Effects of Rice Bran and Wheat Bran on Intestinal Physiology and Small-bowel Morphology in Rats*

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ABSTRACT

The present study was aimed at investigating the nutritional and physiological significance of rice bran as a source of dietary fiber as compared to pectin and wheat bran. The parameters for comparison included hypertrophy and morphology of intestines, stool weights and villus marker enzyme activity. For 6 weeks, 10 Sprague Dawley male rats were given one of six experimental diets: 1% cellulose control (CC), 5% pectin (P5), 5% rice bran (RB5), 10% nce bran (RB10), 5% wheat bran (WB5) or 10% wheat bran (WB10) based on the level of dictary fiber. Among experimental groups, food efficiency ratio and body weight gain was comparable. RB10 increased cecal and colonic tissue weights and content weights of cecum and colon as much as P5 did. Stool weight was positiviely correlated with colonic tissue weight (r = 0.727, P < 0.001), and with colonic content weight (r = 0.647, P < 0.001). Small intestine length increased most in the P5 group, followed by the RB10 group. The scanning electron micrograph of jejunal villi from rice bran groups showed a lcaf-shaped, smooth and regular pattern, whereas that of CC group produced a rather long shape. The wheat bran groups showed an irregular leafshaped pattern, and the pectin group typically produced leaf-shaped villi with surface damage. The activities of villus marker enzymes (maltase and sucrase) were higher in the bran-fed rats than in the control or pectin-fed rats. The results indicate that not only dietary fiber amounts but also fiber sources are closely related to the physiology and morphology of the large and small intestines in rats. Rice bran exerted effects on fecal output and trophic effects on the intestines similar to those of pectin.

KEY WORDS dietary fiber, rice bran, wheat bran, intestinal physiology, intestinal morphology, disaccharidase activity

INTRODUCTION

Rice has been the staple food for thousands of years in Korea. However, the traditional food consumption patterns of Koreans have changed and at the same time, the consumption of dietary fiber has gradually decreased in the last 20 years. As the consumption of rice has decreased, the percentage of dietary fiber taken from rice, which amounted to 50% of total dietary fiber intake in 1970s, became less than 20% in the 1990s.1 Rice bran, which is produced as a by-product of milling rice and 18 used mainly for oil production and livestock feed, is a good source of dietary fiber.23 Compared to wheat bran and oat bran, which have been broadly used as sources of dietary fiber in foods such as breakfast cereals, and whose nutritional and physiological effects have been studied extensively in western countries,3 studies on the effects of rice bran have been negligible.46

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Intestinal epithelium is a very dynamic tissue that can adjust its rates of cell proliferation to adapt to changing digestive demands. Dietary fiber has an overall trophic effect on the intestinal muscle, presumably related to bulk, and on the proliferation of substances in the ileum and colon that are dependent on fermentation and shortchain fatty acid production.7 Soluble dietary fibers act like a sponge, binding water, nutrients, bile acids and carcinogens as they pass along the gastrointestinal tract, which is their primary site of action.⁸ In the presence of soluble fibers, which increase the viscosity of the intestinal contents, physical mixing is replaced by simple diffusion. The rate at which the nutrients appear in circulation is reduced under such conditions, while exposure of the gut surface to the nutrients is increased, triggering the release of regulatory hormones, which stimulate growth of mucosal cells." However, simply characterizing dietary fibers as soluble or insoluble in water is not sufficient to explain their physiological effects.105 Other physicochemical properties of dietary fibers affect their various physiological functions.111 The water-holding capacity of dietary fibers is influenced by the particle size, chemical composition, and structure of dietary fibers.¹²⁾

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The aim of this study was to compare the physiological consequences of two dietary fiber sources, rice bran and wheat bran, on intestinal physiology and morphology. At the same time their effects were compared with those of pectin, which is a typical soluble fiber.

MATERIALS AND METHODS

1. Animals

Sixty male Sprague Dawley rats (Korea Center for Experimental Animals, Taejon) weighing about 150 g each were divided equally into six groups according to average body weight. After the rats were acclimated to the facility for one week while receiving a commercial pellet diet (Jinyang), they were given free access to test diets and water for 6 weeks in individual cages and maintained in a temperature (21℃) and humidity (55%) controlled room. Food intake was measured every other day and body weight once a week. Feces were collected for two days during the last week, weighed and kept frozen at −20℃.

2. Diets

Table 1 represents the composition of the test diets, which were based on AIN-76 diet.¹³ The diets consisted of 60% carbohydrate, 15% protein and 25% fat based on energy. Cholesterol was added at a level of 0.4%, and varying amounts of rice bran oil were added in all diets to make up for the differences in fat content. The amounts of rice bran and wheat bran used to provide a

Table 1. Composition of experimental diets (g / kg diet)

Ingredients	CC	P5	RB5	RB10	WB5	WB10
Casein	150	150	118	85	135	120
Corn starch	483	473	401	292	445	378
Sucrose	168	158	134	97	148	126
Lard	40	40	40	40	40	40
Rice bran oil	75	75	38	2	67	60
AIN mineral mix	40	40	40	40	40	40
AIN vitamin mıx	10	10	10	10	10	10
Cholesterol	4	4	4	4	4	4
α-Cellulose	10	0	0	0	0	0
Pectin	0	50	0	0	0	0
Rice bran	0	0	215	430	0	0
Wheat bran	0	0	0	0	111	222
kcal / 100 g	424	416	409	383	414	394
% Fat	24.4	24.9	25.2	26.4	24.9	26.4
% Protein	14.2	14.4	14 7	15.6	14.5	15.3
% Carbohydrate	61.4	60.7	60.1	58.0	60 6	58.3
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CC: 1% cellulose control P5: 5% pectin group

RB5 rice bran group containing 5% dietary fiber on a dry matter basis RB10: rice bran group containing 10% dietary fiber on a dry matter basis

WB5: wheat bran group containing 5% dietary fiber on a dry matter basis

dietary fiber content of 5% and 10% were calculated according to published data on domestic rice bran^{1,0,15} and data on wheat bran composition from the American Association of Cereal Chemists (AACC). The protein and carbohydrate contents of brans were subtracted from the amounts of casein, corn starch and sucrose in the diets. The pectin diet included 5% pectin and the control diet included 1% cellulose. The Korea Food Research Institute graciously provided the rice bran, and the wheat bran (soft white) was purchased from AACC (St. Paul, Minnesota, USA). Pectin (apple), cellulose, and casein were purchased from SIGMA and AIN-76 mineral mix and AIN-76 vitamin mix form TEKLAD. Rice bran oil and lard were purchased at a local market.

3. Tissue sampling

The rats were anesthetized by diethyl ether inhalation and blood was withdrawn from the abdominal aorta into a syringe containing heparin solution. The abdomen was opened, and the small bowel excised and stripped away from the stomach to the ileocecal valve. From a 1/3 point in the total length of the bowel, a proximal 2 cm segment was resected for a scanning electron microscope (SEM) specimen and another 5-cm segment was also taken. The 5-cm segment was washed with cold saline and blotted with tissue, weighed, and the mucosa was scraped between two slide glasses, weighed and stored at -60° C. The total length of the small intestine was measured. The cecum and colon were excised and weighed and the lengths of the colon were measured after removing the contents.

4. Assay of intestinal disaccharidases activities

Disaccharidases were assayed within 3 months after storage. Mucosa was thawed and homogenized with 20 times the volume of distilled water. An aliquot of musosa homegenate was assayed in accordance with the method of Dahlqvist. The activities of maltase and sucrase were assayed using maltose and sucrose as substrates. One micromole of glucose produced per minute was 1 unit of disaccharidase activity, and the activity was expressed as units/cm intestine and units/g protein (specific activity). Protein was determined by Biuret method using bovine serum albumin as a standard.

5. Observation of morphology of jejunum by SEM

The jejunum species were split longitudinally and pinned flat, mucosal side uppermost in a phosphate buffered

saline (PBS) solution. The tissue was prefixed in a fixation solution (2.6% glutaldehyde, 0.8% paraformaldehyde, 0.1M PBS, pH 7.4) at 4°C for 24 hrs and washed with 0.1M PBS. Then the post fixation was carried out in 1% osmium tetroxide for 2 hrs. Samples were dehydrated with ethanol and isoamylacetate and critical point dried and coated with 300Å gold in a ion coater (Eiko IB5, Japan). Observations were made by scanning electron microscope (Hitachi S-4100, Japan) at 15kv accelerating voltage.

6. Statistical analysis

Group means were compared by the Duncan's multiple range test after preliminary analysis of variance (ANOVA) and differences were considered statistically significant at P<0.05. Correlation among variables was tested using Pearson's correlation.

RESULTS

1. Food intake and weight gain

As shown in Table 2, daily food intake and food efficiency ratios were equal in all 6 groups. Daily consumption of carbohydrates, protein and fat was presumably the same in each group. The similar nutritional status was reflected in the equal weight gains by each group of rats over the 6-week feeding period.

2. Cecal and colonic tissue measurements and fecal mass

The weights of cecal and colonic tissues increased in the pectin-fed and bran-fed rats when compared to the cellulose control group (Table 3). They were the highest in the pectin group, followed by the rice-bran groups. The cecal content weights of P5 and RB10 groups were significantly higher than those of the other groups. On the other hand, the tissue weight of the colon was higroup, WB10 group, WB5 group, and CC group in that order. The colon was longer in pectin-fed and all branfed rats than in the CC group. The cecal content weight of RB10 was the same as that of P5 and significantly higher than those of the other groups. The weight of colonic content of the RB10 group was the highest and those of P5 and all bran-fed groups were significantly higher than that of the CC group. There was a tendency for the levels of RB5 to be lower than those of P5 and higher than those of WB5.

As shown in Table 4, the fecal weight of RB10 was the highest, followed by RB5, WB10, WB5, and P5 and CC. The moisture content of RB10 was the highest, and those of RB10, WB10, and WB5 were significantly higher than that of the CC group. It seems that rice bran increased the fecal bulking effect as compared to pectin and wheat bran. Table 5 shows correlations among variables related to the physiology of the large intestine. Fecal weight was highly correlated with colonic tissue weight (r = 0.727, P < 0.001) and with colonic content (r = 0.647, P < 0.001). Colonic tissue weight was positively correlated with colonic content weight (r = 0.612, P < 0.001).

Table 2. Effect of experimental diets on food intake and weight gain in

Dietar Group	•	Food efficiency ratio	Final weight	Total weight gain
	g / day		g	g/6 weeks
CC	19.94 ± 1.15^{NS}	25.45 ± 2.78^{NS}	345.8 ± 17.9^{N5}	203 ± 21^{N5}
P5	$20\ 25\ \pm 1.20$	24.10 ± 1.80	339.8 ± 11.7	195 ± 12
RB5	$20~02~\pm 1.25$	25.31 ± 1.31	346.3 ± 17.1	203 ± 18
RB10	21.07 ± 1.23	24.29 ± 2.17	347.6 ± 15.9	205 ± 22
WB5	$20\ 12\ \pm\ 1.45$	25.54 ± 2.54	348.4 ± 180	205 ± 15
WB10	2026 ± 1.73	25.14 ± 2.99	347.6 ± 15.9	203 ± 18
Values	s are means \pm SI	D for n = 10	NS not signifi	cant

Table 3. Effect of experimental diets on cecum and colon in rats

Dietary group	Çec	Çecum		Colon		
	Tissue weight	Content weight	Tissue weight	Content weight	Length	
	g g	g	g	g	cm	
CC	$0.541 \pm 0.080^{\circ}$	$2.40 \pm 0.30^{\rm b}$	0.842 ± 0.060^d	$1.50 \pm 0.46^{\circ}$	13.64 ± 1.53^{b}	
P5	0.756 ± 0.103^{4}	3.50 ± 0.56^{a}	0.969 ± 0.114^{lx}	2.13 ± 0.77^{b}	15.10 ± 104^{a}	
RB5	0.612 ± 0.057^{bc}	$2.74 \pm 0.65^{\text{b}}$	1.059 ± 0.254^{b}	$2.38 \pm 0.77^{\rm b}$	$15.21 \pm 0.81^{\circ}$	
RB10	0.690 ± 0.074^{ab}	3.43 ± 0.58^{a}	$1.271 \pm 0.112^{\circ}$	3.31 ± 0.67	$14.81 \pm 0.95^{\circ}$	
WB5	$0.583 \pm 0.075^{\circ}$	$2.59\pm0.34^{\text{b}}$	$0.889\pm0.040^{ m cd}$	$2.29 \pm 0.50^{\circ}$	15.13 ± 1.14^{a}	
WB10	$0.571 \pm 0.074^{\circ}$	2.43 ± 0.35^{b}	$0.958 \pm 0.067^{\mathrm{bol}}$	2.15 ± 0.49^{b}	14.81 ± 0.95^{a}	

Values are means \pm SD for n = 10

Means in column not sharing common superscripts are significantly different (P < 0.05)

ghest in the RB10 group, followed by RB5 group, P5

3. Small-bowel measurement

Total length and 5-cm jejunal weight of small intestine of P5 were the greatest (Table 6). Total lengths of P5 and RB10 were significantly longer than that of the CC

Table 4. Effect of experimental diets on fecal weight and fecal moisture content in rats

Dietary group	Fecal weight	Moisture content
	g / day	%
CC	1.19 ± 0.18^{e}	$20.6 \pm 5.7^{\circ}$
P5	$1.64\pm0.23^{\rm d}$	$26.2\pm8.8^{\rm bc}$
RB5	$2.61 \pm 0.28^{\rm h}$	26.1 ± 5.6^{bc}
RB10	5.15 ± 0.90^{a}	34.1 ± 5.0^{a}
WB5	2.05 ± 0.24^{c}	$27.7 \pm 6.5^{\rm h}$
WB10	2.54 ± 0.17^{b}	$28.9\pm3.9^{\mathrm{ab}}$

Values are means \pm SD for n = 10

Means in column not sharing common superscripts are significantly different (P < 0.05)

Table 5. Correlations among tissue and content weights and fecal mass

	Cecal content	Cecal tissue weight	Colonic content	Colonic tissue weight	Colon length
Fecal mass	0.376**	0.237	0.647***	0.727***	0.444**
Cecal content		0.668***	0.353**	0.418**	0.452***
Cecal tissue weight			0.254	0.359**	0.412**
Colonic content				0.612***	0.627***
Colonic tissue weight					0.478***

^{**}P < 0.01, *** P < 0.001

Table 6. Effect of experimental diets on small intestine in rats

Dietary group	Total length	Jejunal weight	Mucosa
	¢m	g/5 cm jejunum	g / 5 cm jejunum
	$89.6 \pm 5.0^{\circ}$	0.418 ± 0.042^{c}	0.129 ± 0.023^{b}
P5	98.1 ± 8.0^{a}	0 489 <u>+</u> 0.037°	0.233 ± 0.051^a
RB5	$93.3 \pm 6.1^{ m abc}$	0.454 ± 0.028^{abc}	0.237 ± 0.022^{a}
RB10	96.5 ± 3.0^{ab}	$0.467 \pm 0.052^{\mathrm{ab}}$	0.276 ± 0.054^{a}
WB5	$91.7 \pm 4.8^{\text{bc}}$	0.430 ± 0.040^{bc}	0.213 ± 0.031^{a}
WB10	91.2 ± 5.4^{bc}	0.439 ± 0.031 ^{lsc}	0.226 ± 0.037°

Values are means \pm SD for n = 10

Means in column not sharing common superscripts are significantly different (P < 0.05)

group. On the other hand, those of the wheat bran groups were not significantly different from those of the CC group. Mucosal weights of pectin-fed and bran-fed rats were significantly higher than those of the CC group.

4. Morphology of jejunal mucosa

Fig. 1 and Fig. 2 show mucosa surface structures of the jejunum of rats fed different kinds and amounts of dietary fibers for 6 weeks. This was observed using SEM at magnifications of 100 and 300 times. Scanning-electron micrographs of jejunum from the 1% cellulose control rats showed regular long-shaped villi. Feeding pectin or bran had an apparent effect on the ultrastructure of the small intestine. Rats fed pectin had typical leaf-shaped villi. Rats fed rice bran or wheat bran also had leaf-shaped thick villi, but the villi of wheat bran-fed animals had more irregular shapes than rice bran-fed animals. In Figure 2, the apices of the small intestinal villi of rats on a pectin diet had a line of cellular injury.

5. Disaccharidase activity in homogenate of jejunal mucosa

Table 7 shows the activity of maltase and sucrase, which are brush-border marker enzymes. Total activity and specific activity of maltase per cm of jejunum was greatest RB10, followed by RB5, WB10, and WB5. The activity in rats fed bran diets was greater than that of pectin-fed rats and CC rats. The trend of the activity of sucrase and maltase was similar.

DISCUSSION

This study examined and compared the effects of rice bran and wheat bran on intestinal physiology and small-bowel morphology. Their effects were also compared to that of pectin (soluble fiber). Rice bran is known to be a more soluble fiber source than wheat bran.⁵ Data analysis on domestic rice bran proved that rice bran had soluble fiber of less than 2%,² 3.12%,¹⁶ and 3.39%¹⁵ of total

Table 7. Effect of experimental diets on disaccharidase activities of small intestinal mucosa in rats

Dietary group	Ma	Maltase		Sucrase		
	units / cm	units / mg protein	units / cm	units / mg protein		
CC	$1.365 \pm 0.207^{\circ}$	$0.689 \pm 0.164^{\circ}$	$0.207 \pm 0.049^{\circ}$	$0.106 \pm 0.033^{\circ}$		
P5	1.412 ± 0.318^{c}	0.902 ± 0.337^{bc}	$0.250 \pm 0.057^{\circ}$	0.160 ± 0.060^{6}		
RB5	2.622 ± 0.331^a	$1.345\pm0.403^{\circ}$	0.511 ± 0.061^{b}	$0.267\pm0.091^{\circ}$		
RB10	$2.716 \pm 0.739^{\circ}$	1.149 <u>~</u> 0.324 ^{ab}	$0.620 \pm 0.200^{\circ}$	0.258 ± 0.061^{a}		
WB5	1.700 ± 0.296^{bc}	$1.087 \pm 0.321^{\rm eb}$	$0.291 \pm 0.083^{\circ}$	0.184 ± 0.049^{b}		
WB10	2.021 ± 0.443^{b}	$1.293 \pm 0.725^{\circ b}$	$0.419 \pm 0.112^{\rm h}$	0.260 ± 0.134^{a}		

Values are means \pm SD for n = 10

Means in column not sharing common superscripts are significantly different (P < 0.05)

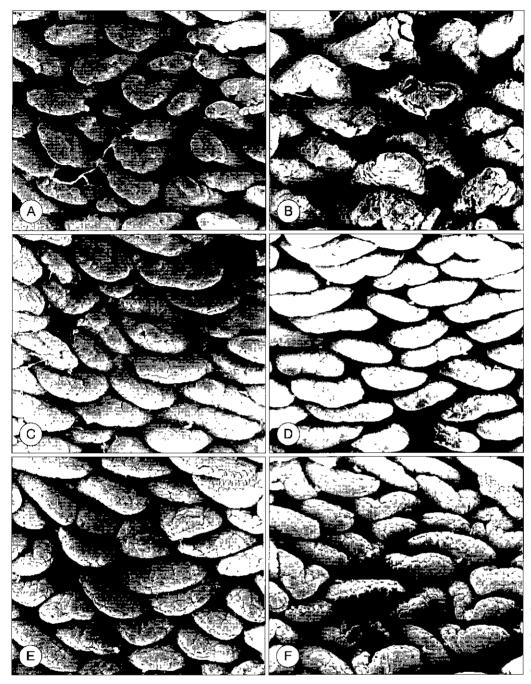


Fig. 1. Scanning electron micrographs of jujunal mucosal surface (×100)

From the top, the photographs are the cellulose control (A). 5% pectin group (B), rice bran group containing 5% dietary fiber (C), rice bran group containing 10% dietary fiber (D), wheat bran group containing 5% dietary fiber (E), and wheat bran group containing 10% dietary fiber (F).

dictary fiber depending on investigators, and major components of fiber like hemicellulose, cellulose, and lignin.²⁾ The reported figures as a percentage of total dietary fiber in domestic rice bran are 26.82%, 25.57%,²⁾ and 22.43%.¹⁴⁾ Wheat bran is relatively unfermented in the colon, mainly because of the highly branched molecular structure of arabinoxylans and the presence of lignin.⁵⁾

Rice bran and wheat bran at the 5% and 10% level of dietary fiber and pectin at the level of 5% did not affect food intake and weight gain for 6 weeks when compared to the control, 1% cellulose diet group. Although incre-

ases in cecal tissue weight were the highest in the pectinfed group, rice bran was more effective than wheat bran in hypertrophy of cecal tissue. However, colonic tissue weight increased the most in rice bran-fed groups, followed by the pectin-fed group and the wheat bran-fed group. At the same time, colonic content and stool weight was highest in rice bran-fed groups. These results were similar to those reported by Gestel *et al.*¹⁵ In their study, fecal nitrogen content and bacterial mass were greatly and significantly increased by rice bran, and to a lesser extent by wheat bran, compared to the fiber-free

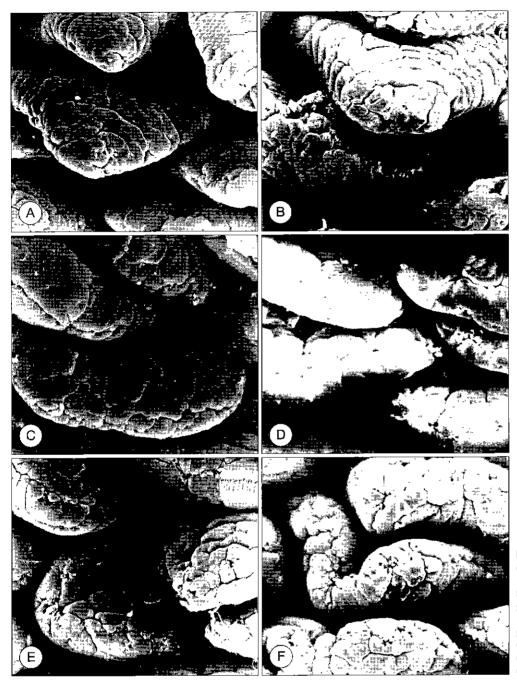


Fig. 2. Scanning electron micrograph of jujunal villi from rats (×300)
From the top, the photographs are the cellulose control (A). 5% pectin group (B), rice bran group containing 5% dietary fiber (C), rice bran group containing 10% dietary fiber (D), wheat bran group containing 5% dietary fiber (E), and wheat bran group containing 10% dietary fiber (F).

control diet. Rice bran produced the greatest stimulation of colonic mucosal growth. The trophic effects of rice bran could be mediated through its fermentation products, namely short-chain fatty acids.¹⁷⁹¹⁸ Jacobs and White¹⁹⁸ observed that the feeding of 20% wheat bran resulted in an increase in mucosal wet weight of the cecum, proximal colon and the distal colon when compared to the control group on the fiber-free diet. This increase in mucosal mass was also associated with the development of cell hyperplasia, as demonstrated by the increase in DNA.¹⁹⁹ As Jacobs stated, these results show

that the modulation of small intestinal mucosal structure and growth by dictary fiber appears to be modulated through alterations in cell proliferation, and that these changes depend not only on the quantity but also the quality of the fiber present in the diet.²⁰⁰ Altered nutriture due to dietary fiber in the intestine causes adaptive responses in the morphology and function of intestinal mucosa.

The weights in cecal tissue and colonic tissue weight were positively associated with stool weight (Table 5). Rice bran was the most effective in increasing stool we-

ight and even more effective than pectin, the soluble fiber. The water-holding capacity (WHC) of dietary fiber has been proposed to be of value in predicting the ability of dietary fiber to alter stool weight.²¹⁾ Also, fecal bulking requires insoluble cell wall components like lignin and cellulose. The increase in intestinal bulk leads to the dilution of luminal contents and the shorter mucosal contact time due to faster transit.²²⁾

We observed that only the pectin and WB10 groups had significantly longer small-bowels and 5-cm jejunum weights than the CC group. Pectin exerted the greatest tropic effect on the jejunum. In our study wheat bran increased neither the total length of the small intestine nor the weight of the 5-cm jejunum as compared to 1% cellulose. Jacob and White obtained similar results. They observed that small intestinal mucosal weight, DNA, and DNA synthesis did not change with wheat bran feeding.¹⁹ Stark et al.23 also observed that pectin feeding led to significant elongation of both the small and large intestines, whereas cellulose only affected colon length. Computerized image analysis of intestinal cross-sections also showed enlarged muscle area in the ileum and midcolon of pectin-fed rats and greater mucosal area in the colon. A similar result was obtained in the ileum of rats fed psyllium or oat bran.24 On the other hand, wheat bran, an insoluble fiber containing cellulose, did not increase smooth muscle thickness in the ileum. The morphological adaptation following soluble fiber feeding may be necessary to provide the extra work to propel a highly viscous gel such as pectin through the length of the intestine.24 Therefore, the increased tissue weight in rats fed rice bran in this study indicates that rice bran may share a property of more viscous soluble fiber.

Dietary fibers have differing effects on different regions of the luminal environment depending on their fermentability. It appears that slowly fermented fibers have a greater influence on the distal environment. McIntyre *et al.*²⁵ observed feces of rats fed 10% wheat bran had total short chain fatty acid levels three times the levels seen with 10% guar gum or 10% oat bran diet. They speculated that highly fermentable oat bran and guar gum are fully fermented in the proximal large bowel and so fail to influence the distal luminal environment because SCFAs are rapidly absorbed. In this study, rice bran had a greater effect on the physiology of the colon than that of the small-bowel or cecum, whereas pectin had the opposite effect.

Undigested soluble polysaccharides form a highly viscous gel in the gastrointestinal tract. Their gel-forming properties are thought to cause damage to the intestinal mucosal structure.²⁶⁾ In our study we observed damaged mucosa in rats fed pectin. On the contrary, rice bran feeding resulted in very regular, compact, and intact mucosa of jejunum. This result is in agreement with the observation that rice bran probably exerts mucosa-protective effects, as did wheat bran to a lesser extent.⁵¹

Maltase and sucrase serve as villus tip marker enzymes. Some studies haveshown that insoluble dietary fiber such as cellulose and bran have no effect on brush-border enzymes. The effects of soluble dietary fibers such as pectins are less straightforward and often contradictory. 277-291 Thomsen and Tasman-Jones investigated the effect of pectin(5%) and cellulose(10%) on jejunal lactase, sucrase, and maltase and found that pectin decreased maltase and sucrase activities but cellulose had no effect. In the present study, we observed that total and specific activities of maltase and sucrase of rice bran-fed groups and, to a lesser extent, wheat bran-fed groups were elevated ascompared to those of the control group and the pectin group.

It seems that pectin exerts its effects on the small-bowel and cecum, whereas brans, especially rice bran, affects the colon both directly and indirectly in ways such as fermentation. We conclude that rice bran, sharing properties of insoluble fiber such as wheat bran, has effects on intestinal physiology similar to those of soluble fiber.

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