Effect of Pectin-Wheat Bran Blends on Rat Blood Lipid and Fecal Responses and on Muffin Quality[1]

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ABSTRACT

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Blends of pectin, a water-soluble fiber, and wheat bran, a rich source of insoluble fiber, were tested for their effect on blood lipid levels and fecal responses in rats. A pectin and bran blend that contained about equal amounts of each component produced a pronounced blood cholesterol-lowering effect in both hypercholesterolemic (cholesterol-fed) and normocholesterolemic rats. In hypercholesterolemic rats, the same blend also lowered liver cholesterol levels quite significantly. This blend produced quite desirable fecal responses in terms of fecal weight, volume, and density. Used in muffins to replace 20% of the flour in the formula, the 1:1 pectin-bran blend increased the total and soluble-fiber contents of the muffins severalfold and reduced their caloric content by 14%.

High blood cholesterol and low fecal bulk are considered the major risk factors in coronary heart disease and colon cancer, respectively. Food and food ingredients high in soluble fiber may lower blood cholesterol (Chen et al 1981, Judd and Truswell 1985), but they usually have a low fecal bulking capacity (Nyman et al 1990, Ranhotra et al 1991). Foods high in insoluble fiber are quite effective in increasing fecal bulk and improving colonic functions (NCI 1985, Anderson 1986, Pilch 1987, Nymen et al 1990), but most do not lower cholesterol (Pilch 1987, Vigne et al 1987). Blending food ingredients high in soluble fiber, such as pectin, with foods high in insoluble fiber, such as wheat bran, may yield a blend with dual functionality: lowering cholesterol and increasing fecal bulk.

Pectin and wheat bran are both readily available commercially. They are economically priced and have been used traditionally in many foods. They were the materials of choice in this study to examine the dual functionality aspects of fiber. Their blends can readily be incorporated into a variety of grain-based foods that are usually not rich sources of soluble fiber.

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MATERIALS AND METHODS

Test Materials and Diets

Wheat bran from a hard red winter wheat was obtained from the pilot mill facilities of the Grain Science Department, Kansas State University, Manhattan, KS. High methoxypectin (unipectine RS pectin) was obtained from Sanofi Bio-Industries, Waukesha, WI. High methoxypectin was chosen, because it was reported to be more effective at lowering cholesterol than low methoxypectin (Judd and Truswell 1985). Compositional information on these materials is presented in Table I.

Eight test diets were prepared: four contained cholesterol (diets A–D) and four did not (diets AA–DD) (Table II). A fiber-free, cholesterol-free diet was also prepared. All diets, except the fiber-

TABLE I
Percent Composition of Bran and Pectin[a]

Measurement	Wheat Bran	Pectin
Moisture	14.3	7.2
Protein (N × 5.7)	14.7	2.0
Ash	6.9	1.5
Fat (acid hydrolysis)	5.6	0.4
Total dietary fiber	47.6	61.0
Insoluble fiber	44.8	0.0
Soluble fiber	2.8	61.0
Carbohydrates	10.9	27.9

[a] As-is basis.

free diet, contained the same level (23.8%) of total dietary fiber, but they differed greatly in the content of soluble fiber (pectin and bran levels differed). All diets were complete in nutrients required by the rats (NRC 1987). Diets were kept frozen and were withdrawn only in amounts needed for daily feeding.

Animals

Eighty-five male, weanling rats of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were randomly assigned to eight groups of 10 rats (fed fiber-containing diets) and one group of five rats (fed fiber-free diet). They were housed individually in mesh-bottomed stainless steel cages in a controlled environment. Although rats consumed adequate amounts of diet, their food intake was restricted to achieve identical intakes between groups. Deionized water was offered *ad libitum*. Body-weight records were maintained.

Blood and Liver Sampling

At the end of weeks 2 and 4, all rats were fasted overnight then lightly anesthetized (under ether); about 2 ml (1 ml at week 2) of blood was withdrawn by cardiac puncture. The blood was allowed to clot, then centrifuged (2,000 rpm, 8 min) to obtain the serum. Lipid analyses were run on the refrigerated serum over the next two days. At the end of week 4, all rats were sacrificed; their livers were removed, blotted dry, weighed, and homogenized. An aliquot of the liver homogenate was used for cholesterol determination.

Fecal Collection and Gut Weight

Feces of rats fed diets AA–DD and the fiber-free diet were collected quantitatively throughout the four-week study period. Collected feces from individual rats were then pooled, air-dried, and weighed. The fecal volumes were determined. Gut weight included the entire gastrointestinal tract after the gut contents were removed and discarded and the gut had been thoroughly washed and blotted dry.

Analytical

The standard AACC methods (AACC 1983) were used to determine moisture, protein, ash, and fat (acid hydrolysis) in bran, pectin, and muffins. Total, insoluble, and soluble fiber were determined by the enzymatic-gravimetric method of Prosky et al (1992). Total and high-density-lipoprotein (HDL) cholesterol in serum were determined enzymatically using kit 352 from Sigma Chemical Co., St. Louis, MO. HDL-cholesterol was determined after phosphotungstic acid precipitation of non-HDL-cholesterol fractions. Serum triglycerides were determined enzymatically using kit 336 from Sigma. Total cholesterol in liver homogenate was determined by the method of Abell et al (1952). Dry fecal volume was determined in a long-stemmed, graduated cylinder, using fine sand as the embedding medium. Density was calculated by dividing fecal dry weights by volume.

Statistical

Mean comparisons were made with Duncan's multiple-range test using the Statistical Analysis System (SAS 1982).

RESULTS AND DISCUSSION

Test Materials and Diets

The water-soluble pectin used in this study contained dextrose as a major added component. The wheat bran was high in total fiber, but it contained very little soluble fiber (Table I). It was tested at a maximum level of 50% (diets A and AA). This level was reduced, through the addition of pectin, to formulate other diets without affecting the total fiber content of these diets (Table II). The average values for total and soluble fiber in the sets of diets B and BB and C and CC represent a ratio typical of the American diet (soluble fiber being about one-third of the total fiber intake). Diets D and DD provided appreciably more soluble fiber.

Diet Intake and Body Weight Gains

With the exception of rats fed diet D, the diet intakes and, thus, the total fiber intakes of rats in other groups were identical. A few rats fed diet D (highest in pectin) lagged slightly behind in diet and, thus, in fiber intake (Table III). Rats fed the highest level of pectin also showed the lowest weight gains, whether or not they were fed cholesterol. Liver weights were nearly twice as high in rats fed cholesterol (diets A–D) than in those not fed cholesterol (diets AA–DD). This may, in part, be due to excessive cholesterol accumulation or fatty infiltration of the liver.

Serum Total and HDL Cholesterol

Feeding cholesterol and cholic acid elevates serum cholesterol levels profoundly (Story et al 1974, Ranhotra et al 1992). This occurred in this study also (diets A–D vs. diets AA–DD). Cholesterol levels were highest, at both weeks 2 and 4, in rats fed the all-bran diet with cholesterol (Table III). The level of 297 mg/dl matched closely with a cholesterol value of 261 mg/dl

TABLE II
Percent Composition of Test Diets[a]

	Diets With Cholesterol				Diets Without Cholesterol			
Measurement	**A**	**B**	**C**	**D**	**AA**	**BB**	**CC**	**DD**
Ingredients								
Wheat bran	50	40	30	20	50	40	30	20
Pectin	...	7.8	15.6	23.4	...	7.8	15.6	23.4
Casein	1.6	2.4	3.2	4.0	1.6	2.4	3.2	4.0
Gluten	1.9	2.8	3.8	4.7	1.9	2.8	3.8	4.7
Soybean oil	9.1	9.6	10.1	10.5	9.1	9.6	10.1	10.5
Cholesterol	1	1	1	1	...	...	...	...
Cholic acid	0.2	0.2	0.2	0.2	...	...	...	...
Constant ingredients[b]	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Sucrose	18.5	16.9	15.2	13.6	19.1	17.5	15.8	14.2
Cornstarch	13.0	14.6	16.3	17.9	13.6	15.2	16.9	18.5
Composition[c]								
Total fiber	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8
Soluble fiber	1.4	5.9	10.4	14.8	1.4	5.9	10.4	14.8
Insoluble fiber	22.4	17.9	13.4	9.0	22.4	17.9	13.4	9.0
Fat	12	12	12	12	12	12	12	12
Protein	10	10	10	10	10	10	10	10
Calories from fat[c]	37	36	36	35	36	35	35	34

[a] Including fiber-free diet containing 6.0% casein, 7.1% gluten, 11.6% soybean oil, 4.7% constant ingredients, 35.3% sucrose, and 35.3 cornstarch.
[b] Contained 1% vitamin mix (American Institute of Nutrition mix 76), 3.5% mineral mix (AIN mix 76) and 0.2% *dl*-methionine.
[c] Calculated values.

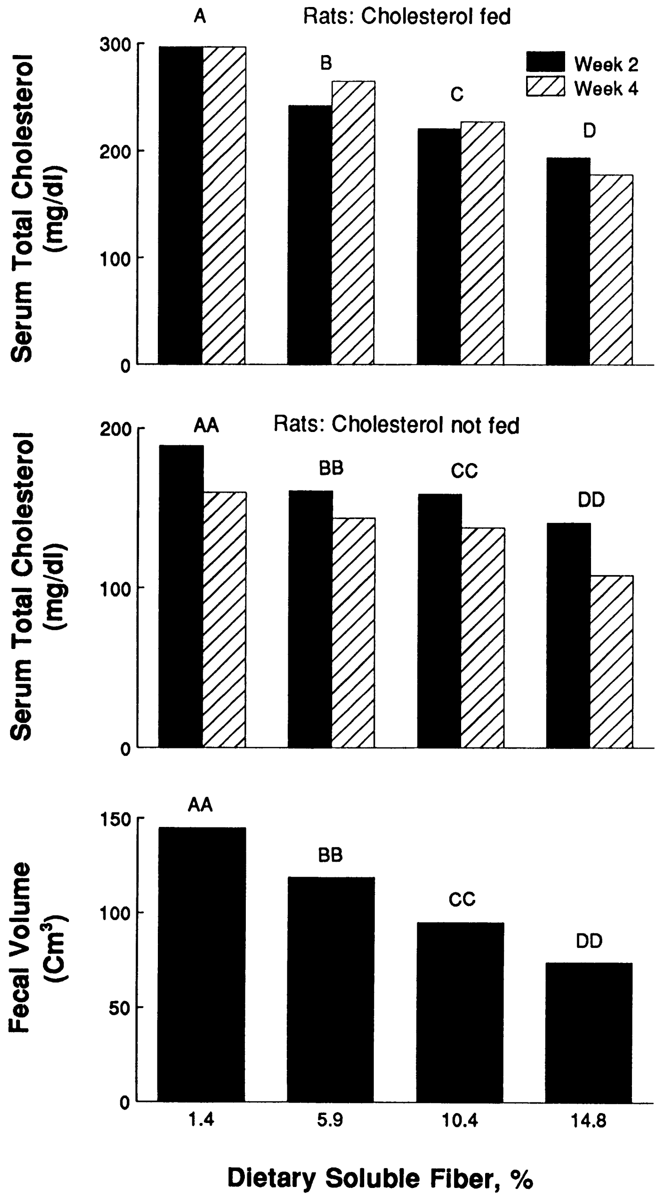

Fig. 1. Serum total cholesterol and fecal volume responses in rats fed pectin and wheat bran blends containing different levels of soluble fiber. A–D, test diets containing cholesterol; AA–DD, cholesterol-free test diets.

recently reported (Rooney et al 1992) in young adult rats fed cholesterol and wheat bran for three weeks. When bran in the diet was increasingly replaced with pectin, serum cholesterol levels fell (Table III, Fig. 1). The bran and pectin blend containing nearly equal amounts of each component affected serum cholesterol levels quite favorably, whether or not cholesterol was added to the diet. Cholesterol-lowering effect may have been even more pronounced at still higher levels of pectin in the blend than tested. Pectin is reported to lower cholesterol by interfering with cholesterol and bile-acid absorption or through inhibition of hepatic cholesterol synthesis as mediated by short-chain fatty acids produced in the colon. (Leveille and Sauberlich 1966, Kay and Truswell 1977, Kelley and Tsai 1978, Chen et al 1981, Judd and Truswell 1985, Ahrens et al 1986).

In rats, serum HDL-cholesterol is the major cholesterol fraction. High HDL-cholesterol levels provide protection against heart disease (Pilch 1987). In both sets of diets (A–D and AA–DD), HDL-cholesterol, as percent of total cholesterol, tended to increase as bran was replaced with increased amounts of pectin (Table III).

Serum Triglycerides

Elevated serum triglyceride levels are viewed by some as an independent risk factor in heart disease (Austin 1991). Triglyceride levels were lower in rats fed cholesterol than in those not fed cholesterol. The physiological significance of this is difficult to explain. Wheat bran appeared to lower triglyceride levels initially (week 2), as observed also by Vigne et al (1987), but this effect did not persist (Table III).

Liver Cholesterol

Liver cholesterol levels in rats fed cholesterol-free diets (diets AA–DD) were low and typified values reported in other studies (Ranhotra et al 1990), but levels were elevated in rats fed diets containing cholesterol (Table III). Increasing the amount of pectin substituting for bran caused significant ($P < 0.05$) progressive lowering of liver cholesterol, a response similar to that noted for serum cholesterol and also reported by others (Leveille and Sauberlich 1966, Judd and Truswell 1985).

Intestinal Responses

Fiber-free diets, as the current study also revealed, produce very little fecal bulk. Over the four-week period, rats fed the fiber-free diet produced only 6 g of dry feces (Table IV). In rats fed the fiber-containing diets, those fed the bran-based diet (diet AA) had the highest fecal bulk and volume (Table IV, Fig. 1). As more pectin was progressively substituted for bran, fecal weights and volumes decreased proportionally. However, both

TABLE III
Serum and Liver Responses in Rats Fed Various Pectin and Bran Blends for Four Weeks[a]

Measurement	Diets							
	A	B	C	D	AA	BB	CC	DD
Fiber intake, g								
Total fiber	60.2	60.2	60.2	59.2	60.2	60.2	60.2	60.2
Soluble fiber	3.5	14.9	26.2	36.9	3.5	14.9	26.2	37.5
Body weight gain, g[b]	55 ± 5 c	55 ± 4 c	56 ± 3 c	48 ± 5 d	61 ± 3 a,b	65 ± 2 a	63 ± 5 a	59 ± 4 b,c
Liver weight, g	6.5 ± 0.5 a	6.4 ± 0.2 a	6.4 ± 0.5 a	5.8 ± 0.7 b	3.3 ± 0.2 d	3.4 ± 0.3 c,d	3.5 ± 0.3 c,d	3.8 ± 0.3 c
Serum total cholesterol, mg/dl								
Week 2	297 ± 78 a	242 ± 60 b	221 ± 44 b,c	194 ± 31 c,d	189 ± 7 c,d	161 ± 15 d,e	159 ± 11 d,e	141 ± 10 e
Week 4	297 ± 73 a	265 ± 58 a,b	227 ± 32 b	178 ± 45 c	160 ± 13 c	144 ± 18 c,d	138 ± 14 c,d	108 ± 13 d
Serum triglycerides, mg/dl								
Week 2	39 ± 6 e	48 ± 8 d,e	46 ± 8 e	57 ± 12 c,d	60 ± 7 b,c	68 ± 9 b	68 ± 12 b	89 ± 17 a
Week 4	58 ± 10 b,c	54 ± 7 b,c,d	47 ± 10 d	51 ± 15 c,d	75 ± 10 a	76 ± 13 a	66 ± 10 a,b	62 ± 12 b,c
Serum HDL cholesterol, % of total cholesterol								
Week 2	10 ± 4 e	12 ± 6 e	15 ± 5 e	28 ± 10 d	53 ± 5 c	63 ± 7 b	72 ± 8 a	69 ± 5 a
Week 4	8 ± 3 e	8 ± 3 e	12 ± 4 e	24 ± 9 d	63 ± 4 c	66 ± 5 b,c	70 ± 6 b	79 ± 3 a
Liver cholesterol, mg/g	137 ± 12 a	126 ± 9 b	100 ± 11 c	82 ± 7 d	3 ± 0 e	3 ± 0 e	3 ± 1 e	3 ± 0 e

[a] Values are average ± SD (8–10 rats per diet). Within a row, means not sharing the same letter are significantly different ($P < 0.05$). Diets A–D contained cholesterol; diets AA–DD did not.
[b] Initial body weight: 50 ± 5 g.

TABLE IV
Intestinal Responses in Rats Fed Various Pectin and Bran Blends for Four Weeks[a]

Measurement	Diet AA	Diet BB	Diet CC	Diet DD
Fecal amounts[b]				
Dry weight, g	61 ± 2 a	51 ± 2 b	45 ± 4 c	42 ± 4 d
Dry volume, cm^3	145 ± 12 a	119 ± 9 b	95 ± 6 c	74 ± 7 d
Density, g/cm^3	0.42 ± 0.02 c	0.43 ± 0.01 c	0.47 ± 0.04 b	0.57 ± 0.03 a
Wet-to-dry feces ratio				
Week 1	2.3 ± 0.2 a	2.3 ± 0.2 a	2.5 ± 0.3 a	2.4 ± 0.3 a
Week 3	1.9 ± 0.2 a	1.8 ± 0.2 a,b	1.7 ± 0.1 b,c	1.6 ± 0.2 c
Intestinal weight, g[c]	4.5 ± 0.4 b	4.4 ± 0.2 b	5.0 ± 0.4 a	5.3 ± 0.4 a

[a] Values are average ± SD (8–10 rats per diet). Within a row, means not sharing the same letter are significantly different ($P < 0.05$).
[b] In the fiber-free diet: dry weight, 6 ± 1 g; dry volume, 6 ± 1 cm^3; dry density, 1.00 ± 0.08, g/cm^3.
[c] Entire gastointestinal tract. Intestinal weight of rats fed the fiber-free diet, 3.9 ± 0.4 g.

TABLE V
Muffin Formula and Composition

Measurement	Muffin Control	Muffin Test
Formula, %		
Bread flour	100	80
Pectin + bran blend	...	20
Constant ingredients[a]	76.75	76.75
Water	70	95
Scores		
External (maximum, 30)	25.25	23.75
Internal (maximum, 70)	57.25	57.25
Total (maximum, 100)	82.50	81.00
Average value, per muffin		
Width, cm	6.8	6.9
Height, cm	5.8	5.2
Weight, g	61.8	61.6
Composition, per muffin		
Moisture, g	15.6	18.1
Protein, g	3.0	2.6
Ash, g	1.4	1.5
Fat, g	2.9	1.8
Total fiber, g	1.0	3.6
Insoluble fiber, g	0.6	1.6
Soluble fiber, g	0.4	1.9
Carbohydrates, g	37.9	34.0
Energy, calories	190	163

[a] Granulated sugar, 50%; dry honey, 10%; salt, 2%; baking soda, 2.5%; baking powder (BL-60), 2.25%; and shortening, 10%.

measurements were still much higher than those of the fiber-free diet. Fecal density was the lowest in rats fed the all-bran diet, signifying a greater fecal bulk. Densities of the other three diets (diets BB–DD) were only a little higher, but they were still quite remote from the density value of 1.0 obtained for the fiber-free diet.

The water-holding capacity of feces appeared to change as animals adapted to the fiber-containing diets; wet-to-dry fecal ratios (established on freshly voided fecal pellets) suggest a decrease in this capacity (week 1 vs. week 3) with time and also with a decrease in the level of bran in the diet.

Feeding of high-fiber diets has been shown to cause intestinal cell proliferation and, thus, enlargement of the gut. This occurred, as indicated by gut weights, in this study also (gut weight on fiber-free diet, 3.9 g) (Table IV). The high-pectin diets (diets CC and DD) triggered significantly ($P < 0.05$) greater cell proliferation than did the low-pectin (diet BB) or pectin-free diet (diet AA). This may be due to more extensive fermentation of pectin in the gut, or it may be because the gels the pectin forms in the gut need propelling, thus stimulating cell growth or proliferation.

Product Making

Viewing all physiological responses collectively, it seems that the bran and pectin blend which contained nearly equal amounts of each component (diets D and DD), would promote quite favorable blood and liver cholesterol responses as well as fecal responses. This blend was used to replace 20% wheat flour (higher replacement produced unsatisfactory product) in a traditional muffin formula (Table V). The resultant product scored nearly as high as the control product. It contained about four times more total fiber, five times more soluble fiber, and 14% fewer calories. This blend may be suitable for a variety of other bakery products.

CONCLUSIONS

Fiber sources high in insoluble fiber, such as wheat bran, and soluble fiber, such as pectin, usually evoke different physiological responses. Combining the two in an appropriate ratio may provide a duality of response. This study revealed that wheat bran and pectin can be combined in near equal amounts for this purpose.

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