Decreasing Effect of Chitosan on the Apparent Fat Digestibility by Rats Fed on a High-fat Diet

Keiji DEUCHI, Osamu KANAUCHI, Youji IMASATO, and Eiichi KOBAYASHI

Applied Research Center, Research and Development Dept., Kirin Brewery Co. Ltd., 3 Miyahara. Takasaki, Gunma 370-12, Japan
Received February 24, 1994

We investigated the effects of various dietary fibers or their likenesses on the apparent fat digestibility by rats fed on a high-fat diet. Each of 23 different fibers was added at 5% (w/w) to a purified diet containing 20% (w/w) corn oil. The rats were fed these diets for 2 weeks, and the feces were collected from each animal during the last 3 days. When compared with cellulose (control), 10 of the tested fibers significantly increased the fecal lipid excretion. Among these fibers, chitosan markedly increased the fecal lipid excretion and reduced the apparent fat digestibility to about a half relative to the control. The apparent protein digestibility was not greatly affected by chitosan. The fatty acid composition of the fecal lipids closely reflected that of the dietary fat. These results suggest that chitosan has potency for interfering with fat digestion and absorption in the intestinal tract, and for facilitating the excretion of dietary fat into the feces.

The beneficial effects of dietary fiber have attracted strong attention. These benefits are not only recognized as being a reduction in the energy density of a diet[1] and an increase in the stool weight or in the frequency of defecation,[2] but also as a preventive measure against disorders prevalent in the lower intestinal tract, e.g., diverticulitis or colon cancer.[3] The effects of dietary fiber on lipid metabolism have been extensively studied, and there are many reports concerned with their effects on serum and liver lipids.[4-6] There is, however, little information on the total lipids excreted into the feces concomitantly with the ingestion of dietary fiber. Slavin and Marlett[7] have reported that ingesting cellulose did not affect the fecal lipid excretion. De Scrijver *et al*,[8] suggested that the apparent protein digestibility was reduced to some extent by the intake of oat bran, but the apparent fat digestibility not as much. Pectin stimulated the discharge of fecal steroids?) but affected the total lipid excretion to a lesser extent.[10] It is generally accepted that apparent fat digestibility is not as much affected by dietary fiber as that of protein.[11,12] If certain dietary fibers can reduce only the fat digestibility, a reduced caloric intake will be achieved by their ingesting without affecting protein nutrition. In this study, we investigated the effect of various fibers on the apparent digestibility of both fat and protein in rats fed with high-fat diets.

Materials and Methods

Fiber. The fiber samples used in this experiment were purchased commercially (Table I). Polypropylene and kapok were reduced to pieces less than 1 .0 mm long by using laboratory cutters to make their size the same as that of the other water-insoluble fibers.

Animals *and diets.* Male Sprague-Dawley rats weighing approximately 150 g were obtained from Japan SLC (Hamamatsu, Japan). They were individually housed in metabolic cages in a room kept at 22±1 C with a 12-h light and dark cycle (lighting from 8:00 a.m. to 8:00 p.m.). The rats were assigned to 23 groups of 5 to 6 rats each and were fed *ad hintum* on a control diet (containing 5% cellulose as the fiber source, its composition being given in Table II) for 5 days before the start of the experiment. Subsequently, each group was fed on the stated diet (cellulose was replaced by various fibers) for 14 days. The rats were allowed free access to the respective diets and drinking water. During

Table I. Dietary Fibers and Likenesses Tested

Fiber	Product name	Supplier
Water-insoluble		
Cellulose	CELLULOSE POWDER	Oriental Yeast co., Japan
Chitin	CHITIN 500	Seikagaku Co., Japan
Chitosan	CHITOSAN 500	Seikagaku Co., Japan
Cholestyramine	DOWEX 1-X2	Muromacbi Kagaku Kogyo Kaishu
CMC	CMC	Wako Pure Chemical Industries
Kapok	OIL CATCHER	Kakui Co., Kagoshima, Japan
Polypropylene	OIL BLOTTER	Mitsui Petro-chemical Industries
Water-soluble Acacia	ARABIC GUM	Suzu Funmatsu Yakujin co.
Agar	JP PBS-6	Ina Syokuhin Kogya Co.
Carrageen	SATIAGUM B	Sanofi Bio-Industries Co.
Furcellaran	NEO SOFT FR-I	Taiyo Kagaku Co.
Guar	GUARPAK PF-20	Dainippon Pharmaceuti-cal co.
HPC	NISSO HPC M	Nippon Soda Co.
HPMC	65SH 400	Shin-Etsu Chemical Co.
Karaya	NEO SOFT KR	Taiyo Kagaku Co.
Konjak-mannan	PROPOLE PA	Shimizu Chemical Co.
Locust bean	NEO SOFT L	Taiyo Kagaku Co.
MC	SM-400	Shin-Etsu Chemical Co.
Pectin	PECTIN	Wako Pure Chemical Industries
PGA	KIMILOID MV	Kimitsu Chemical Industries
Sodium alginate	KIMILOID l-3	Kimitsu Chemical Industries
Tamarind	GLYLOID 2-A	Dainippon Pharmaceuti-cal Co.
Tragaeanth	JP TRAGACANTH	Suzu Funmatsu Yakuhin co.

CMC, carboxymethyl cellulose; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; MC, methylcellulose; PGA, propylene glycol alginate.

Table II. Composition of the Experimental Diets

Constituent	Content (%)
Casein	20.0
Corn oil	20.0
Mineral mixture'	3.5
Vitamin mixture'	1.0
Choline chloride	0.2
Sucrose	50.3
Fiber'	5.0
Total	100.0

. AIN-76 ™ formula[24]

' Details are shown in Table I.

Cellulose was used for the control diet, and one type of fiber was used for each experimental diet.

the experimental period (14 days), the food intake was measured daily and the rats were weighed every 3 days. and were weighed every 3 days.

Analytical methods. The feces excreted during the last 3 days of the experimental period were collected every day and stored at -80°C. After lyophilization, the fecal samples were pulverized and weighed before analysis. Fecal lipids were determined gravimetrically by a modification of the Saxon method.[13] A portion of the lyophilized feces (about 1.5 g) was suspended in 1 ml of distilled water and 9 ml of cone. HCI in a sealed glass tube kept at 50°C for 30 min under nitrogen gas. The lipid fraction was then extracted with 40 ml of diethyl ether and determined gravimetrically after a completely removing the solvent. The apparent fat digestibility was obtained from the equation [(intake of lipid - fecal lipid)/intake of lipid x 100]. In addition, the fatty acid composition and nitrogen content of the feces from each rat fed on the diets containing cellulose, chitin, chitosan and propylene glycol alginate (PGA) were determined. To do this, the fecal lipid was extracted from 1.5 g of lyophilized feces with 20 ml of chloroform-methanol (2: 1, v/v) according to the method of Folch et *al.*[14] Undecanoic acid (Wako Pure Chemicals) was added as an internal standard. After the solvent had been evaporated under a nitrogen stream, the residual lipid was treated with BF, methanol to obtain fatty acid methyl esters, before gas chromatographic measurements were performed with a Shimadzu GC-14A gas-liquid chromatograph (GLC). Analytical conditions: column, 50m x 0.25 mm I.D., packed with CP-Sil 88 (Gasukuro Kogyo); column temperature, 80 - 130°C (10°C/min), 130 -175°C (4°C/min), 175 - 2 10°C (3°C/min); injection and detection temperatures, 200°C and 250°C respectively, detector, FID; carrier gas, helium; flow rate, 40 (ml/min). The fatty acid composition of the corn oil used as the fat source in all the diets was also analyzed by GLC. The nitrogen content in the feces was determined by the Kjeldahl method, using a KJELTEC AUTO 1030 analyzer (Nibon General Co.). The apparent protein digestibility was calculated in the same way as that already mentioned for lipids according to the equation [(intake of nitrogen from casein-fecal nitrogen)/intake of nitrogen from casein x 100].

Statistical analysis. The data were analyzed by the Wilcoxon-Mann-Whitney test to evaluate whether or not there was a significant difference at $p<0.05$ between the cellulose (control) group and each other fiber group.

Results

The effects of ingesting various fibers on the fecal weight, fecal lipid amount, and apparent fat digestibility are summarized in Table III. Compared to cellulose as the control, chitosan and kapok increased the fecal weight. However, acacia, cholestyramine, furcellaran, guar, karaya, konjak-mannan, locust bean, pectin, sodium alginate, tamarind, and tragacanth all significantly decreased the fecal weight. With respect to the

Table III. Effects of Various Dietary Fibers on Fecal Lipid Excretion

	Dry weight of feces (g/3days)	Total lipid in feces (mg/3days)	Apparent digestibility %
Water-insoluble			
Cellulose (control)	4.86±1.33	578.8±3 11.5	94.9±2.1
chitin	3.41io.95	428.8±136.6	95.7±1.0
chitosan	8.95±3.58	5380±2824	50.8±21.6
Cholestyramine	2.18±0.17	3 10.7±44.2	97.fk0.6
CMC	4.39±0.42	419.9±81.5	96.5±0.6
Kapok	7.55±0.64	924.0±89.1	91.7±1.1
Polypropylene'	5.04±0.5 1	530.4±108.3	95.8±0.6
Water Soluble			
Acacia	1.77a.69	351.8±118.5	96.4±0.4
Agar	3.81±0.32	284.2±34.5	97.2±1.5
Carrageen	5.07±0.73	1109±277.6*	90.4±1.5
Furcellaran	2.69±0.65**	446.6±157.1	9 5 . a . 9
Guar	1.82±0.38**	556.5±119.7	94.0±1.7
HPC	4.06±0.42	152.9±26.4**	98.5±0.3**
HPMC	4.53±0.25	269.3±122.1	97.4±1.2
Karaya	3.32±0.76*	491.9±145.4	95.1±1.5
Konjak-mannan	1.95±0.19**	537.9±84.7	94.8±0.6
Locust bean	1.82±0.23**	614.5±166.9	94.0±1.8
MC	3.76±0.38	128.5±16.2**	98.7±0.1**
Pectin	1.22±0.32**	168.0±54.0**	92.6±1.9
PGA	5.75±1.18	1641±452.0**	83.4±3.0**
Sodium Alginate	2.14±0.39**	727.5±194.5	9 1.9±2.2
Tamarind	1.65±0.18**	364.3±58.1	96.5±0.5
Tragacantha	3.02±0.99	837.8k348.5	92.8±2.3

Values are the means ± SD for 5 to 12 (control) rats; . and . * indicate significant differences form the control at $p<0.05$ and $p<0.01$, respectively based on the Wilcoxon Mann-Whitney test. Apparent fat digestibility considered equal to the difference between the intake and fecal output of lipids.

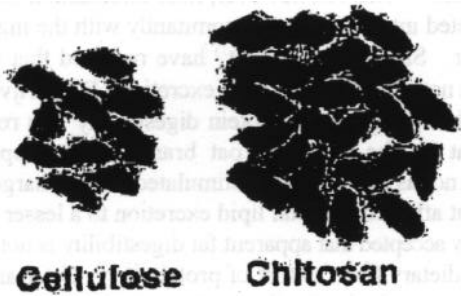

Fig. Appearance of Feces,
These feces were collected on the final day from one rat fed on cellulose and from one fed on chitosan as a fiber for 14 days.

water-insoluble fibers, a reduction of the apparent fat digestibility was found in the chitosan-and kapok-receiving groups. Of the water-soluble fibers, carrageen and PGA reduced the apparent fat digestibility. In particular, chitosan markedly increased the fecal mass as well as the fecal lipid excretion without inducing diarrhea, although the fecal bulk was rather large, oily and yellowish in appearance as shown in the figure.

We then investigated the effect on the apparent protein digestibility of ingesting chitosan and PGA, which were the most effective regarding fecal lipid excretion among the large number of water-insoluble and -soluble fibers tested so far (Table IV). When chitin, chitosan cellulose and PGA are compared with one another, there are no significant differences in food intake among these groups. Consequently, chitosan and PGA intake were proved to

Table IV. Food intake. Body Weight Gain and Apparent Protein Digestibility in Rats Fed on Four Different Rinds of Fiber for 14 Days

	Food intake (g/rat/day)	Body weight gain (g/14 days)	Apparent protein digestibility (%)
Cellulose (cont)	18.5 ± 0.8	92.4 ± 11.7	95.5 ± 0.7
chitin	17.5 ± 1.5	88.0 ± 5.7	92.4 ± 1.0"
chitosan	17.5 ± 1.2	76.1 ± 10.9'	84.6 ± 2.1 '
PGA	16.6 ± 2.5	83.2 ± 7.4	94.0 ± 1.9

Values are the means ± SD for 5 to 12 (control) rats; . and " indicate significant differences from the control at p CO.05 and p <0.0 1, respectively, based on the Wilcoxon-Mann-Whitney test.

Table V. Fatty Acid Compositions of Fecal Linids

	Fatty acid (mg/3 days)			
	16:0	18:0	18:1	18:2
Cellulose (control)	40.6 ± 11.0	20.5 ± 5.5	45.2 ± 17.3	22.4 ± 9.8
chitin	40.8 ± 4.1	34.4 ± 5.7**	66.0 ± 16.1*	23.2 ± 5.0
Chitosan	538.3 ± 263.9**	161.7 ± 72.4**	1411 ± 761.1**	1992 ± 899.2**
PGA	135.3 ± 17.4**	8 4 . 5 ± 11.1**	180.3 ± 36.9**	132.4 ± 31.9**

Values are the means ± SD for 5 to 12 (control) rats: * and ** indicate significant differences from the control at p <0.05, and p <0.0 1, respectively, based on the Wilcoxon-Mann-Whitney test.

Table VI. Fatty Acid Ratio of Feces and Corn Oil

	Fatty acids (%)				
	16:0	18:0	18:1	18:2	Total
Cellulose (control)	8.5 ± 4.0	4.2 ± 1.9	10.2 ± 6.1	49 ± 307	27.8 ± 14.0
chitin	10.4 ± 3.6	8.5 ± 2.5*	15.7 ± 2.1	5.7 ± 1.8	40.4 ± 9.2
chitosan	10.1 ± 1.3	3.1 ± 0.5	25.7 ± 4.6**	38.9 ± 6.1**	77.8 ± 8.0**
PGA	8.5 ± 1.3	5.3 ± 1.0	11.3i2.1	8.3 ± 1.9	33.4 ± 5.0
Corn oil	10.6	2.3	28.1	52.9	93.9
(B - A)	10.7	3.1	28.6	45.6	88.0

A, mean amount of fecal fatty acid in the control; B, amount of fecal fatty acid from each rat in the chitosan group; T, total amount of fecal fatty acid from each rat in the chitosan group; T, mean total amount of fecal fatty acid in the control. Values are the means ± SD for 5 to 12 (control) rates: * and ** indicate significant differences from the control at p <0.05, and p <0.0 1, respectively, based on the Wilcoxon-Mann-Whitney test

cause a significant decrease in the apparent protein digestibility, although a lesser extent than with the apparent fat digestibility. The fatty acids compositions of the fecal lipids from the four groups are shown in Table V. The fecal content of these fatty acids was much higher in the chitosan- and PGA-receiving group than in the control group. When we compared their fatty acid composition ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) with that of corn oil, we found out that both were in principle very similar (Table IV).

Discussion

The results of the present investigation made clear that chitosan had potent ability to increase lipid excretion into the feces. Some recent in vitro studies [15-17] have demonstrated that chitosan could bind much more bile acids than cholestyramine to the extent of several times its original weight under optimal conditions. Many investigators [6, 15, 18, 19] have described that chitosan brought about a high hypocholesterolemic effect, probably due to the increased excretion of bile acids into the feces. However, there are few reports referring to the ability of chitosan to excrete dietary fat into the feces. Nauss *et al.* [16] have suggested that a soluble form of chitosan would be able to interfere with intraluminal lipid absorption through the interaction with micelle formation or emulsification of lipids in the enteric phase. Ikeda *et al.* [4] have offered a similar suggestion on the basis of their study on fully emulsified oil with chitosan and sodium taurocholate. On the other hand, it has also been suggested that dietary fiber would not necessarily limit the *in* vivo absorption of dietary fat, although it did delay the rate of fat absorption.[20] It is therefore noteworthy to find that chitosan had strong ability to increase the fecal lipid excretion in vivo, and that the fatty acid composition of the fecal lipid fairly well reflected that of the dietary fat. According to our analyses, 93.9% of the corn oil was composed of four major fatty acids ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$), while the total content of these four major fatty acids was obtained as 77.8% of the fecal lipid from the chitosan receiving rats. When the amount of fatty acids excreted was adjusted by subtracting the mean amount of fecal fatty acid in the cellulose-receiving group as "endogenous" from that in the chitosan-receiving group, 88.0% of the additional fecal lipid was composed of the four major fatty acids (Table VI). The content ratio of the major fatty acids in the feces of rats fed on chitosan faithfully reflected that of corn oil. Therefore, we consider that dietary fat was excreted in the feces when the rats were fed with chitosan.

On the other hand, the increase in the fecal excretion of protein was considerably lower than that of the lipids in this experiment, despite the previous observation that lipid absorption was not as much affected by dietary fiber as protein absorption. [11, 17] Chitosan is an N-deacetylated product of chitin that is a (1-4)-linked 2-acetamido-2-deoxyβ-o-glucan. Both chitin and chitosan contain nitrogen in their structure. Hirano *et al*,[21] have reported that the digestibility of chitin and chitosan varied from 25% to 38% and from 4 1% to 83%, respectively, in rabbits according to their adaptation to the feeding conditions, and that chitosan was more digestible than chitin. In this study, we have assumed that all the fecal nitrogen originated from dietary casein so as not to overestimate the protein absorption. Obviously, undigested chitin or chitosan excreted in the feces affected the nitrogen content and apparent protein digestibility in the rats. Therefore, the degree of dietary protein absorption by the rats fed on chitin or chitosan may be much higher than the values given in Table IV. This suggests that chitosan specifically inhibited the digestion and absorption of dietary fat. We used sucrose as the sole carbohydrate source, because 1) many studies about dietary fiber or chitosan have used sucrose as the carbohydrate (energy) source in accordance with the AIN-76™ formula, [15, 18, 22-24] and 2) we were worried that unabsorbed starchy fragments would affect the apparent fat digestibility instead of the fiber. If chitosan served as a flocculant for starch in the gut, the ability of chitosan to excrete dietary fat into the feces would be affected by feeding it together with starch. This possibility must be clarified in the future.

As shown in Table IV, feeding chitosan resulted in a low body weight gain We do not consider that such a low weight gain was caused by growth retardation due to any toxicity of chitosan, because it is widely accepted that chitosan is a natural product with very low toxicity.[7] We have also confirmed that the epididymal fat pad was half-reduced in weight by ingesting chitosan, as will be described in a subsequent paper.[26] We think that such a low weight gain is mainly attributable to a reduction in food efficiency due to increased fecal lipid excretion, even though an absorptive obstruction of protein and other nutrients such as vitamins and minerals must also be considered to some extent.

We have confirmed in this study that cholestyramine did not affect the fecal lipid excretion, and that kapok and polypropylene that are both capable of forming a hydrophobic environment in the intestinal tract also had little effect. In addition, by taking into consideration the properties of chitin, these results may suggest that chitosan elicited its effect by its unique solubility, rather than by any ability to combine with bile acids. With regard to the mechanism, we consider that the chitosan dissolved in the stomach to form an emulsion with intragastric oil droplets and would begin to precipitate in the small intestine at pH 6.0-6.5. As the numerous chains of polysaccharides start to aggregate, they would entrap fine oil droplets in their matrixes, pass through the lumen and empty into the feces. These features imply that a suitable chitosan intake would be useful to control ovemutrition by fat and to prevent adult disease.

References

1) J.J. Beereboom, *Crit.* Rev. *Food Sci. Nutr.*, 11, 401-413 (1979).
2) J.H. Cummings, M.J. Hill, D.JA. Jenkins, J.R. Pearson, and H.S. Wiggins, Am. *J. Clin. Nutr.* 29, 1468-1473 (1976).
3) D. Burkitt, Cancer, 28, 3-13 (1971)
4) I. Ikeda, Y. Tomari, and M. Sugano, *J. Nutr.*, 119, 1383-1387 (1989).
5) V.M. Martinez, R.K. Newman, and C.W. Newman, *J. Nutr.*, 122, 1070-1076 (1992).
6) CD. Jennings, K. Boleyn, S.R. Bridges, and P.J. Wood, *Proc. Soc. Exp. Biol.* ***Med.,*** 189, 13-20 (1988).
7) J.L. Slavin and J.A. Marlett, *J. Nutr.*, 110, 2020-2026 (1980).
8) R. de Schrijver, D. Fremaut, and A. Verheyen, *J. Nutr.*, 122, 1318-1324 (1992).
9) R.M. Kay and A.S. Truswell, Am *J. Clin. Nutr.*, ***30,*** 171-176 (1977).
10) M. Stasse-Wolthuis, H.F.F. Albers, J.G.C. van Jeveren, J.W. de Jong, J.G.A. Hautvast, R.J.J. Hermus, M.B. Katan, W.G. Btydon, and M.A. Eastwood, Am. *J. Clin. Nutr.*, 33, 1745-1756 (1980).
11) J.H. Cummings, Am. *J. Clin. Nutr.*, ***3*** 1, S21-29 (1978).
12) J.L. Kelsay, *Am. J. Clin. Nutr.*, 31, 142-159 (1978).
13) ***K.*** Ito, ***Medical Technology*** (in Japanese), 8, 1252-1256 (1980).
14) J. Folch, M. Lee, and H.S. Stanley, *J.* ***Biol. Chem.,*** 226, 497-509 (1957).
15) M. Sugano, T. Fujikawa, Y. Hiratsuji, K. Nakashima, N. Fukuda, and Y. Hasegawa, *Am. J. Clin. Nutr.*, 33, 787-793 (1980).
16) J. L. Nauss, J.L. Thompson, and J. Nagyvary, ***Lipids,*** 18, 714-719 (1983).
17) G.V. Vahouny, R. Tombes, M.M. Cassidy, D. Kritchevsky, and L.L. Gallo, ***Lipids,*** 15, 1012-1018 (1980).
18) M. Sugano, S. Watanabe,A. Kishi, M. Izume, and A. Ohtakara, ***Lipids,*** 23, 187-191 (1988).
19) J.J. Nugyvary, M.L. Falk, M.L. Hill, M.L. Schmidt, A.K. Wilkins, and E.L. Bradbury, *Nutr.* ***Rep. Int.,*** 20, 677-684 (1979).
20) G.V. Vahouny, S. Satchithanandam, I. Chen, SA. Tepper, D. Kritchevsky, F.G. Light-foot, and M.M. Cassidy, Am. *J. Clin. Nutr.*, 47, 201-206 (1988).
21) S. Hirano. C. Itakura, H. Seino, Y. Akiyama, I. Nonaka, N. Kanbara, and T. Kawakami, *J. Agric.* ***Food Chem., 38,*** 1214-1217 (1990).
22) II. Takeda, A. Nakajima, and ***S.*** Kiriyama, ***Biosci, Biotech. Biochem.,*** 56, 551-555 (1992).
23) K. Tsuji, T. Ichikawa, N. Tanabe, S. Abe, S. Tarui, and Y. Nakagawa, ***Nippon*** *Nōgeikagoku Kaishi* (in Japanese), 66, 1241-1246 (1992).
24) American Institute of Nutrition, *J.* Nua., 107,1340-l 348 (1977).
25) D. R. Landes and WA. Bough, ***Bull.*** *Environ. Contam. Toxicol.* 15, 555-561 (1976).
26) 0. Kanauchi, K. Deuchi, Y. Imasato and E. Kobayashi, ***Biosci. Biotech. Biochem.*** 58, 1617-1620 (1994).