72. Influence of Dietary Konjac Mannan on Fecal Microflora in F344 Rats

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Epidemiologic (Burkitt, 1975; Trowell, 1973) and animal model (Wilson *et al.*, 1977; Watanabe *et al.*, 1979; Reddy, 1981) studies suggest that dietary fibers play an important role in colon carcinogenesis. The protective effect of the dietary fiber, such as wheat bran (Wilson *et al.*, 1977), pectin (Watanabe *et al.*, 1979), and citrus fiber (Reddy *et al.*, 1981) on chemical carcinogenesis in the colon is claimed to be due to the increased fecal weight, shortened transit time, suppressive adsorption or dilution of colon contents including any carcinogens and promoters which may be present in the alimentary tract (Burkitt *et al.*, 1972). On the other hand, some intestinal bacteria have been known to produce detrimental substances to the host from dietary fat or protein, such as ammonia, toxic amines, H_2S , phenols, possibly carcinogens and promoters (Drasar and Hill, 1974). It has been postulated that the effect of diets on colon carcinogenesis may be mediated through alternation in the composition or metabolism of intestinal microflora (Wynder *et al.*, 1969; Reddy *et al.*, 1975).

We have previously reported the inhibitory effect of Konjac mannan (KM) on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in F344 rats (Mizutani and Mitsuoka, 1983). This study was to examine the effect of dietary KM on the composition of fecal microflora and fecal pH in F344 rats.

Material and methods. Inbred male F344 rats (Shizudokyo, Hamamatsu) weighing 120–130 g at 5 week of age were divided randomly into three groups of 9 rats each and maintained on a basal control diet or diets containing 5 and 10% KM (Shimizu Chemical Co., Mihara) as shown in Table I. All animals were housed in wire-bottomed cages in a temperature ($24\pm 1^\circ C$) and humidity ($60\pm 5\%$)-controlled room and given food and water *ad libitum*, and weighed weekly for 10 weeks.

Fecal microflora were analyzed by culturing the fresh feces at week 7 after the beginning of the experiment. Processing of the fecal specimens was made by previously described methods (Mitsuoka *et al.*, 1965; Mitsuoka *et al.*, 1976). The fresh feces was aseptically collected, weighed and diluted serially tenfold in the prerduced diluent. The diluted samples were spread on the surface of nonselective and selective agar plates, and incubated at $37^\circ C$ aerobically or anaerobically as shown in Table II. Bacterial colonies growing on agar plates were counted and then observed microscopically after Gram staining. Identification of the bacterial groups was made on the basis of colony morphology, cell morphology, and arrangement of cells.

The pH of the fresh feces from each of 8 rats in KM and control diet groups was also measured. The fresh feces was suspended in 4 volumes of

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Table I. Percentage composition of diets

	Control diet*	5% KM diet**	10% KM diet**
Casein	20.0	19.0	18.0
DL-Methionine	0.3	0.285	0.27
Corn starch	51.5	48.925	46.35
Dextrose	13.0	12.35	11.7
Alphacel	5.0	4.75	4.5
Konjac mannan	0	5.0	10.0
Corn oil	5.0	4.75	4.5
Mineral mixture	3.5	3.325	3.15
Vitamine mixture	1.5	1.425	1.35
Choline chloride	0.2	0.19	0.18

* This diet was based on American Institute of Nutrition standard reference diet with modification. In this modified diet, the amount of vitamin mix and the amount and source of carbohydrate were varied. Choline chloride was also substituted for choline bitartrate. ** Konjac mannan was added to the control diet to concentrations of 5% and 10%.

Table II. Media and culture methods

Medium	Organisms enumerated usually	Incubation method	Incubation time (days)
Non-selective media			
M10 agar	Fastidious anaerobes	Plate-in-bottle	5
EG agar	Anaerobes	Steel wool jar replaced air with CO ₂	3
BL agar	Anaerobes		
TS blood agar	Aerobes	Aerobic condition	1-2
Selective media			
BS agar	<i>Bifidobacterium</i>	Steel wool jar replaced air with CO ₂	3
ES agar	<i>Eubacterium</i>		
NBGT agar	Bacteroidaceae		
VS agar	<i>Veillonella</i>		
NN agar	<i>Clostridium perfringens</i>		
LBS agar	<i>Lactobacillus</i>	Aerobic condition	1
DHL agar	Enterobacteriaceae		2
TATAC agar	<i>Streptococcus</i>		
PEES agar	<i>Staphylococcus</i>		
P agar	Yeasts and molds		

M10: Medium 10. EG: Eggerth-Gagnon. BL: Glucose-blood liver. TS: Trypticase soy. BS: *Bifidobacterium* selective. ES: *Eubacterium* selective. NBGT: Neo-mycin-brilliant green-taurocholate-blood. VS: *Veillonella* selective. NN: Neo-mycin-Nagler. LBS: *Lactobacillus* selective. DHL: Deoxycholate hydrogen sulfide lactose. TATAC: Tetrazolium-azido-thallium-acridine orange-crystal violet. PEES: Phenylethyl alcohol-egg yolk-*Staphylococcus* 110. P: Potato dextrose.

distilled water. A reading was taken for the fecal suspensions, using pH meter (Model HG-3, DKK, Tokyo).

Results and discussion. The weight gain in 5 and 10% KM diet groups was significantly lower than that in a control diet group (Fig. 1). There was no difference in the weight gain between 5 and 10% KM groups through the experiment. The inhibition of colon tumorigenesis may be ascribed to the caloric restriction (Tannenbaum, 1959; Ross and Bras, 1965), because growth retardation was shown in the present results. However, Fleiszer and his colleagues (1978) suggested that reduced energy intake alone could not account for the significantly protective effect of dietary bran on colonic carcinoma. We have previously reported that alteration of colonic microflora, especially increase in *Bifidobacterium* by dietary KM cannot be overlooked in the inhibitory effect of KM on liver tumorigenesis in C3H/He male mice (Mizutani and Mitsuoka, 1980).

As shown in Table III, bacterial counts of *Bifidobacterium* in the 10% KM

Fig. 1. Growth curves of F344 rats maintained on a basal control diets containing 5 and 10% KM.

diet group were significantly higher than those in control diet group ($p < 0.001$). In contrast, the counts of *Clostridium*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* were significantly lower in the 10% KM group than in the control group ($p < 0.01$, $p < 0.05$, $p < 0.001$, $p < 0.01$ respectively). In the 5% KM group, *Streptococcus* and *Staphylococcus* were also significantly lower in the counts compared to the control group ($p < 0.01$).

The 10% KM-fed rats also yield a lower pH of feces: fecal pH was 6.66 ± 0.26 (Means $\pm$ SD, $n=8$) as compared to 6.95 ± 0.04 (Mean $\pm$ SD, $n=8$) in the control group ($p < 0.05$). This result indicated that an elevation of the acidity of the feces was consistent with increase in *Bifidobacterium* population.

It has been hypothesized that alkaline fecal pH increases the risk of colon cancer (Burkitt, 1981; Thornton, 1981). Wheat bran, as an undigested dietary component, provides bacteria with substrate which may be fermented into short-chain fatty acids thus lowering colonic pH (Cummings *et al.*, 1976). Acidification of colonic contents could, in turn, be inhibitory to bile acid degradation (Aries and Hill, 1970). The hypothesis has been supported by the findings that dietary wheat bran lower the colonic pH (Jacobs and Lupton, 1982), and that lactulose lowered fecal pH and subsequently suppressed DMH-induced colon tumor in rats (Samelson *et al.*, 1985). Dietary KM may act in a similar manner to these findings in the colon. The present study suggested that the inhibitory effect of dietary KM on DMH-induced colon carcinogenesis may be mediated through alteration of the fecal microflora with the significant multiplication in *Bifidobacterium* population in rats.

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Table III. Fecal microflora of F344 rats maintained on a control diet or diets containing 5% and 10% Konjac mannan

Bacterial group	Control diet (A)	5% KM diet (B)		
Total	10.5±0.3	10.5±0.3		
Enterobacteriaceae	6.5±0.4 ^{††} (100) ^{†††}	6.7±0.9 (100)		
<i>Streptococcus</i>	7.7±0.4 (100)	7.1±0.3 (100)		
<i>Staphylococcus</i>	5.6±0.4 (100)	4.8±0.5 (86)		
<i>Corynebacterium</i>	5.9±0.4 (100)	5.7±0.3 (100)		
<i>Bacillus</i>	4.3±0.6 (86)	3.8±0.1 (43)		
<i>Lactobacillus</i>	8.4±0.5 (100)	8.9±0.8 (100)		
<i>Bifidobacterium</i>	7.4±1.1 (57)	7.9±1.9 (100)		
<i>Eubacterium</i>	9.5±0.4 (100)	9.3±0.5 (100)		
Bacteroidaceae	10.0±0.4 (100)	10.1±0.3 (100)		
<i>Clostridium</i>	10.0±0.5 (100)	9.5±0.7 (100)		
Peptococcaceae	9.4±0.5 (100)	9.7±0.4 (100)		
Fusiform bacteria	8.4±0.7 (43)	9.0±0.1 (29)		

Bacterial group	10% KM diet (C)	Significance [†]		
		A:B	A:C	B:C
Total	10.7±0.1	.	.	.
Enterobacteriaceae	6.8±1.1 (100)	.	.	.
<i>Streptococcus</i>	7.1±0.6 (100)	**	*	.
<i>Staphylococcus</i>	4.1±0.8 (100)	**	***	.
<i>Corynebacterium</i>	5.1±0.4 (100)	.	**	**
<i>Bacillus</i>	3.8 (29)	.	.	.
<i>Lactobacillus</i>	8.9±0.8 (100)	.	.	.
<i>Bifidobacterium</i>	10.4±0.3 (100)	.	***	**
<i>Eubacterium</i>	9.3±0.3 (71)	.	.	.
Bacteroidaceae	9.9±0.2 (100)	.	.	.
<i>Clostridium</i>	9.3±0.3 (100)	.	**	.
Peptococcaceae	9.8±0.3 (100)	.	.	.
Fusiform bacteria	9.1±0.8 (71)	.	.	.

[†] Bacterial counts were analyzed statistically by t-test. *p<0.05, **p<0.01, ***p<0.001. . Not significant. ^{††} Values are expressed as means±SD of log bacterial counts per g feces (7 rats). ^{†††} Values are expressed as frequency of occurrence (%) for 7 rats (no significant differences among all groups by χ^2 -test).

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