

# Effect of Wheat Bran Fiber and Bran Particle Size on Fat and Fiber Digestibility and Gastrointestinal Tract Measurements in the Rat

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## ABSTRACT

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The effect of wheat bran (AACC hard red) and bran particle size on fat and fiber digestibility and gastrointestinal tract measurements were investigated with diets containing 5.7–10.7% dietary fiber. Fifty-six male weanling Sprague-Dawley rats were randomly assigned to four diets containing 5% cellulose (C5); 10.5% cellulose (C10); 21.5% coarse (2 mm) wheat bran (CB); or 22.2% fine (0.5 mm) wheat bran (FB) in a six-week study. Dietary fiber digestibilities were significantly different ( $P < 0.05$ ) among treatment diets (CB > FB > C5 > C10) but there was no effect in fat digestibility among treatments. High-fiber diets fed to rats resulted in significantly greater wet and dry fecal weights than low-fiber diets. Bran diets resulted in significantly higher fecal moisture than cellulose diets. Cecum lengths increased significantly with bran diets

compared with cellulose diets. The CB diet resulted in significantly higher stomach weights than with cellulose diets. Stomachs were heavier and cecal lengths were greater with bran diets than with cellulose diets; however, a high-cellulose diet resulted in increased colon weight. Except for higher fiber digestibility of coarse bran, bran particle size had no significant effects. Healthful effects of wheat bran may be associated with gastrointestinal morphology and function. Fecal bulking and decreased intestinal transit time can prevent constipation and may dilute or reduce absorption of toxic or carcinogenic metabolites, thus improving gastrointestinal health and lowering the risk of tumor development and cancer.

Increased consumption of dietary fiber is reported to be beneficial in protecting against colon cancer (Burkitt 1971; Cummings et al 1976), as well as in lowering plasma cholesterol and lipids (Ranhotra et al 1977; Jenkins et al 1979; Moore et al 1985). McIntyre et al (1993), Govers et al (1999), and Compher et al (1999) reported that there is reduced risk of colon cancer with wheat bran diets. A review by Brodribb (1980) indicates that wheat bran, a common source of dietary fiber, is effective in preventing diverticular disease. Efforts to clarify these beneficial effects led to investigations of the influence of dietary fiber particle size on various physiological responses. Brodribb and Groves (1978), Heller et al (1980), and Wrick et al (1983) reported greater stool weights with coarse bran than with fine bran in human subjects; these were attributed to higher fecal moisture. However, Mongeau and Brassard (1985) observed no significant effect of bran particle size on fecal moisture in rats. Cummings (1978) and Ali et al (1981) observed more fecal fat with high fiber diets, but concluded that the increase in fecal fat was nutritionally nonsignificant. However, Mongeau et al (1986) reported an increase of 68% in fecal fat when bran was included in a rat diet.

This study was undertaken to investigate the influence of wheat bran and bran particle size on dietary fiber and fat digestibilities as well as on digestive tract organ lengths and weights.

## MATERIALS AND METHODS

Fifty-six male weanling Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) were kept in a controlled environment at  $21 \pm 1^\circ\text{C}$ , 65% rh, with 12-hr automatic illumination (6 A.M. to 6 P.M.) daily. Animals were housed in individual stainless steel, wire-bottom cages that were equipped with an automatic water system for drinking and flushing. After being fed the AIN-76A diet for seven days, animals were randomly assigned to four treatment groups with 14

rats per group. Two bran treatments were designed to correspond in fiber content to a high ( $\approx 10\%$ ) dietary fiber diet for humans. Based on an initial determination of dietary fiber (Prosky et al 1988) in bran and cellulose, diets were formulated to contain 10% dietary fiber (a high-cellulose control and two bran treatments). The fourth diet was low-cellulose (5%) AIN-76A and used as a reference treatment. Diets contained 5% cellulose (C5), 10.5% cellulose (C10), 21.5% coarse (through 2-mm screen; mean particle size 0.684 mm) American Association of Cereal Chemists (AACC) hard red wheat bran (CB), or 22.2% fine (through 0.5-mm screen; mean particle size 0.341 mm) AACC hard red wheat bran (FB). Analyses showed that dietary fiber content of the diets was 5.7, 10.7, 10.7, and 10.0% for C5, C10, CB, and FB, respectively.

The two mean particle sizes of wheat bran used were chosen to compare the effects of distinctly different particle sizes, as well as to approximate the particle size range of processed wheat bran in commercial use. Cellulose particle size was 0.08–0.10 mm; no larger particle size of cellulose was commercially available. Composition of the diets is given in Table I. Diet ingredients were obtained

TABLE I  
Composition of Diets

Ingredient	Diet (g/kg, dry matter) <sup>a</sup>			
	C5	C10	CB	FB
HR wheat bran	...	...	215	222
Corn starch	300	245	135	128
Cellulose	50	105	...	...
Sucrose	350	350	350	350
Casein	200	200	200	200
Corn oil	50	50	50	50
Mineral mix <sup>b</sup>	35	35	35	35
Vitamin mix <sup>b</sup>	10	10	10	10
DL-Methionine	3	3	3	3
Choline bitartrate	2	2	2	2
Dietary fiber <sup>c</sup>	57	107	107	100

<sup>1</sup> Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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<sup>a</sup> C5 = 5% cellulose, C10 = 10.5% cellulose, CB = 21.5% AACC hard red (HR) wheat bran milled through a 2-mm screen, and FB = 22.2% HR wheat bran milled through a 0.5-mm screen. Mean particle size distribution expressed as bran remaining on a given screen, mesh size (mm) as percent of total bran sample. Distribution for the 2-mm bran (mean, 0.684 mm): 1.4, 2; 1.0, 26; 0.72, 34; 0.51, 23; 0.36, 8; and 0.19, 7 (mm, %); and for the 0.5-mm bran (mean, 0.341 mm): 0.46, 1; 0.39, 58; 0.33, 22; 0.24, 10; and 0.15, 9 (mm, %).

<sup>b</sup> American Institute of Nutrition 1980 (vitamin A = 4,000 IU/kg and vitamin E = 50 IU/kg diet).

<sup>c</sup> Analyzed by the procedure Prosky et al (1988).

from Dyets, Bethlehem, PA, and wheat bran was from the AACC, St. Paul, MN.

Total feed consumption was measured, fresh feed was provided three times per week, and animals were weighed twice per week.

Three 48-hr total cumulative fecal collections were made beginning on days 9, 23, and 37 of the experimental period, which corresponded to animal ages of 37, 51, and 65 days, respectively. Fecal collection steel trays lined with paper towels were used for fecal collection. Spilled diet contaminating any fecal pellet was carefully removed. To reduce fecal moisture loss, fecal samples were picked up at 7:00 A.M. and 3:00 P.M. each day of the collection periods and stored at 4°C in sealed plastic bags. Total fecal samples for each period were weighed, then stored at -70°C until they were freeze-dried for analysis.

Diet samples were analyzed for total dietary fiber by the procedure of Prosky et al (1988). Freeze-dried fecal samples were milled through a 0.85-mm screen and analyzed for fiber by the same procedure used for diets, without modification. Apparent dietary fiber digestibilities were calculated as apparent dietary fiber digestibility = (dietary fiber intake - dietary fiber excretion)/dietary fiber intake. Diet and feces were analyzed for crude fat (AOAC 1980).

After 42 days, animals were fasted for 16 hr and anesthetized with methoxyflurane; heart, liver, kidney, renal fat, and epididymal fat were collected. Organs were rinsed in saline, blotted dry with cheesecloth, and weighed. The digestive tract was removed, fat and ligaments were trimmed away with scissors, and stomach, small

intestine, cecum, and colon were separated. Lumens of these organs were flushed with saline, and organs were blotted dry and weighed. Lengths of the small intestine, cecum, and colon were measured. All procedures were performed with care to prevent stretching of the organs.

Data were analyzed using Duncan's new multiple range test (Steel and Torrie 1960). A value of  $P \leq 0.05$  was considered the criterion of significance.

## RESULTS AND DISCUSSION

Initial weights ( $105 \pm 2$  g, mean  $\pm$  SEM) after seven days of equilibration on the AIN-76A diet were similar in all experimental groups. After consuming treatment diets for 42 days, full-fed final weights ( $384 \pm 9$  g), weight gains ( $279 \pm 8$  g), feed intake ( $903 \pm 20$  g), and feed/gain ( $3.2 \pm 0.1$  g/g) were similar in all treatment groups. Considering that cellulose is poorly digestible, cellulose diets had lower caloric density than bran diets. Similar feed-gain data suggest that all treatment groups had reached mature weights and the feed intake and weight gain differences observed during the earlier part of the study disappeared by the conclusion of the study. Similar observations were reported by Mongeau et al (1986). At the conclusion of the study, fasting body weights ( $366 \pm 9$  g), heart ( $1.2 \pm 0.1$  g), liver ( $10.5 \pm 0.4$  g), kidney ( $3.0 \pm 0.1$  g), renal fat ( $8.3 \pm 0.6$  g), and epididymal fat ( $4.9 \pm 0.4$  g) weights were also similar in all groups of rats fed the treatment diets. Body and

**TABLE II**  
Gastrointestinal Tract Weights and Lengths in Rats Fed Hard Red (HR) Wheat Bran or Cellulose Diets for Six Weeks<sup>a</sup>

Diet <sup>b</sup>	Weight (g)				Length (mm)		
	Stomach	Cecum	Small Intestine	Colon	Cecum	Small Intestine	Colon
C5	1.60c	0.65	4.50	1.31b	4.41b	122.39	17.43
C10	1.64bc	0.63	4.54	1.55a	4.40b	125.99	18.76
CB	1.84a	0.71	4.30	1.30b	4.68a	126.06	18.01
FB	1.77ab	0.71	4.49	1.19b	4.89a	125.93	18.17
SEM	0.06	0.04	0.27	0.07	0.09	1.81	0.68

<sup>a</sup> Rat weights = wet weights. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> C5 = 5% cellulose, C10 = 10.5% cellulose, CB = 21.5% AACC hard red (HR) wheat bran milled through a 2-mm screen, and FB = 22.2% HR wheat bran milled through a 0.5-mm screen;  $n = 14$ , except for HR bran, 2 mm, where  $n = 13$  (one animal died).

**TABLE III**  
Fecal Output and Moisture Content of Feces in Rats Fed Hard Red (HR) Wheat Bran or Cellulose Diets for Six Weeks<sup>a</sup>

Diet <sup>b</sup>	Period 1 (37 days)			Period 2 (51 days)			Period 3 (65 days)			Mean Moisture (%) <sup>c</sup>
	Wet (g)	Dry (g)	Moisture (%)	Wet (g)	Dry (g)	Moisture (%)	Wet (g)	Dry (g)	Moisture (%)	
C5	1.5b	1.2c	20.3b	2.3b	1.7c	23.2ab	2.2b	1.7b	20.8b	21.4b
C10	2.8a	2.3a	18.8b	3.8a	3.0a	20.8b	2.7a	3.0a	19.3b	19.7b
CB	2.8a	2.0b	26.3a	3.7a	2.7b	27.1a	4.1a	2.8a	31.3a	28.2a
FB	2.9a	2.1ab	25.8a	3.8a	2.7b	28.3a	4.0a	2.8a	28.8a	27.6a
SEM	0.1	0.1	1.9	0.1	0.1	2.0	0.1	0.1	2.2	1.2

<sup>a</sup> Each collection period was two days; days in parentheses = animal age; wet and dry feces measured in g/day. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> C5 = 5% cellulose, C10 = 10.5% cellulose, CB = 21.5% AACC hard red (HR) wheat bran milled through a 2-mm screen, and FB = 22.2% HR wheat bran milled through a 0.5-mm screen;  $n = 14$ , except for HR bran, 2 mm, where  $n = 13$  (one animal died).

<sup>c</sup> Mean for periods 1, 2, and 3.

**TABLE IV**  
Dietary Fiber Intake, Fecal Excretion, and Apparent Digestibility in Rats Fed Hard Red (HR) Wheat Bran or Cellulose Diets for Six Weeks<sup>a</sup>

Diet <sup>b</sup>	Period 1 (37 days)			Period 2 (51 days)			Period 3 (65 days)			Mean Digest (%) <sup>c</sup>
	Intake (g)	Excretion (g)	Digest (%)	Intake (g)	Excretion (g)	Digest (%)	Intake (g)	Excretion (g)	Digest (%)	
C5	1.2c	0.8c	31.4c	1.5c	1.2c	21.0c	1.3c	1.1c	14.3c	21.9c
C10	2.3a	1.9a	18.7d	2.9a	2.5a	15.1d	2.6a	2.4a	7.6d	13.8d
CB	2.1b	1.2b	43.3a	2.7b	1.5b	42.7a	2.5ab	1.6b	37.5a	41.1a
FB	2.1b	1.3b	37.1b	2.6b	1.6b	40.6a	2.3b	1.6b	32.4b	36.9b
SEM	0.1	0.1	1.5	0.2	0.1	1.8	0.2	0.1	1.9	1.6

<sup>a</sup> Each collection period was two days; days in parentheses = animal age; fiber intake and excretion measured in g/day. Digest = apparent digestibility. Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

<sup>b</sup> C5 = 5% cellulose, C10 = 10.5% cellulose, CB = 21.5% AACC hard red (HR) wheat bran milled through a 2-mm screen, and FB = 22.2% HR wheat bran milled through a 0.5-mm screen;  $n = 14$ , except for HR bran, 2 mm, where  $n = 13$  (one animal died).

<sup>c</sup> Mean apparent digestibility for periods 1, 2, and 3.

organ weight data suggest that animals had reached their mature adult body and organ weights at 70 days of age when these measurements were made.

Digestive tract organ weights (residue free) and small intestine, cecum, and colon lengths in rats fed wheat bran or cellulose diets are shown in Table II. Stomach weights in the rats fed bran diets (CB and FB) were significantly higher ( $P < 0.05$ ) than those in rats fed low-cellulose diet (C5); values for rats fed coarse bran diet (CB) were also significantly higher than those fed high-cellulose diet (C10). Higher stomach weights with bran diets may suggest healthier development and functioning of the stomach when fed cereal brans, and coarse bran appears to be more beneficial than fine bran. Increased stomach weights with higher consumption of neutral detergent fiber in pigs have been observed (Stanogias and Pearce 1985). Small intestine weights and lengths were similar among all rats. Stanogias and Pearce (1985) observed significantly higher weights but not lengths of small intestines of pigs fed high fiber diets. This difference in small intestine weights may be due to species difference or relative maturity differences because rats in the present study were mature and pigs in Stanogias and Pearce (1985) were growing at the conclusions of their study. Cecum lengths, but not weights, were significantly higher in rats fed bran diets than in those fed cellulose diets. Stanogias and Pearce (1985) reported a significant increase in length of the cecum in pigs fed high-fiber diets. Increased cecal length with bran diets suggests greater storage and fermentation of cereal bran diets in the cecum compared with cellulose diets. Calculated cecal weight per length for C5, C10, CB, and FB diets fed animals was 1.47, 1.43, 1.52, and 1.45 g/cm, respectively, suggesting that increased cecal length with bran diets was due to thinning or stretching of the tissue. Higher fermentation of cereal brans could result in greater production of butyric acid which has been suggested to lower the risk of colon cancer with wheat bran diets. In a 40-week study, Watanabe et al (1979) observed a significantly lower incidence of tumors induced by azoxymethane with a 15% wheat bran diet compared with the control diets in F344 rats. McIntyre et al (1993) reported significantly fewer tumors in rats with dimethylhydrazine when fed a diet containing 10% wheat bran. High butyrate production with wheat bran was associated with protection against large bowel cancer. Kestell et al (1999) have reported significant retardation of the metabolism of the carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline in rats by a 10% wheat bran diet. The wheat bran diets did not significantly influence colon weights or lengths. However, Jacobs and Schneeman (1981) found increased proximal and distal colonic weights in rats fed 20% wheat bran compared with rats fed a fiber-free diet. Omaye and Chow (1984) reported an increase in proximal colon lengths in rats fed a 20% bran diet compared with rats fed a 5% bran diet. Differences in colon length in response to wheat bran as observed by Omaye and Chow (1984) are difficult to explain. The variability in colon measurements between the present study and those reported by Jacob and Schneeman (1981) may be due to comparison of wheat bran diets with fiber-free control in their study. Rats fed the high-cellulose diet (C10) had significantly higher colon weights than those fed the other three diets. Calculated colon weight per length for C5, C10, CB, and FB diets fed animals was 0.75, 0.83, 0.72, and 0.66 g/cm, respectively, suggesting that increased colon weight with the C10 diet was due to thicker tissue in rats fed this diet. Similarly, Oku et al (1982) observed a significant increase in colon weights in rats fed a 20% cellulose diet compared with rats fed a fiber-free diet. Prizont (1987) suggested that suppressed tumor development and increased colon weight in the rat with the poorly digestible 20% cellulose diet may relate to improved colon health by dilution of the toxic metabolites.

Fecal output for three 48-hr periods from rats fed wheat bran or cellulose diets is given in Table III. High-fiber diets (C10, CB, and FB) resulted in significantly higher wet as well as dry fecal weights than low fiber diet (C5) for all three collection periods.

For collection periods 1 and 2, fecal dry weights with the high-cellulose diet (C10) were also significantly higher than those with the coarse bran diet. Average fecal moisture with bran diets for combined periods 1, 2, and 3 in rats fed bran diets was significantly higher than those fed cellulose diets. Similar feed intake but higher fecal moisture and dry weight with bran diets suggests reduced transit time because it reduces time for reabsorption of water by the gastrointestinal tract. Reduced transit time and higher fecal output could result in greater dilution of the toxic metabolites, factors which may lead to a healthier gastrointestinal tract and lowered incidence of tumors and cancer of the large intestine. With a wheat bran diet, reduced uptake of the carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline as well as decreased transit time through the gastrointestinal tract have been observed (Sjodin et al 1992). There were no significant differences in wet or dry fecal weights between the two particle sizes of wheat bran tested. Brodribb and Groves (1978) and Heller et al (1980) reported significantly higher wet fecal weight with coarse bran than with fine bran in human subjects. Our results are similar to those of Mongeau and Brassard (1985), who observed no effect of particle size on fecal moisture in rats. In their study, Mongeau and Brassard (1985) report high fecal moisture (48%) from rats fed 15–25% bran diets. In the present study, fecal moisture of only 28% was observed from rats fed 21–22% bran diets. This discrepancy may be attributable, in part, to differences in fat and starch content of the diets or different fecal collection procedures.

Dietary crude fat digestibility values for the three collection periods were similar for all diet groups; values ranged from 92 to 95%, with the mean crude fat digestibility of 94%. Similarly, Cummings (1978) and Ali et al (1981) concluded that an increase in fecal fat observed in bran diets was nutritionally insignificant because it resulted in a small decrease in fat absorption in human subjects. Cadden (1986) reported similar fat digestibility values for 0% fiber versus 20% bran diets in rats. However, Mongeau et al (1986) reported a 68% increase in fecal fat excretion with wheat bran supplementation. Higher fat diets (10%) may be responsible for the difference because lower fat diets (5%) were used in the present study.

Dietary fiber intake, excretion, and digestibility in rats fed bran or cellulose diets for six weeks are presented in Table IV. Dietary fiber digestibility was significantly different among treatments for all collection periods (CB > FB > C5 > C10), except for period 2, when differences between CB and FB were nonsignificant. Intake and excretion values were significantly higher for rats fed C10 than those fed bran diets for all three collection periods, except during period 3, when fiber intakes were similar for rats fed C10 and CB diets. Higher intake with the high cellulose (C10) diet may be due to lower caloric density in this since dietary fiber digestibility of this diet only 14%. Mean dietary fiber digestibility for all three periods were significantly different among treatments. Apparent dietary fiber digestibility decreases from period 1 to period 3 for C5, C10, CB, and FB diets were 54, 59, 13, and 13%, respectively. Decrease in dietary fiber digestibility with time may indicate a decrease in digestibility in mature rats compared with growing animals. Digestibility of dietary fiber in bran diets was markedly greater than fiber digestibilities in cellulose diets. Disappearance of bran from fecal content may be due to its fermentation in the cecum and large intestines with the possible production of short chain fatty acids including butyrate, which is believed to be responsible for the beneficial effect of wheat bran. Munakata et al (1995) observed that shortening of the transit time through the cecum and colon with an increase of fecal volume and suppression of fat absorption all participate in the mechanism of the inhibitory action of wheat bran on carcinogenesis of the large intestine in rats. Govers et al (1999) observed that inclusion of wheat bran in the diet decreased fermentation in the cecum and that there was more fermentation in the proximal colon, resulting in significantly higher butyrate and lower ammonia in the distal

colon, which may contribute to the modulation of colon cancer risk. Compher et al (1999) reported that a wheat bran diet resulted in significantly higher fecal butyrate and fewer aberrant crypt foci in the proximal and distal colon of F344 rats. Kritchevsky (1999) reported in a review article that a wheat bran diet resulted in 40% lower incidence of colon tumors in rats compared with those fed a cellulose diet.

The results of the present study indicated that dietary fiber is digestible (14–41%) in the rat, and the digestibility varies significantly with the type and level of dietary fiber fed as well as with the particle size of dietary fiber from bran. Significantly lower digestibility of fine bran diet compared with coarse bran diet was unexpected and difficult to explain. Gastrointestinal tract organ measurements also were affected by changes in type and amount of dietary fiber, as evidenced by the increase in stomach weight and cecal length with bran diets and increased colon weight with the high-cellulose diet.

Digestibility of nutrients may be affected by increased consumption of dietary fiber, but the current findings showed only a small, nonsignificant decrease (3%) in apparent fat digestibility with wheat bran. This may be due to the fat content of wheat bran, which may not be readily digestible.

Dietary fiber from fine bran did not differ from that of coarse bran in its effect on gastrointestinal tract organ measurements, fecal wet or dry weights, or fat digestibility; however, reducing the particle size of bran significantly lowered the apparent digestibility of dietary fiber in bran in rats. Increasing dietary fiber consumption resulted in increased fecal weights, with feces from bran diets holding more water than feces from cellulose diets. Healthful effects of wheat bran may be associated with gastrointestinal morphology and function. Fecal bulking and decreased intestinal transit time can prevent constipation and may dilute or reduce absorption of toxic or carcinogenic metabolites, thus improving gastrointestinal health and lowering the risk of tumor development and cancer.

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