Effects of Semi-purified Dietary Fibers Isolated from *Lagenaria siceraria*, *Raphanus sativus* and *Lentinus edodes* on Fecal Steroid Excretions in Rats

Yasuko SANNOUMARU,* Jun SHIMIZU, Kahoru NAKAMURA,¹
Takashi HAYAKAWA,² Toshichika TAKITA,
and Satoshi INNAMI**

Department of Nutrition, Faculty of Agriculture, Tokyo University
of Agriculture, Setagaya-ku, Tokyo 156, Japan

¹Department of Nutrition, Junior College of Agriculture, Tokyo University
of Agriculture, Setagaya-ku, Tokyo 156, Japan

²Department of Utilization of Biological Resources, Gifu University,
Gifu 501-11, Japan

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Summary Rats were fed diets, each of which contained 5% of the semi-purified dietary fiber (DF) preparations from three kinds of foods, continuously for a total of 7 weeks. The fat level was varied, and cholesterol (Chol) was added toward the end of the experimental period. After 7 weeks, decreases of serum and liver Chol concentrations were observed in the Shiitake (*Lentinus edodes*) group when compared to the cellulose (CP) group. In the absence of Chol, fecal excretions of several secondary bile acids and total bile acids were affected by the type of DF, showing an increase particularly in the Shiitake group. Total neutral steroid excretions were decreased in the test DF groups compared to the CP group at both fat levels, and were increased by Chol supplementation in all of the test DF groups. It was demonstrated that the effects of the three DF preparations on fecal steroid excretions somewhat differed depending on the dietary factors. In the absence of Chol, the lithocholic acid (LCA)/deoxycholic acid (DCA) ratio was significantly lowered in the test DF groups compared to the CP group by the fat level elevation, and was affected by the interaction of fiber with fat. This ratio responded more intensely to the dietary factors than the two other indices (composition ratios of fecal bile acids). However, this response was lost in the presence of Chol. These results suggest that further studies are warranted to examine if the intense response of the LCA/DCA ratio to the tested DF preparations and fat in the present study will also be shown to other DF

* Present address: The Skylark Food Science Institute, Chiba 261, Japan

** To whom correspondence should be addressed.

and what significance it has in evaluating the function of DF.

Key Words semi-purified dietary fibers, fecal steroid excretion, bile acids, neutral sterols, lithocholic acid/deoxycholic acid ratio

Since epidemiological studies by Reddy and Wynder (1,2) and Hill (3) demonstrated that there was a significant positive correlation between the fecal bile acid (BA) concentration and the incidence of colon cancer, BA metabolism in the intestine has been a matter of growing concern. A large number of subsequent animal experiments (4-6) and *in vitro* studies (7-9) suggest that cholesterol (Chol) or BA, or their metabolites, act as co-carcinogenic or co-mutagenic promoters. Not only the total excretion of BA but also the type of BA and the ratio of secondary BA to total BA have therefore been considered important. Subsequently, Wilpart and Roberfroid (9) and Owen et al. (10,11) hypothesized that the ratio of lithocholic acid (LCA) to deoxycholic acid (DCA) in feces is a more sensitive index for the risk of colon cancer.

The quality and quantity of lipids (12-14) and the type and quantity of dietary fibers (DF) (15-17) are known to be the dietary factors which modify fecal BA excretion. The quantity of dietary Chol is also a potent factor (18,19). The effects of DF on the composition of fecal BA differ depending on the type of DF and experimental conditions, and do not always show similar spectra. In recent years, attention has been directed toward the interaction of dietary fats and DF (20), leading to studies on their effects on BA metabolism (21,22). However, their results are not necessarily concordant because of the effect of the type of DF, and the data on this subject are therefore scarce.

In the present study, we selected the semipurified preparations extracted from three kinds of foods which are popular DF sources in Japan and have not yet fully been studied regarding variations of fecal steroid profiles. Then, we carried out experiments in succession in order to investigate what effects the increase of dietary fats would have on the response of fecal steroids in rats fed DF preparations with different hemicellulose composition and how the addition of Chol to the diet would modify the composition of fecal steroids under DF influence.

MATERIALS AND METHODS

Preparation and composition of DF. The DF samples used in the study were obtained from the following foods: 1) Kanpyo (dried white flower gourd, *Lagenaria siceraria* STANDLEY var. *hispida* HARA) (produce of Tochigi Prefecture, Suyama Co., Ltd.), 2) Kiriboshi daikon (Kiriboshi, dried Japanese radish strips, *Raphanus sativus* L.) (produce of Miyagi Prefecture, Yamaki Y Co., Ltd.) and 3) Shiitake, dried (a kind of fungus, *Lentinus edodes* (BERK) Sing.) (produce of Ohita Prefecture, Yamada Shoten Co., Ltd.). The samples were soaked in water for 30 min, squeezed lightly and cut into fine strips (hard tips of Shiitake stems were

removed). The cut samples were freeze-dried in a vacuum, powdered with a coffee mill and passed through a 30-mesh sieve. DF was extracted from the powdered samples by the modified AOAC enzymatic gravimetric method (23). The alcohol precipitates were filtered through a Buchner funnel, washed with alcohol and acetone, and air-dried. General components of the samples were routinely determined and the DF compositions were analyzed according to the Southgate method (24). The results are shown in Table 1, indicating semi-purified preparations. There were no marked differences in the DF content of roughly 85% among the three samples although there were some differences in hemicellulose fraction.

Animals, diet composition and rearing method. Male Sprague-Dawley strain rats aged 4 weeks (Clea Japan, Inc., Tokyo) were used in the study. They were housed individually in stainless steel cages. The animal room was maintained at $22 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, and a light/dark cycle of 12 h (lighting: 8:00–20:00). After rats were fed the AIN-76TM basal diet modified with lard instead of corn oil for one week, those without any abnormality in growth were divided into four groups of six rats each, and were given the experimental diets. The basal diet (control) was composed of 25% casein, 5% lard, 49.8% sucrose, 3.5% mineral mixture (AIN-76TM), 1.0% vitamin mixture (AIN-76TM), 0.2% choline bitartrate and 5% cellulose.

The DF preparations were added at the level of 5% to each experimental diet. Rats were fed the diets for a total of 7 weeks, with 5% lard in the first 3 weeks, 10% lard for the following 2 weeks and 10% lard and 0.5% of Chol in the last 2 weeks. The differences arising from alteration in the dietary ingredients in the experimental period (4th to 7th weeks) were adjusted with sucrose.

During the 7-week experimental period, feces were collected for the last 5 days in the 3rd, 5th and 7th weeks and weighed before and after vacuum freeze-drying to determine the water content. Feces were powdered and kept at -30°C until analysis. The animals were starved for 7 h from 6:00 to 13:00 on the last day of the experiment and sacrificed by cardiocentesis under pentobarbital anesthesia. The organs including the digestive tract were weighed, and serum was obtained. The liver was perfused with ice-cooled physiological saline injected under pressure from the portal vein to remove blood and weighed, and 0.5 g of the tissue was sampled for

Table 1. Composition of semi-purified dietary fiber samples (%).

	Kanpyo	Kiriboshi	Shiitake
Moisture	3.2	3.8	3.6
Protein	9.7	6.0	7.2
Ash	3.2	4.5	0.9
Total fiber	83.9	85.7	88.3
Water soluble hemicellulose	9.7	3.0	2.7
Water insoluble hemicellulose	22.6	29.3	36.9
Cellulose	50.0	51.1	45.9
Lignin	1.6	2.3	2.7

extracting lipids with a chloroform : methanol (2 : 1) mixture on the day of sacrifice.

Measurements of serum and liver lipids and fecal steroids. Serum total cholesterol (TC), triglycerides (TG) and phospholipids (PL) and liver TG and PL were determined using the kits available from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and liver TC by the Zak method (25).

Fecal BA and neutral sterols (NS) were measured by gas chromatography according to the method of Grundy et al. (26). Extracted fecal lipids were enzymatically deconjugated and saponified to separate them into BA and NS fractions. They were then subjected to methylation and trifluoroacetylation using an acetyl chloride : methanol (1 : 20) mixture and trifluoroacetic anhydride (TFA; Wako Pure Chemical) for use as analytical samples. The internal standard substances used for BA and NS analyses were respectively nor-deoxycholic acid (3,12-dihydroxy-nor-cholanic acid) synthesized by the method of Riegel et al. (27) and cholestanone (Sigma Chemical Co., MO, USA). A Shimadzu GC 12A (Shimadzu Co., Kyoto, Japan) attached with a flame ionization detector was used for gas chromatography. The analytical conditions were as follows: BA: column, 1.5% AN-600 on 100–120 mesh Gaschrom Q (1.6 m × 3 mm ID, Japan Chromato Industry, Tokyo); column temperature, 225°C; injector and detector temperature, 250°C; carrier gas, N₂ (flow rate, 65 ml/min); NS: column, 3% SP-2401 on 100–120 mesh Supelcoport (1.6 m × 2.6 mm ID, Supelco, PA, USA); column temperature, 220°C; injector and detector temperature, 250°C; carrier gas, N₂ (flow rate, 35 ml/min).

Statistical processing. Individual group comparisons were made using Duncan's new multiple range test ($p < 0.05$). Data were also analyzed by two-way ANOVA using the SAS computer package. A separate analysis was carried out to determine the significance of fiber, fat or fiber × fat (weeks 3 and 5), and fiber, Chol or fiber × Chol (weeks 5 and 7). This procedure was adopted to minimize the influence of aging as much as possible.

RESULTS

Body weight gain, food intake, liver weight, and serum and liver lipid concentrations

The results are shown in Table 2. Hardly any intergroup differences were observed in body weight gain, food intake and liver weight for the entire feeding period. Serum TC and PL concentrations were generally high in the Kanpyo group and low in the Kiriboshi and Shiitake groups when compared to the CP group. The values for the Kiriboshi and Shiitake groups were significantly lower than those in the Kanpyo group. Liver TC concentrations in the Shiitake group were significantly lower than those of the CP group as well as the other two DF groups.

Fecal weight and water content

The results are shown in Table 3. Compared to the CP group, the fecal wet and dry weights showed significant decreases in all the measurement weeks in all of

Table 2. Body weight gain, food intake, liver weight and serum and liver lipid concentrations in rats fed on the experimental diets for 7 weeks.¹

Items	Cellulose	Kanpyo	Kiriboshi	Shiitake
Body weight gain (g/day)	6.33±0.22	6.27±0.16	5.73±0.24	5.92±0.14
Food intake (g/day)	20.9±0.41	20.5±0.31	19.6±0.78	20.1±0.49
Liver (g)	18.8±0.98	19.7±0.92	17.1±0.92	17.9±0.74
Serum (mg/dl)				
Total cholesterol	129±11.9 ^{ab}	160±14.9 ^a	111±4.6 ^b	104±8.6 ^b
Triglycerides	347±78.7	443±57.8	288±37.8	306±40.8
Phospholipids	199±20.6 ^{ab}	215±13.3 ^a	168±8.8 ^b	168±12.5 ^b
Liver (mg/g)				
Total cholesterol	19.0±1.2 ^a	24.2±2.5 ^a	22.8±2.2 ^a	13.7±0.7 ^b
Triglycerides	64.9±4.8 ^a	70.0±4.7 ^{ab}	87.8±9.1 ^b	85.6±8.1 ^{ab}
Phospholipids	31.0±1.5 ^a	33.2±1.7 ^{ab}	36.9±1.0 ^b	31.8±1.4 ^a

¹ M±SEM (n=6): means in a same line with different superscript letter are significantly different ($p < 0.05$).

the DF groups, but the fecal water content showed a significant increase in all week in the Kanpyo and Shiitake groups. The fat level affected only the fecal dry weight while Chol addition affected the fecal weight as well as the fecal water content. Fat, however, affected neither these parameters nor the interaction of Chol addition and DF.

Fecal steroid excretions

Tables 4 and 5 show the changes in fecal BA excretions in rats fed the experimental diets. In the absence of Chol, the different types of DF produced differences in the excretions of BA per 1 g of feces excluding CA and ω -muricholic acid (ω -MCA), the excretions in the Shiitake group being particularly increased. The analysis of BA excretions per day in the absence of Chol revealed that DCA was strongly affected by the type of DF and the fat level while total BA showed the effect of the type of DF but not of the fat level. Chol addition significantly elevated the concentration of each BA as well as that of total BA and increased the excretion per day in all the groups. Intergroup differences were observed except in the LCA concentration. However, no interaction was observed between fiber and fat or fiber and Chol (excluding ω -MCA).

Table 6 shows the relation between DF and the ratios of BA. The secondary/total BA ratio was affected by fiber but more intensely by the fat level. Chol addition exerted no effect. There was no interaction between fiber and fat or between fiber and Chol. The CDCA/CA ratio was lowered in the DF groups compared to the CP group and with elevation in the fat level. Chol addition raised the ratio in the DF groups. The LCA/DCA ratio was also decreased in the DF groups compared to the CP group at either of the fat levels, and the decrease was also produced by elevation in the fat level. In contrast, Chol addition raised the

Table 3. Fecal wet weight and moisture content and its statistical analysis data.¹

Items	Week	Cellulose	Kanyo	Kiriboshi	Shiitake	Pooled SEM	Tow-way ANOVA (<i>p</i> value)			
							Fat 5% and 10% ²	Chol(−) and (+) ³		
Fecal wet weight (g/day)										
	3	1.60 ^{a1}	1.19 ^b	1.19 ^b	1.34 ^b	0.07	Fiber	0.0002	Fiber	0.0005
	5	1.96 ^a	1.77 ^{ac}	1.38 ^b	1.47 ^{bc}	0.07	Fat	NS	Chol	0.0062
	7	2.25 ^a	2.11 ^a	1.61 ^b	1.96 ^{ab}	0.08	Fiber×fat	NS	Fiber×Chol	NS
Fecal dry wieght (g/day)										
	3	1.38 ^a	0.87 ^b	0.96 ^b	0.93 ^b	0.03	Fiber	0.0001	Fiber	0.0001
	5	1.65 ^a	1.22 ^b	1.12 ^b	1.09 ^b	0.04	Fat	0.0001	Chol	NS
	7	1.74 ^a	1.39 ^b	1.24 ^b	1.28 ^b	0.04	Fiber×fat	NS	Fiber×Chol	NS
Fecal moisture (%)										
	3	13.3 ^a	26.5 ^b	19.2 ^c	29.6 ^b	0.77	Fiber	0.0001	Fiber	0.0001
	5	15.6 ^a	30.0 ^b	19.1 ^a	25.9 ^{ab}	1.07	Fat	NS	Chol	0.0037
	7	22.2 ^a	31.6 ^b	22.7 ^a	34.9 ^b	1.31	Fiber×fat	NS	Fiber×Chol	NS

¹ Means (*n* = 6) in a same line with different superscript letters are significantly different (*p* < 0.05).² Overall *p* values from two-way ANOVA for differences by fiber, fat or fiber × fat (data analysis of weeks 3 and 5).³ Overall *p* values from two-way ANOVA for differences by fiber, Chol or fiber × Chol (data analysis of weeks 5 and 7). NS, not significant; Chol, cholesterol.

Table 4. Effect of semi-purified dietary fibers on fecal bile acids and its statistical analysis data.¹

Bile acid	Week	mg/g dry feces			Two-way ANOVA (<i>p</i> value)		
		Cellulose	Kanpyo	Kiriboshi	Shitake	Pooled SEM	Fat 5% and 10% ² Chol(-) and (+) ³
Lithocholic acid	3	0.09 ^{ab1}	0.07 ^a	0.09 ^{ab}	0.18 ^b	0.01	Fiber 0.001 Fiber NS
	5	0.06 ^a	0.09 ^a	0.10 ^a	0.17 ^b	0.01	Fat NS Chol 0.0001
	7	0.37	0.45	0.55	0.96	0.09	Fiber × fat NS Fiber × Chol NS
Deoxycholic acid	3	0.11 ^a	0.31 ^a	0.38 ^{ab}	0.62 ^b	0.02	Fiber 0.0001 Fiber 0.0001
	5	0.22 ^a	0.51 ^b	0.67 ^{bc}	0.81 ^c	0.04	Fat 0.0001 Chol 0.0001
	7	0.79 ^a	1.17 ^{ab}	1.56 ^b	1.92 ^b	0.11	Fiber × fat NS Fiber × Chol NS
α -Muricholic acid	3	0.07 ^a	0.11 ^{ab}	0.10 ^{ab}	0.19 ^b	0.01	Fiber 0.001 Fiber 0.014
	5	0.05 ^a	0.06 ^a	0.09 ^a	0.16 ^b	0.01	Fat NS Chol 0.0001
	7	0.43	0.25	0.33	0.88	0.08	Fiber × fat NS Fiber × Chol NS
Hyodeoxy cholic acid	3	0.81	1.21	1.29	1.57	0.10	Fiber 0.0148 Fiber 0.0147
	5	0.52 ^a	1.07 ^{ab}	1.21 ^b	0.87 ^{ab}	0.10	Fat 0.0334 Chol 0.0004
	7	0.78 ^a	3.65 ^{ab}	4.35 ^b	2.22 ^{ab}	0.47	Fiber × fat NS Fiber × Chol NS
Cholic acid	3	0.06	0.05	0.03	0.04	0.01	Fiber NS Fiber 0.0128
	5	0.04	0.04	0.06	0.10	0.01	Fat NS Chol 0.0002
	7	0.21 ^{ab}	0.12 ^a	0.15 ^a	0.42 ^b	0.04	Fiber × fat NS Fiber × Chol NS
β -Muricholic acid	3	0.11 ^a	0.29 ^a	0.23 ^a	0.65 ^b	0.05	Fiber 0.0003 Fiber 0.0003
	5	0.06 ^a	0.10 ^{ab}	0.21 ^{bc}	0.28 ^c	0.02	Fat 0.0091 Chol 0.0001
	7	0.52 ^a	0.48 ^a	1.00 ^{ab}	1.65 ^b	0.11	Fiber × fat NS Fiber × Chol NS
ω -Muricholic acid	3	0.18	0.25	0.17	0.19	0.03	Fiber NS Fiber 0.0002
	5	0.11	0.14	0.13	0.27	0.05	Fat NS Chol 0.0001
	7	1.21	0.37	0.26	0.40	0.05	Fiber × fat NS Fiber × Chol 0.0001
Total bile acids	3	1.79 ^a	2.80 ^a	2.62 ^a	3.96 ^b	0.12	Fiber 0.0001 Fiber 0.0045
	5	1.33 ^a	2.27 ^b	2.65 ^b	2.88 ^b	0.13	Fat 0.0052 Chol 0.0001
	7	4.60 ^a	6.94 ^{ab}	8.63 ^b	8.86 ^b	0.57	Fiber × fat NS Fiber × Chol NS

¹ Means (*n* = 6) in a same line with different superscript letters are significantly different (*p* < 0.05).^{2,3} See footnote to Table 3.

Table 5. Effect of semi-purified dietary fibers on fecal bile acids and its statistical analysis data.¹

Bile acid	Week	Cellulose			Pooled SEM			Two-way ANOVA (<i>p</i> value)		
		Kanpyo	Kiriboshi	Shitake	mg/g dry	Shitake	Pooled SEM	Fat 5% and 10% ²	Chol(-) and (+) ³	
Lithocholic acid	3	0.13 ^{ab1}	0.06 ^a	0.09 ^a	0.18 ^b	0.01	0.01	Fiber	Fiber	NS
	5	0.11 ^a	0.11 ^a	0.11 ^a	0.19 ^b	0.01	0.01	Fat	Chol	0.0001
	7	0.64	0.61	0.67	1.11	0.09	0.09	Fiber × fat	Fiber × Chol	NS
Deoxycholic acid	3	0.16 ^a	0.27 ^{ab}	0.37 ^b	0.60 ^c	0.03	0.03	Fiber	Fiber	0.0015
	5	0.36 ^a	0.62 ^{ab}	0.75 ^b	0.88 ^b	0.05	0.05	Fat	Chol	0.0001
	7	1.36 ^a	1.59 ^{ab}	1.94 ^{ab}	2.31 ^b	0.12	0.12	Fiber × fat	Fiber × Chol	NS
α -Muricholic acid	3	0.09 ^a	0.10 ^a	0.10 ^a	0.17 ^b	0.01	0.01	Fiber	Fiber	0.0078
	5	0.07 ^a	0.07 ^a	0.10 ^{ab}	0.17 ^b	0.01	0.01	Fat	Chol	0.0001
	7	0.74 ^{ab}	0.36 ^a	0.41 ^a	1.06 ^b	0.08	0.08	Fiber × fat	Fiber × Chol	NS
Hyodeoxy cholic acid	3	1.12	1.02	1.20	1.50	0.11	0.11	Fiber	Fiber	0.0238
	5	0.84	1.28	1.20	0.95	0.09	0.09	Fat	Chol	0.0001
	7	1.36 ^a	4.89 ^b	5.15 ^b	2.67 ^{ab}	0.53	0.53	Fiber × fat	Fiber × Chol	NS
Cholic acid	3	0.09	0.04	0.03	0.03	0.01	0.01	Fiber	Fiber	NS
	5	0.07	0.04	0.07	0.10	0.01	0.01	Fat	Chol	0.0001
	7	0.37	0.17	0.19	0.51	0.05	0.05	Fiber × fat	Fiber × Chol	NS
β -Muricholic acid	3	0.15 ^a	0.26 ^a	0.22 ^a	0.56 ^b	0.04	0.04	Fiber	Fiber	0.0071
	5	0.09 ^a	0.12 ^{ab}	0.27 ^{ab}	0.31 ^b	0.03	0.03	Fat	Chol	0.0001
	7	0.88 ^a	0.71 ^a	1.28 ^{ab}	2.17 ^b	0.17	0.17	Fiber × fat	Fiber × Chol	NS
ω -Muricholic acid	3	0.25	0.23	0.17	0.20	0.04	0.04	Fiber	Fiber	0.0001
	5	0.19	0.18	0.16	0.28	0.06	0.06	Fat	Chol	0.0001
	7	2.11 ^a	0.52 ^b	0.33 ^b	0.49 ^b	0.08	0.08	Fiber × fat	Fiber × Chol	0.0001
Total bile acids	3	2.48 ^a	2.43 ^a	2.48 ^a	3.72 ^b	0.15	0.15	Fiber	Fiber	NS
	5	2.16 ^a	2.73 ^{ab}	2.85 ^{ab}	3.12 ^b	0.14	0.14	Fat	Chol	0.0001
	7	7.96	9.46	10.5	10.8	0.59	0.59	Fiber × fat	Fiber × Chol	NS

¹ Means (*n* = 6) in a same line with different superscript letters are significantly different (*p* < 0.05).^{2,3} See footnote to Table 3.

Table 6. Effect of semi-purified dietary fibers on fecal bile acid parameters and its statistical analysis data.¹

Parameter	Week	Cellulose	Kanyo	Kiriboshi	Shiitake	Pooled SEM	Two-way ANOVA (<i>p</i> value)		
							Fat 5% and 10% ²	Chol(-) and (+) ³	
Secondary/total bile acids	3	0.67	0.65	0.73	0.65	0.02	Fiber	0.0364	Fiber
	5	0.69 ^{a1}	0.79 ^b	0.79 ^b	0.74 ^{ab}	0.01	Fat	0.0005	Chol
	7	0.69	0.79	0.75	0.60	0.03	Fiber × fat	NS	Fiber × Chol
CDCA/CA ⁴	3	7.68 ^a	5.71 ^{ab}	4.88 ^b	4.19 ^b	0.34	Fiber	0.0004	Fiber
	5	3.29 ^a	2.85 ^{ab}	2.45 ^{ab}	1.98 ^b	0.16	Fat	0.0001	Chol
	7	3.30	4.04	4.13	2.74	0.26	Fiber × fat	NS	Fiber × Chol
LCA/DCA ⁴	3	0.86 ^a	0.24 ^b	0.27 ^b	0.28 ^b	0.06	Fiber	0.0001	Fiber
	5	0.30 ^a	0.19 ^b	0.16 ^b	0.21 ^b	0.02	Fat	0.0013	Chol
	7	0.46	0.38	0.37	0.50	0.03	Fiber × fat	0.0096	Fiber × Chol

¹ Means (*n* = 6) in a same line with different superscript letters are significantly different (*p* < 0.05).^{2,3} See footnote to Table 3.⁴ CDCA, chenodeoxycholic acid and its metabolites; CA, cholic acid and its metabolites; LCA, lithocholic acid; DCA, deoxycholic acid.

Table 7. Effect of semi-purified dietary fibers on fecal neutral sterols and its statistical analysis data.¹

Neutral sterols	Week	mg/day				Two-way ANOVA (<i>p</i> value)			
		Cellulose	Kanpyo	Kiriboshi	Shiitake	Pooled SEM	Fat 5% and 10% ²		
Coprostanol	3	4.76	3.78	3.98	3.19	0.40	Fiber	NS	Fiber
	5	9.89 ^a	4.89 ^b	3.62 ^b	3.35 ^b	0.54	Fat	NS	Chol
	7	17.5	15.7	15.3	15.2	1.78	Fiber × fat	NS	Fiber × Chol
Cholesterol	3	2.93 ^{ab}	3.03 ^{ab}	3.73 ^a	2.34 ^b	0.21	Fiber	NS	Fiber
	5	5.36 ^a	4.37 ^{ab}	4.16 ^{ab}	3.13 ^b	0.34	Fat	0.0037	Chol
	7	44.8	41.7	35.7	37.7	2.80	Fiber × fat	NS	Fiber × Chol
Cholestanol	3	1.44 ^a	1.07 ^{ab}	1.37 ^a	0.88 ^b	0.07	Fiber	0.0001	Fiber
	5	3.58 ^a	1.76 ^b	1.56 ^b	1.33 ^b	0.19	Fat	0.0001	Chol
	7	1.70	0.86	1.05	1.16	0.14	Fiber × fat	0.0057	Fiber × Chol
Total neutral sterols	3	11.1	9.85	11.4	8.55	0.52	Fiber	0.0001	Fiber
	5	23.9 ^a	15.1 ^b	12.6 ^b	10.7 ^b	0.97	Fat	0.0001	Chol
	7	70.3 ^a	62.6 ^{ab}	55.3 ^b	57.2 ^b	1.87	Fiber × fat	0.0028	Fiber × Chol

¹ Means (*n* = 6) in a same line with different superscript letters are significantly different (*p* < 0.05).
^{2,3} See footnote to Table 3.

LCA/DCA ratio but no difference was observed among the DF groups. While there was an interaction between fiber and fat, no interaction was seen between fiber and Chol.

Table 7 shows major NS excretions in feces per day. The excretion of cholestanol significantly decreased or showed a decreasing tendency in all the DF groups compared to the CP group. The excretion increased in all the DF groups when the fat level was elevated. There was an interaction between fiber and fat. Exactly the same tendency was observed in the total NS excretions. Although Chol addition increased the excretion of each NS, the effect of DF was seen only in the excretions of cholestanol and total NS.

DISCUSSION

Since the same animals were successively used in the experiments, changes in lipid metabolism by aging should be considered the effects of Chol metabolism (28, 29) and fecal steroid excretions (30, 31). According to Uchida et al. (31), however, such changes occur over a prolonged period of time and any changes taking place in 8 to 12 weeks are minimal, with no changes in serum Chol. We also observed no significant elevations in plasma Chol from the beginning of the experiment up to the 5th week (data not included), and therefore judged that the effect of aging, if any, over the 7-week experimental period was very small. We nevertheless minimized such effects as much as possible in the statistical analysis of changes over time.

Fecal wet weight per day in the test DF groups was significantly decreased in any of the measurement weeks when compared to the CP group. This is presumably because, for one, the test DF levels were different from the CP group, and for another, the DF preparations were more easily decomposed and fermented by intestinal bacteria than cellulose because of their relatively high hemicellulose content. However, significant increases in the fecal water content were observed in both the Kanpyo and Shiitake groups compared to the CP and Kiriboshi groups. The reason for this is not clear but it may be attributed to the difference in their chemical structures.

A larger number of BA showed significance when excretions were evaluated per 1 g of feces than when evaluated per day. The effect of fecal output is presumably involved in this difference. The fat level had the effect on the fecal dry weight but not on the total BA excretions per day.

Excretions of individual BA differed depending on the type of DF and on the absence/presence of Chol, and showed no clear tendency. There was, however, one noteworthy phenomenon; excretions of LCA, DCA, α -MCA, β -MCA and total BA in the Shiitake group increased significantly or showed an increasing tendency compared to those in the CP group in any week of measurement.

Although the reason for the increase in excretion of these BA in the Shiitake group is not clear at present, it is highly likely that hemicellulose, which is abundant in the Shiitake DF, induced a change in the intestinal environment, which in turn

produced a change in Chol or BA metabolism. The involvement of eritadenine is assumed to be minimal but cannot be ruled out completely since its content in the DF preparation was not determined.

The ratio of secondary BA to total BA was affected more strongly by the fat level than by the type of DF, and not by Chol addition. Reddy et al. (15) reported that pectin, carrageenan and alfalfa significantly increased total BA excretions compared to cellulose whereas no change was produced by wheat bran and that the proportion of CA metabolites was markedly larger than that of CDCA metabolites only in animals fed carrageenan.

Story (16) reported that the excretions of mainly CDCA derivatives were increased in rats fed DF such as cellulose, oat bran, corn bran and alfalfa. Thomas et al. (32) observed that the proportion of CDCA derivatives was larger than CA in pectin-fed rat feces. According to Vahouny et al. (17), alfalfa, guar gum, psyllium and wheat bran lowered and pectin and mixed fiber raised the CDCA/CA ratio compared to cellulose. In our study, the excretion of CDCA metabolites and CA metabolites both tended to increase. The CDCA/CA ratio was lowered in the test DF groups compared to the CP group.

As discussed above, the fecal excretion of secondary BA was affected by the difference in the type of DF and the results of some experiments on the same type of DF differed among researchers (experimental conditions).

In the present study, the LCA/DCA ratio of feces in the test DF groups was lowered compared to that in the CP group and was affected by the fat level, showing the interaction between fiber and fat. Chol addition produced higher LCA/DCA ratios in all the groups excluding the CP group, but no difference was observed between the CP and test DF groups. Gallaher and Franz (21) studied the relation between three kinds of wheat bran and the corn oil level and reported that the fecal LCA/DCA ratio was affected by neither the type of DF nor the fat level. They (22) also studied the relation between the corn oil level and oat bran, rye bran, barley bran and sugar beet fiber, and found that the fiber type affected the LCA/DCA ratio independent of the fat level. It is known that Chol supplementation promotes CDCA metabolism (18,19). It is therefore considered that Chol addition to DF, with its LCA/DCA lowering action, raises this ratio and masks any difference that may exist among DF.

The present study revealed that the LCA/DCA ratio, one of the several indices showing characteristics of fecal BA, responds well to the DF tested. As reported by Gallaher et al. (21,22), this response seems to differ by the type of DF. The significance of the response of the LCA/DCA ratio in evaluating the function of DF needs to be clarified. Further studies are warranted to elucidate the relation between the composition and ratios of fecal steroids and the type of DF in association with colon cancer risks.

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