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Interactions of Lipid Metabolism and Intestinal Physiology with *Tremella fuciformis* Berk Edible Mushroom in Rats Fed a High-Cholesterol Diet with or without Nebacitin

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Male adult Wistar rats were randomly divided into six groups in a 2 × 3 factorial design and fed diets containing different levels of *Tremella fuciformis* Berk (TFB) dietary fiber (0, 5, or 10%) and 1 g of cholesterol/100 g of diet with or without 0.7% Nebacitin for 4 weeks. TFB contained 6.2% soluble dietary fiber and 57.3% insoluble dietary fiber. The results showed that the serum LDL-cholesterol, hepatic total cholesterol, and triglyceride levels were significantly decreased ($P < 0.05$) in the rats fed diets with TFB content with or without Nebacitin. However, the serum total cholesterol, VLDL-cholesterol, and triglyceride levels were significantly decreased ($P < 0.05$) by Nebacitin. In feces, the presence of TFB (T5, T10, AT5, and AT10) in the diet significantly increased the total neutral steroids and bile acid excretions and undigested fiber concentrations as compared to T0 or AT0. In the small intestine, the Nebacitin diets increased the weights of both cecum and colon−rectum contents and lowered short-chain fatty acid (SCFA) concentrations of serum and cecal contents more than no Nebacitin diets did. It was suggested that the hypocholesterolemic effect of TFB dietary fiber may be mediated by the increase in fecal neutral steroids and total bile acids excretion and the increase in SCFA productions. The TFB edible mushroom dietary supplement altered the intestinal physiology of the rats.

KEYWORDS: Intestinal physiology; lipid metabolism; short-chain fatty acids (SCFA); *Tremella fuciformis* Berk

INTRODUCTION

From ancient times, edible mushrooms have been widely used in Chinese cuisine and are known for their pharmaceutical effects in Chinese herbal medicine (*1*). The Heterobasidiae edible mushrooms, a subclass of Basidiomycetes, the fruiting bodies of *Tremella fuciformis* Berk (TFB), namely White-jelly-leaf, are the most popular. In our laboratory, the TFB total dietary fiber content was found to be about 63.5%, which made TFB a very high dietary fiber resource.

Dietary fiber is an important factor that can affect plasma lipids so as to lower the risk of diseases (*2*). Increased blood lipid and serum cholesterol concentrations contribute to the etiology of cardiovascular diseases (*3*). McIntyre et al. (*4*) demonstrated that different fibers have different regional effects on fermentation-related indexes and on tumor formation in rat models, with insoluble non-starch polysaccharides showing an inhibitory effect on tumorigenesis. Non-starch polysaccharide fermentations by the colonic microflora produced short-chain fatty acids (SCFA), including acetate, propionate, and butyrate, which played an important role in maintaining the health and integrity of the colonic epithelium (*5*). Chen et al. (*6*) proposed that the hypochloesterolemic effects of propionate produced by the colonic microflora might be related to altering rat hepatic cholesterol synthesis. It had been estimated that SCFA absorption from the fiber fermentations might provide 5−30% of the daily energy requirements (*7*, *8*). Furthermore, insoluble non-starch polysaccharides increase the luminal bulk contents, which will dilute dietary toxins and carcinogens, increase the transit of digesta through the colon, and, in turn, reduce the contact time of harmful compounds with the colonic mucosa.

Dietary fiber and related compounds such as oligosaccharides (*9*) and resistant starches (*10*, *11*) produce SCFA in rats. Govers et al. (*12*) reported that the combined consumptions of resistant starch and insoluble non-starch polysaccharides contributed to the dietary modulation of colon cancer risk. Very little work has been conducted on the investigation of the effects of TFB edible mushroom dietary fiber levels on rats in altering lipid metabolism and intestinal physiology with or without addition of antibiotics (the former will inhibit colonic microflora fermentations). In this study, we fed rats three levels of TFB edible mushroom diets containing a higher cholesterol level (1 g/100 g diet), with or without Nebacitin (bacitracin and

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Table 1. Composition of *Tremella fuciformis* Berk[a]

moisture	11.63 ± 0.05
ash	5.63 ± 0.05
crude fat	1.34 ± 0.02
crude protein[b]	8.06 ± 0.32
dietary fiber	
total	63.55 ± 0.78
soluble	6.24 ± 0.08
insoluble	57.30 ± 0.09
nitrogen-free extract[c]	9.79 ± 0.45

[a] All values are means ± SEM (n = 3), in units of grams per 100 g. [b] Crude protein was calculated as %N × 4.38 (Crisan and Sand, 1978). [c] Calculated as the differences between 100 and the sums of moisture, protein, fat, ash, and total dietary fiber contents.

Table 2. Composition of the Test Diet[a]

	No ANTB			ANTB		
ingredient	T0	T5	T10	AT0	AT5	AT10
corn starch[b]	638	568	499	638	568	499
casein[b]	200	191	182	200	191	182
soybean oil[b]	100	100	100	100	100	100
mineral mixture[b,c]	35	35	35	35	35	35
vitamin mixture[b,c]	10	10	10	10	10	10
Tremella fuciformis Berk		79	157		79	157
DL-methionine[b]	3	3	3	3	3	3
choline bitartrate[b]	2	2	2	2	2	2
cholic acid[b]	2	2	2	2	2	2
cholesterol	1	1	1	1	1	1
Nebacitin[d]				+	+	+

[a] Diet abbreviatons: No ANTB, diet without Nebacitin; ANTB, diet with Nebacitin; T0, dietary fiber-free diet; T5, 5% TFB dietary fiber diet; T10, 10% TFB dietary fiber diet; AT0, dietary fiber-free diet + 0.7% Nebacitin; AT5, 5% TFB dietary fiber diet + 0.7% Nebacitin; AT10, 10% TFB dietary fiber diet + 0.7% Nebacitin. All values are given in units of grams per kilogram of diet. [b] Cornstarch was purchased from Roquette Freres Lestrem USP (Lestren, France). Soybean oil was procured from Tung Yi Co. (Taipei, Taiwan). Casein, AIN-76 mineral mixture, and AIN-76 vitamin mixture were procured from ICN Biochemicals (Costa Mesa, CA). DL-Methionine, choline bitartrate, cholic acid, cholesterol, neomycin sulfate, and bacitracin were procured from Sigma Chemical Co. (St. Louis, MO). *Tremella fuciformis* Berk (Maoshuo No. 1) was provided by the Taiwan Agricultural Research Institute. [c] AIN (Bieri et al.1977, 1980). [d] Nebacitin: bacitracin and neomycin sulfate (2:1, w/w).

neomycin sulfate, 2:1, w/w), to investigate the effects of TFB on the following parameters: cholesterol concentration in serum and liver, small intestine length, weights of mucosa, colon−rectum, and cecum, SCFA concentrations in serum and cecal contents, and fecal moisture in rats.

MATERIALS AND METHODS

Animals and Feeding. Male Wistar rats (Animal Center of Taiwan University Medical College, Taipei, Taiwan) weighing about 260 g each were housed individually in wire-bottomed stainless steel cages in a temperature- and humidity-controlled room (at 22 °C) with a 12-h light/dark cycle, with free access to food and water. All animal experimental procedures followed the published guidelines (*13*). The rats were then divided randomly into six groups. Six semipurified experimental diets were designed, differing only in the amount of TFB and with or without Nebacitin. The approximate compositions of TFB are shown in **Table 1**. The six treatment group diets were divided on the basis of three different levels of TFB contents, as shown in **Table 2**. Each treatment group contained seven rats. The groups are defined as follows. For Nebacitin-free diet (No ANTB): (1) T0, dietary fiber-free diet; (2) T5, 5% TFB dietary fiber diet; and (3) T10, 10% TFB dietary fiber diet. For Nebacitin-containing diet (ANTB): **(**4**)** AT0, dietary fiber-free diet + 0.7% Nebacitin; **(**5**)** AT5, 5% TFB dietary fiber diet + 0.7% Nebacitin; and **(**6**)** AT10, 10% TFB dietary fiber diet + 0.7% Nebacitin. The *Tremella fuciformis* Berk (TFB) (Maoshuo No. 1), in dehydrated form, was provided by the Taiwan Agricultural Research Institute. After being cleaned with water, TFB samples were cooked in boiling water [water/TFB (w/w) = 1:1] and dried under 60 °C hot air for 2 h. The contents of all three diets were then ground and sifted using a 0.42-mm-diameter mesh analytical sifter. The TFB flour was treated with α-amylase, protease, and amyloglucosidase in vitro to mimic the digestion of TFB in the intestine of the rats. The residues were then loaded onto aluminum studs and coated with gold for 3 min at 8 mA under a pressure of 13.3 Pa. The samples with or without enzyme treatments were scanned by scanning electron microscopy and examined using a Hitachi model S-2400 SEM (Tokyo, Japan).

Analytical Methods. The contents of the diets were homogenized, put in plastic bags, tightly sealed, and refrigerated at 4 °C. Each group of rats (n = 7/group) was fed for 4 weeks. During the 4-wk feeding periods, feces were collected once every day in the last 2 wk and stored at −20 °C until analysis. At the end of the experimental periods and after 18 h of fasting, the rats were anesthetized with 1 g/L sodium pentobarbital and dissected. Blood was collected from the abdominal aorta, incubated at room temperature for 45 min, and centrifuged at 4000g for 15 min. The serum was then stored in a freezer at −70 °C. The small intestine, cecum, and colon−rectum were excised, rinsed in 9 g/L NaCl solution, and weighed (using filter paper to remove water from the organs). The length of the small intestine was measured by hanging with a 2 g weight. The mucosal tissue was removed from the small intestine and weighed. Moisture, ash, crude fat, and crude protein contents of TFB and fecal moisture content were analyzed according to the AOAC method (*14*). Total dietary fiber, soluble dietary and insoluble dietary fiber, and undigested fecal fiber were analyzed according to the method of Prosky et al. (*15*). The serum triglyceride concentrations were determined according to the methods of Mcgowan et al. (*16*). The total serum cholesterol concentrations were determined using the Richmond method (*17*). For the determinations of HDL-cholesterol concentrations, phosphotungstic acid and magnesium were added to the serum, which resulted in VLDL and LDL precipitations. After centrifugation at 4000g for 15 min, the upper liquid, containing HDL, was measured using the Richmond method (*17*).

Heparin and sodium citrate were added to the serum, causing LDL to precipitate. The serum was then centrifuged for 15 min at 4000g. The upper liquid contained VLDL. The LDL-cholesterol concentration was calculated by subtracting the cholesterol content of the upper liquid (VLDL) from the total cholesterol level using the Richmond method (*17*).

The frozen liver was thawed, and lipids were extracted according to the method of Folch et al. (*18*) as follows. A 1.5-g liver tissue sample was cut and extracted with 20 mL of chloroform/methanol = 2:1 (v/v). After the addition of 4 mL of 0.5 g/L $CaCl_2$ (w/v) solution, the extracts was collected in a sample flask and stored. The liver lipid extracts were accurately measured into a glass tube with a screw top to determine the liver triglyceride content according to the method of Soloni (*19*). Triolein was used to plot a triglyceride standard curve. The liver extracts were pretreated for liver total cholesterol according to the method of Carlson and Goldfarb (*20*) and determined by the method of Richmond (*17*).

Fecal lipid was extracted according to the method of Carlson and Goldfarb (*20*). After concentration by vacumm, the Richmond enzymatic method (*17*) was used to determine the total fecal neutral steroid concentrations. The method of Mashige et al. (*21*) was used to determine the fecal bile acids.

The concentrations of SCFA were measured using a gas−liquid chromatograph using serum and cecal content samples after ethanolic extractions (*22*).

Statistical Analysis. Data of differences among treatment group means were assessed by two-way ANOVA (SAS Institute, Cary, NC). Group means were considered significantly different at $P < 0.05$, as determined by Duncan's new multiple range test.

RESULTS

Diets and SEM. The daily food intake during the experimental period did not differ among groups [19.3 g/(rat·d), pooled

Table 3. Serum and Liver Lipid Concentrations and Liver Relative Weight in Rats Fed Different Amounts of Dietary Fiber of *Tremella fuciformis* Berk Diets with or without Nebacitin[a]

	No ANTB			ANTB			pooled	ANOVA[c]		
diet[b]	T0	T5	T10	AT0	AT5	AT10	SEM	fiber	ANTB	fiber × ANTB
serum										
total cholesterol, mmol/L	3.66a	3.54a	2.68b	2.71b	2.69b	2.10c	0.38	ns	0.0260	ns
VLDL-cholesterol, mmol/L	0.95a	0.96a	0.56b	0.47b	0.65b	0.37c	0.14	ns	0.0009	ns
LDL-cholesterol, mmol/L	1.74a	1.46b	0.88c	1.39b	1.13bc	0.91c	0.16	0.0089	ns	ns
HDL-cholesterol, mmol/L	0.84a	0.84a	0.84a	0.75a	0.70a	0.65a	0.06	ns	ns	ns
triglyceride, mmol/L	0.68a	0.68a	0.57ab	0.43b	0.48b	0.38c	0.06	ns	0.0010	ns
liver										
relative weight,[d] g	4.29a	4.13ab	3.88c	3.99b	3.80c	3.58d	0.07	0.0001	0.0001	ns
total cholesterol, mmol/g	3.05a	2.59b	1.99c	2.87ab	2.54b	1.96c	0.13	0.0001	ns	ns
triglyceride, mmol/g	1.39a	1.07b	0.79c	1.26ab	1.17b	0.95c	0.10	0.0029	ns	ns

[a] Values are means ± SEM ($n = 7$). Within a row, values with different letters are significantly different ($P < 0.05$, Duncan's multiple range test). [b] Abbreviatons: No ANTB, diet without Nebacitin; ANTB, diet with Nebacitin; T0, dietary fiber-free diet; T5, 5% TFB dietary fiber diet; T10, 10% TFB dietary fiber diet; AT0, dietary fiber-free diet + 0.7% Nebacitin; AT5, 5% TFB dietary fiber diet + 0.7% Nebacitin; AT10, 10% TFB dietary fiber diet + 0.7% Nebacitin. [c] Results were analyzed by two-way ANOVA to determine the effect of fiber and the effect of antibiotics (ANTB). Differences of $P < 0.05$ were considered significant for main treatment effects and their interaction. ns, not significant ($P > 0.05$). [d] Liver relative weight: (liver weight/body weight) × 100.

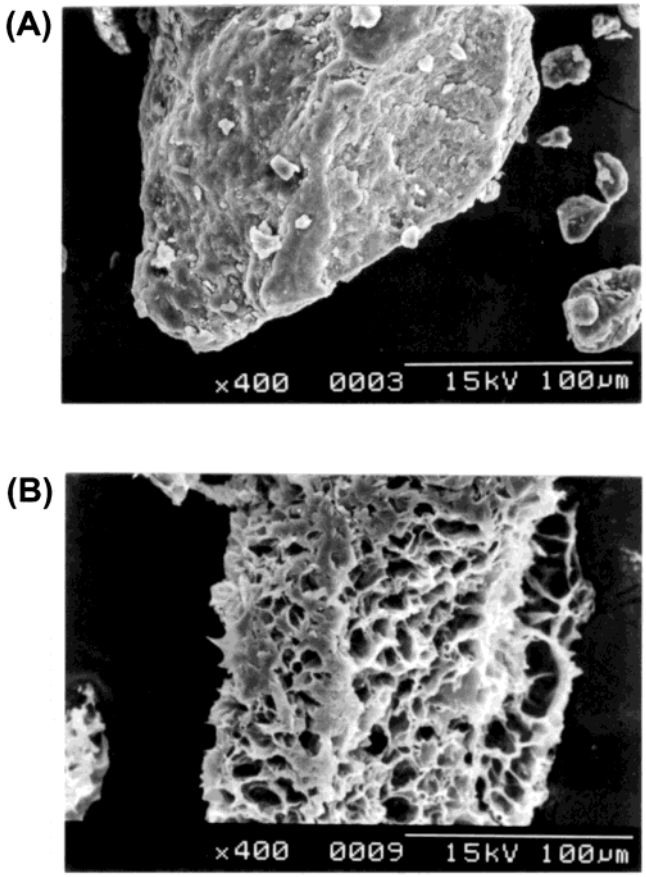

Figure 1. Scanning electron micrograph of *Tremella fuciformis* Berk flour (A) without or (B) with enzyme treatments (400× actual size).

SEM 0.26 g]. Weight gains among different experimental groups were also unaffected by the diets with different levels of TFB (151.3 g, pooled SEM 6 g). The TFB contained 6.2% soluble dietary fibers and 57.3% insoluble dietary fibers, which was a very high dietary fiber resource (**Table 1**). **Figure 1** shows SEM photographs (400×) of TFB without (**Figure 1A**) and with (**Figure 1B**) enzyme digestion. After enzyme digestion, the residue of TFB was sponge-like and porous, compared with the compacted and irregular size of untreated TFB residue. The diet content was a modified AIN-76 diet (*23*, *24*; **Table 2**) in which carbohydrates accounted for 63 g/100 g, protein for 20 g/100 g, and fat for 10 g/100 g. Casein and soybean oil were the major nutritional elements of each diet treatment.

Serum and Liver Lipid Concentrations. Serum LDL-cholesterol ($P = 0.0089$), hepatic total cholesterol ($P = 0.0001$), and triglyceride ($P = 0.0029$) levels were significantly decreased ($P < 0.05$) in those rats fed diets with TFB content with or without Nebacitin. Total cholesterol, VLDL-cholesterol, and triglyceride concentrations in serum and hepatic relative weight were decreased by the Nebacitin treatment as compared with the counterpart group ($P < 0.05$), and the group fed the AT10 diet had lower relative liver weight than the other groups (**Table 3**).

Total Neutral Steroids and Bile Acid Excretions. In feces, the presence of TFB (T5, T10, AT5, and AT10) in the diet significantly increased the total neutral steroids and bile acid excretions and undigested fiber concentrations as compared to T0 and AT0. No significant differences in total fecal bile acid levels were observed between the AT5 and AT10 diets. In the small intestine, the ANTB diets increased the weights of both cecum and colon−rectum contents more than No ANTB diets did (**Table 4**). The length and mucosal weights of the small intestine and fecal moisture of rats ($P = 0.0591$, 0.0006, and 0.0377) increased with increasing dietary TFB levels in diets with or without Nebacitin (**Table 4**).

SCFA Concentrations. The SCFA concentrations of acetate and propionate in the serum increased with increasing dietary TFB levels ($P = 0.0084$ and 0.0017) and were significantly lower in rats that consumed the diet containing Nebacitin ($P = 0.0003$ and 0.0018) (**Table 5**). In the cecal content, acetate and propionate concentrations increased with increasing dietary TFB ($P = 0.0044$ and 0.0001) and were significantly lower in rats that consumed the diet containing Nebacitin ($P = 0.000$ and 0.0001) (**Table 5**). Butyrate was not found in serum and cecal contents of rats fed ANTB diets; however, butryate was increased in cecal contents with increasing dietary TFB levels in rats fed No ANTB diets.

DISCUSSIONS

In this study, we demonstrated that rat serum LDL-cholesterol and hepatic total cholesterol and triglyceride concentrations were reduced with increased dietary mushroom TFB levels as rats were fed higher cholesterol (1 g/100 g diet) diets. The TFB mushroom diet altered the intestinal physiology of the rats.

The mushroom, *Tremella fuciformis* Berk (TFB), contains 6.24% soluble dietary fiber and 57.3% insoluble dietary fiber.

Table 4. Length of Small Intestine, Mucosal Weight, Cecal Weight, Colon–Rectum Weight, Fecal Moisture, Fecal Undigested Fiber, Fecal Total Neutral Steroid, and Bile Acid Excretions in Rats Fed Different Amounts of Dietary Fiber of *Tremella fuciformis* Berk Diets with or without Nebacitin[a]

	No ANTB			ANTB			pooled	ANOVA[c]		
diet[b]	T0	T5	T10	AT0	AT5	AT10	SEM	fiber	ANTB	fiber × ANTB
small intestine										
length, cm	124.20b	127.92ab	135.77a	126.39ab	129.04ab	130.15ab	7.24	0.0591	ns	ns
mucosal weight, g	1.02c	1.93b	2.78a	1.27bc	1.70bc	1.71bc	0.22	0.0006	ns	0.0461
cecal weight, g	1.00d	2.21c	2.71b	2.08c	3.03b	3.58a	0.21	0.0001	0.0001	ns
colon–rectum weight, g	0.99c	1.15c	1.49ab	1.27b	1.56a	1.59a	0.08	0.0010	0.0020	ns
feces										
moisture, %	40.03c	45.34b	53.29a	38.90c	45.27b	49.28ab	3.59	0.0377	ns	ns
undigested fiber, g/100 g dm	7.12d	17.34c	25.80b	14.36c	29.98a	32.68a	0.39	0.0001	0.0001	0.0001
total neutral steroids, μmol/dm	109.07c	154.89b	183.59a	91.76c	140.20b	143.35b	10.62	0.0001	0.0012	ns
total bile acids, μmol/dm	15.55c	18.50c	25.39b	14.15c	30.97a	28.60a	2.93	0.0001	0.0201	0.0204

[a] Values are means ± SEM ($n = 7$). Within a row, values with different letters are significantly different ($P < 0.05$, Duncan's multiple range test). [b] Abbreviatons: No ANTB, diet without Nebacitin; ANTB, diet with Nebacitin; T0, dietary fiber-free diet; T5, 5% TFB dietary fiber diet; T10, 10% TFB dietary fiber diet; AT0, dietary fiber-free diet + 0.7% Nebacitin; AT5, 5% TFB dietary fiber diet + 0.7% Nebacitin; AT10, 10% TFB dietary fiber diet + 0.7% Nebacitin. [c] Results were analyzed by two-way ANOVA to determine the effect of fiber and the effect of antibiotics (ANTB). Differences of $P < 0.05$ were considered significant for main treatment effects and their interaction. ns, not significant ($P > 0.05$).

Table 5. Short-Chain Fatty Acid Concentration of Serum and Cecal Content in Rats Fed Different Amounts of Dietary Fiber of *Tremella fuciformis* Berk Diets with or without Nebacitin[a]

	No ANTB			ANTB			pooled	ANOVA[c]		
diet[b]	T0	T5	T10	AT0	AT5	AT10	SEM	fiber	ANTB	fiber × ANTB
serum										
acetate, μmol/L	146.02bc	177.57ab	194.82a	124.64c	130.74c	151.52bc	8.45	0.00841	0.0003	ns
propionate, μmol/L	14.43ab	15.65ab	23.14a	nd	13.52b	14.67ab	3.12	0.0017	0.0018	ns
butyrate, μmol/lL	3.45a	3.59a	5.77a	nd	nd	nd	0.80			
cecal content										
acetate, μmol/L	30.85ab	31.26ab	35.14a	2.99d	17.07c	25.62bc	2.74	0.0044	0.0001	ns
propionate, μmol/L	13.41b	15.18b	23.02a	1.12d	5.92c	6.15c	1.38	0.0001	0.0001	ns
butyrate, μmol/L	1.49b	1.73b	2.32a	nd	nd	nd	0.44			

[a] Values are means ± SEM ($n = 7$). Within a row, values with different letters are significantly different ($P < 0.05$, Duncan's multiple range test). nd, not detected. [b] Abbreviatons: No ANTB, diet without Nebacitin; ANTB, diet with Nebacitin; T0, dietary fiber-free diet; T5, 5% TFB dietary fiber diet; T10, 10% TFB dietary fiber diet; AT0, dietary fiber-free diet + 0.7% Nebacitin; AT5, 5% TFB dietary fiber diet + 0.7% Nebacitin; AT10, 10% TFB dietary fiber diet + 0.7% Nebacitin. [c] Results were analyzed by two-way ANOVA to determine the effect of fiber and the effect of antibiotics (ANTB). Differences of $P < 0.05$ were considered significant for main treatment effects and their interaction. ns, not significant ($P > 0.05$).

Insoluble dietary fiber accounted for 90.1% of the TFB total dietary fiber. The energy sources of the diet were decreased with increasing insoluble dietary fiber content. However, in this study, there was no significant difference in weight gain among the six treatment groups (data not show). The TFB used in this study was observed to be effective in lowering both serum total cholesterol and LDL-cholesterol concentrations (**Table 3**). Since none of the serum HDL-cholesterol concentrations were affected, the reductions in total serum cholesterol by the TFB diets were mainly attributable to lower LDL-cholesterol concentrations. The TFB diets also significantly lowered the liver total cholesterol concentrations (**Table 3**). The prevention of excess cholesterol accumulations in the liver and the significant lowering of the total serum cholesterol and LDL-cholesterol concentrations by TFB diets could be due to a reduction in cholesterol absorption. Consequently, the increase in fecal neutral steroid and bile acid level output found in the rats fed the mushroom diets was consistent with the above assumptions, with or without Nebacitin treatment (**Table 4**). Enhancing fecal bile acid excretion caused increased hepatic synthesis of bile acids and liver depletion of cholesterol in the rats, which results in a higher rate of cholesterol synthesis and reduced serum cholesterol concentrations (*26*). The bile acid excretions in the feces were increased by diets containing Nebacitin. This might be the result of inhibition of bacteria in the intestine and colon that convert glycine- and taurine-conjugated bile acids into free bile acids following fiber fermentation (*27*).

This mushroom was fermented in the large intestine to produce SCFA, including acetate, propionate, and butyrate, which reduced serum and liver cholesterol concentrations. This result was the same as the report (*11*) that the resistant rice starch was fermented by intestinal microfola to produce propionate. The diets containing Nebacitin (AT5 and AT10 diets) inhibited the intestinal microfola fermentations to produce SCFA compared to the T5 and T10 diets, respectively (**Table 5**), which was consistent with the higher undigested fiber contents in feces of rats fed ANTB diets (**Table 4**). Therefore, the higher unfermented dietary TFB in AT5 and AT10 diets (compared to T5 and T10 diets) might combine with bile acids (*28*) and result in lower serum total cholesterol concentrations (**Table 3**) and higher total bile acid excretions in feces (**Table 4**). Dietary propionate was reported to inhibit the synthesis of fatty acids in the liver, probably through competition with lactate (*6*), thereby lowering the triacylglycerol secretions in rats. Chen et al. (*6*) proposed that lowered plasma cholesterol concentrations were produced by inhibiting hepatic cholesterogenesis via propionate formed through large-bowel fermentation.

The successive 5% increased TFB in T5 and T10 diets compared to the T0 diets caused a total 33−58 μmol/L increase of acetate and propionate in serum. Because TFB dietary fiber could generate SCFA by microfola fermentations, Suzuki and Kajuu (*29*) pointed out that SCFA inhibited the synthesis of hepatic triglycerides and, therefore, reduced serum lipids. Nishina and Freedland (*30*) demonstrated that propionate could

inhibit the activity of pyruvate dehydrogenase in the liver and thus reduce the synthesis of fatty acids. Hara et al. (*31*) pointed out that a decrease in the hepatic cholesterol synthesis rate mainly contributed to the lowering of plasma cholesterol in rats on the SCFA diet.

The lengths and mucosal weights of the small intestine were larger in rats fed the T10 diets than other groups. This might be the results of SCFA production in the colon by the fermentation of dietary TFB. Friedel and Levine (*32*) reported that SCFA infusion into the colon produced significantly greater mucosal height and mucosal DNA. Koruda et al. (*33*) found that the fiber polysaccharides stimulated mucosal cell mitotic activity in the intestine. This might be the same phenomenon leading to higher cecum and colon−rectum weights of rats fed T5 and T10 diets compared to those fed T0 diets in terms of the higher SCFA production.

The TFB mushroom comprised high indigestible non-starch polysaccharides of $\beta(1\rightarrow3)$-glucans and glucuronoxylomannan (*25*, *34*). The viscous and gel-forming properties of soluble dietary fiber such as $\beta(1\rightarrow3)$-glucans could lower cholesterol absorption by inhibiting the formation of micelles in the rat small intestine (*35*, *36*). The anionic charged functional groups in glucuronoxylomannan found in the mushroom might also interfere with the absorptions of cholesterol from the digestive tract of the animal in a way similar to that in ion-exchange resins (*37*). From SEM observations, the mimic of intestinal tract digestion by enzyme treatments of TFB in vitro also showed porous and sponge-like structures (**Figure 1**), similar to the observation with ion-exchange resins (*37*). This might be the result of the higher fecal moisture content in rats fed the higher T10 diets as compared to the other groups (**Table 4**). However, different dietary fiber have different properties and structures as well as various physical and chemical properties.

In summary, the dose−response relationships in this rat model showed that TFB dietary fiber could reduce the serum LDL-cholesterol and hepatic cholesterol concentrations. The hypocholesterolemic effects of TFB dietary fiber might be mediated by the increase of fecal neutral steroids and total bile acid excretions and the increase of SCFA productions. The TFB mushroom diet altered the intestinal physiology of the rats.

ABBREVIATIONS USED

SCFA, short-chain fatty acids; SEM, scanning electron microscope; T0, dietary fiber-free diet; T5, 5% TFB dietary fiber diet; T10, 10% TFB dietary fiber diet; AT0, dietary fiber-free diet + Nebacitin; AT5, 5% TFB dietary fiber diet + Nebacitin; AT10, 10% TFB dietary fiber diet + Nebacitin; No ANTB, diet without Nebacitin; ANTB, diet with Nebacitin; TFB, *Tremella fuciformis* Berk.

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