
coprostanol	45.72±8.06*	37.45±9.21^{a}	46.48±4.71^{b}	51.58±7.24^{b}	60.72±4.78^{c}
cholesterols	47.14±7.13*	32.87±6.01^{a}	46.52±11.39^{b}	53.58±1.77^{b}	62.88±8.39^{c}
Small intestinal contents					
Dry weight(g)	0.29±0.18*	0.23±0.56^{a}	0.27±0.54^{ab}	0.28±0.64^{ab}	0.30±0.28^{b}
Total bile acids(μmol)	1.31±0.29*	0.98±0.28^{a}	1.19±0.24^{a}	1.49±0.11^{b}	1.58±0.16^{b}
Cecal tissue					
wet weight (g)	2.30±0.31*	2.07±0.33^{a}	2.96 ±0.75^{b}	3.09±1.03^{b}	3.25±0.62^{b}
Area (cm^2)	14.46±2.41	15.95±3.17^{a}	19.78±1.57^{ab}	19.15±5.26^{ab}	26.07±7.16^{b}
Cecum contents					
Wet weight (g)	2.01±0.20*	1.70±0.30^{a}	2.83±0.61^{c}	2.56±0.82^{bc}	2.73±0.61^{bc}
Moisture (g/100g)	80.80±2.62	82.66±1.13^{a}	84.63±1.17^{b}	85.25±0.56^{bc}	87.05±1.71^{c}
pH	8.40±0.54	8.49±0.50^{a}	7.98±0.33^{b}	8.18±0.37^{ab}	8.31±0.27^{ab}
Free ammonia （μg/g）	71.53±14.06*	149.19±21.4^{a}	41.31±11.00^{b}	43.81±14.41^{b}	57.95±26.49^{b}

All data are expressed as means ± SD, n=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. $^{a, b, c}$ Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

Table 6

Effects of particle size of cellulose on the SCFAs of cecum content in ovariectomized rats.

	Sham	OVX			
		OVX-CON	OVX-OC	OVX-MCC	OVX-CNC
Acetic acid (μmol/g)	12.84±0.36*	12.10±0.29[a]	12.64 ±0.75[ab]	12.81±0.25[b]	12.50±0.55[ab]
Propionic acid (μmol/g)	3.65±0.22*	3.33±0.18[a]	3.73±0.24[b]	3.82±0.23[b]	3.76±0.20[b]
Isobutyric acid (μmol/g)	4.89±0.09*	4.80±0.07[a]	4.87±0.77[a]	4.91±0.04[b]	4.90±0.08[b]
n-butyric acid (μmol/g)	4.16±0.26	4.01±0.08[a]	4.30±0.41[a]	4.42±0.35[b]	4.29±0.28[a]
Total SCFA (μmol/g)	25.54±0.85*	24.24±0.18[a]	25.53±1.47[b]	25.96±1.26[b]	25.44±1.09[b]

All data are expressed as means ± SD, n=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. [a, b] Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

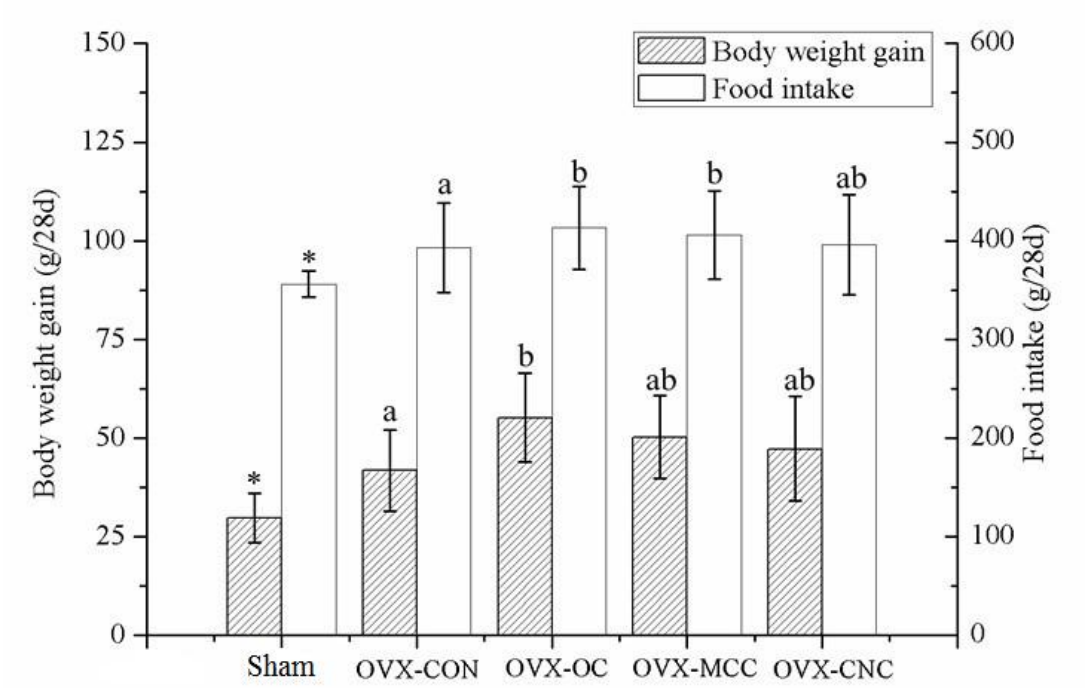

Fig. 1 Effects of particle size of cellulose on body weight gain and food intake in ovariectomized rats. All data are expressed as means ± SD, n=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. [a, b]Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

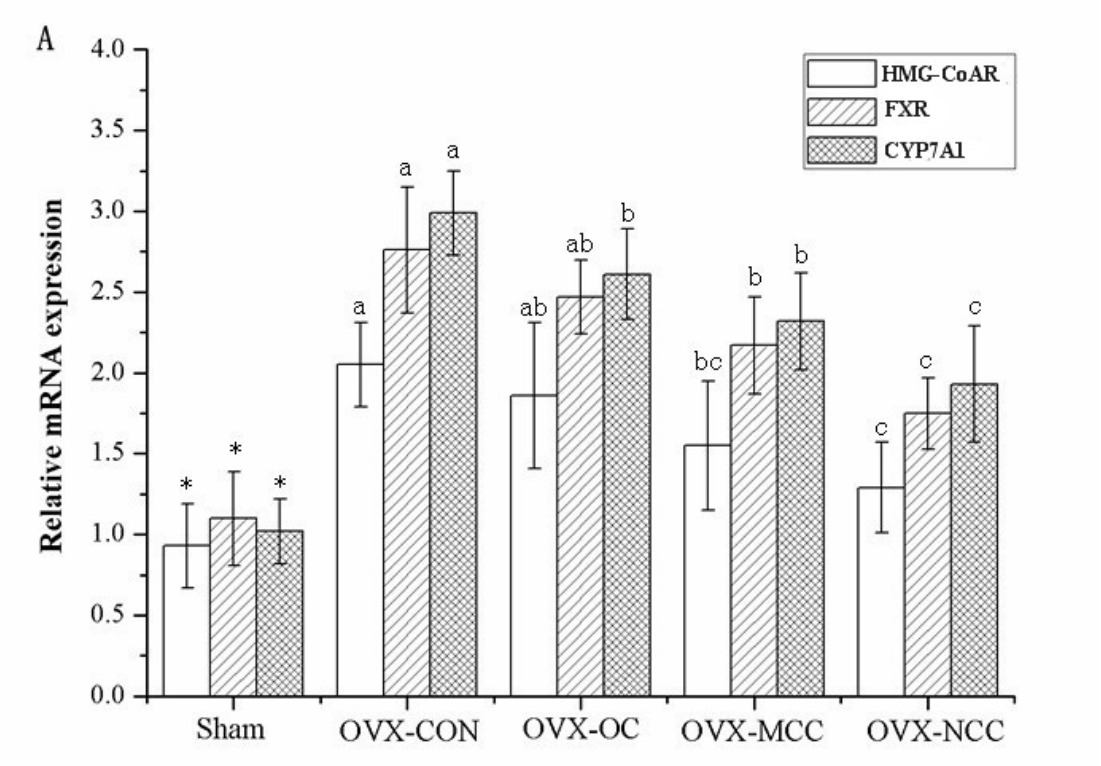

Fig.2 Effects of cellulose particle size on the (A) hepatic and (B) ileac genes expression. All data are expressed as means ± SD, *n*=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. [a, b, c] Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).

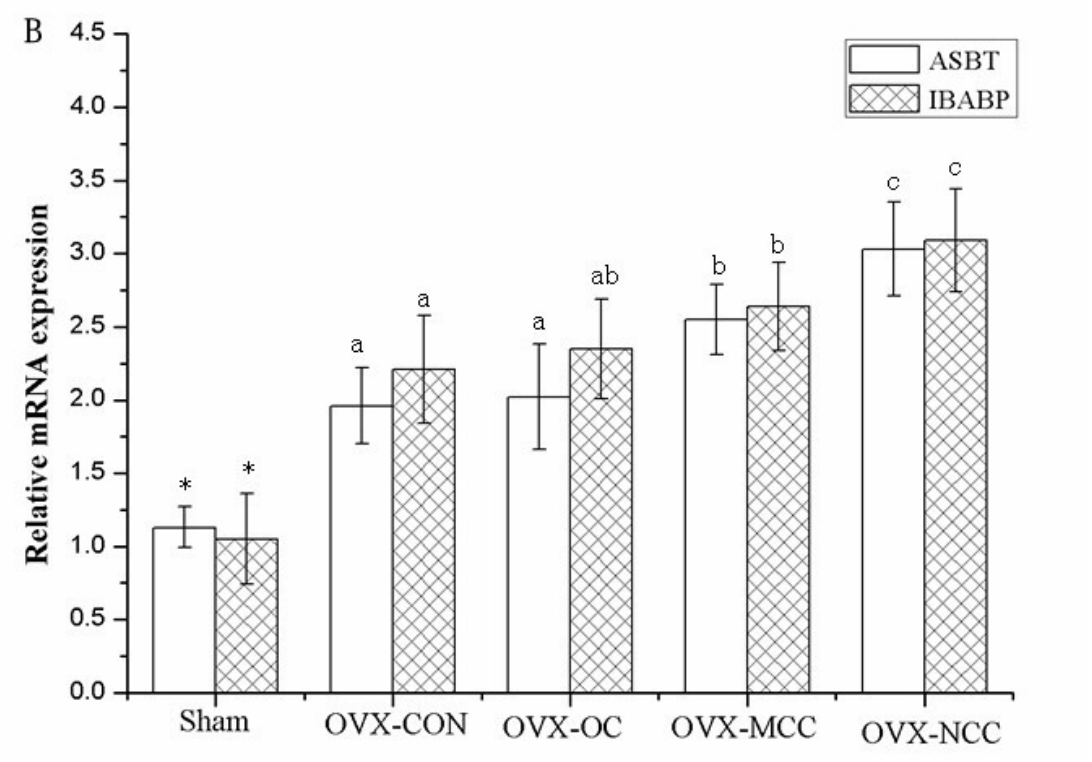

Fig.2 Effects of cellulose particle size on the (A) hepatic and (B) ileac genes expression. All data are expressed as means ± SD, *n*=8. * Means are significantly different ($P < 0.05$) than the means in the OVX-CON group. [a, b, c] Means with different superscript letters are significantly different among the ovariectomized rats ($P < 0.05$).