Possible Health Benefits of Sorghum and Millet Bran

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ABSTRACT

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The effects of sorghum and millet bran on blood and liver cholesterol levels and colonic physiology were examined using 110 male Sprague-Dawley rats. A white sorghum (low tannin),a brown sorghum (high tannin) and a pearl millet were decorticated at the 8% level and the bran fractions were analyzed for insoluble, soluble and total dietary fiber. A basal fiber-free diet containing cholesterol and cholic acid to induce hypercholesterolemia was uniformly diluted with the following fiber supplements to achieve 6% TDF by weight: cellulose, pectin, wheat bran, oat bran, American blend, white sorghum bran, brown sorghum bran and millet bran. After 21 days on the diets, the animals were sacrificed and the following measurements were taken: serum and liver cholesterol, cecal surface area, colonic pH, and dry fecal weight. Serum cholesterol values of brown sorghum and white sorghum brans were not significantly different from cellulose or wheat bran. The millet bran resulted in significantly lower serum cholesterol levels comparable to pectin. The American blend resulted in the lowest cholesterol level of any diet. Liver cholesterol values resulted in a similar pattern, the sorghum, millet and wheat brans having significantly ($p < .05$) higher values than the American blend, pectin and oat

bran. In the colon, the sorghum and millet were excellent bulking agents, did not increase cecal surface area or decrease pH and thus had a positive effect. The presence of tannins in the brown sorghum seem to have a positive effect as the brown sorghum bran had the heaviest fecal dry weight, smallest cecal surface area and shortest gut length. The American blend was not an effective bulking agent and increased cecal surface area. Therefore the American blend lowered cholesterol levels significantly but did not have a positive influence in the colon. It may be concluded that sorghum brans have potential to be used as bulking agents however they do not lower cholesterol. Millet bran may be capable of lowering cholesterol and exerting a positive effect on the colon but confirmation of these results is needed.

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INTRODUCTION AND LITERATURE REVIEW

In the early seventies, Burkitt and Trowell (1-3) hypothesized that many of the diseases prevalent in developed countries were due to a lack of dietary fiber in the diet. In the last two decades, research has shown that dietary fiber can influence the development of two of the United States' leading causes of deaths: coronary heart disease (CHD) and colon cancer.

Coronary heart disease costs Americans an estimated 78 billion dollars annually and over a million lives (4). In addition to high blood pressure and smoking, high blood cholesterol is a major modifiable risk factor associated with coronary heart disease (5). Colon cancer results in the second highest mortality rate among all cancer cases in the United States (6). Although the causes of these diseases are multifactorial, evidence from epidemiological studies in human populations and animals suggests that dietary factors such as dietary fiber play an important role in the development of these diseases (7-10).

Definition of Fiber

Dietary fiber is defined as the polysaccharides and lignin not digested by the enzymes of the human digestive tract (11). Dietary fibers are not created equally; each different source of fiber has its own unique physicochemical properties.

However; in general, dietary fiber can be divided into two broad categories: water soluble and insoluble.

Effects of Soluble Fiber

In the gastrointestinal tract, soluble fibers attract water to form gels. These gels delay gastric emptying and absorption of nutrients in the small intestine. Once soluble fiber reaches the large intestine, it is fermented by the colonic microflora. In this process, the microflora break down the polysaccharides to produce energy, gases (CO_2, CH_4, and H_2), and short chain fatty acids (acetate, propionate and butyrate)(12). Since the soluble fibers are completely degraded by the microflora, they do not increase fecal bulk effectively.

In general, soluble fibers lower high blood cholesterol levels (13). There are three proposed mechanisms through which soluble fibers decrease cholesterol levels. 1) The formation of gels traps dietary cholesterol, rendering it unavailable for absorption in the small intestine. 2) Soluble fibers interfere with the enterohepatic recirculation of cholesterol-derived bile acids and thus more cholesterol must be used to replace the lost bile acids. 3) The fermentation of the soluble fiber in the colon produces the short chain fatty acid propionate which inhibits HMG coA reductase, the key regulatory enzyme in cholesterol biosynthesis. Cholesterol is probably lowered by a combination of these mechanisms, depending on the specific fiber utilized.

In the colon, a study by Jacobs and Lupton found that pectin and oat bran decrease colonic pH, increase cell proliferation and increase carcinogenesis (14). Therefore, soluble fibers lower cholesterol levels but may enhance colon cancer.

Effects of Insoluble Fiber

Insoluble fibers are not gel formers and are poorly fermented by the colonic microflora; thus, it continues along the colon to add bulk to the feces. Although insoluble fibers did not lower cholesterol levels, they are generally excellent bulking agents and are protective against colon cancer (14).

Fiber Intake

Recent guidelines by the National Cancer Institute suggest a daily intake of 25-30 grams/day of dietary fiber with an upper limit of 35 grams/day (15). According to Anderson et al (16), the average American consumes 12 g fiber/day, which is a complex mixture of different types and sources of fibers. Therefore, Americans should be looking for ways to add fiber to their diets. Many cereal grain fibers such as wheat bran and oat bran have been physiologically characterized as to their effects on blood lipids and colonic functions. Sorghum and millet have not.

Description of Sorghum and Millet

Sorghum (Sorghum bicolor L. Moench) is a unique cereal grain due to its drought resistance and adaptability to tropical conditions. Because of this characteristic, it is mainly utilized in the arid regions of the world as a subsistence and cash crop where other cereal grains are unable to grow.

Millets are small seeded, annual cereal grasses adapted to hot, dry climates. Pearl millet is the most widely grown of all millets and is one of the most drought tolerant of all cereals.

Uses of Sorghum and Millet

In arid regions of the world such as Africa and India, millet and sorghums are used as staple food sources. Sorghum and millet provide 90% of the food energy requirements in the hot, dry Sahelian region of Africa (17). It can not be stessed enough that sorghum and millet are of much greater importance in the diets of poor people in the semi-arid tropics than wheat is in the diets of Americans. Sorghum and millet are utilized in food such as: unfermented breads such as chapati and roti in India and tortillas in Mexico and Latin America; fermented breads such as kisra and injera in East Africa; porridges in Africa, India, Mexico and Latin America, and alcoholic beverages such as beer in West Africa. Sorghum and millet are also utilized as snack foods worldwide, eaten puffed and popped (18).

Sorghum Production

Sorghum ranks fifth in total production of cereal grains. The major production areas are in Asia and Africa, although the United States (concentrated in Kansas, Texas, Nebraska and Missouri) produces about 30% of the total world production (19). In the United States, millet and sorghum are mainly utilized as livestock feed ingredients.

Sorghum and millet may be the cereal grains of the future because of their ability to produce under semi arid conditions unsuited for other cereals. If the greenhouse effect becomes a reality, sorghum would be well suited to grow under the warm, hot, dry conditions. Another situation which may increase the production of millet and sorghum is the disappearance of underground water reservoirs in the midwest. Unless crops are suited to dryland conditions, production may be abandoned due to water scarcity or cost (20).

Nutritional Value of Sorghum and Millet

Sorghum is classified as high and low tannin types. Tannins are condensed polyphenols which protect the kernel against insect, bird and microorganism attacks. Responsible for the red to brown color in high tannin sorghums, tannins decrease the nutritional value of the sorghum because of an undigestible tannin-protein complex (21).

Sorghum and millet have proximate composition, amino acid content and nutritional value similar to corn. It is well recognized that sorghum protein digestibility is slightly lower than any other major cereal grain (22,23). The protein quality of sorghum is limited by low lysine content.

Sorghum and Millet Fiber

Sorghum and millet kernels are composed of three main anatomical parts: pericarp (outer layer), endosperm (storage tissue), and germ (embryo). In general, millet kernels resemble sorghum kernels except that they are a third of the size and have a higher germ:endosperm proportion. The dietary

fiber is usually located in the pericarp: sorghum contains between 7 and 10% total dietary fiber.

According to Knudsen and Munck, the majority of the fiber in sorghum is insoluble (86%) while only a small portion is soluble (14%). Sorghum contains only 1% of soluble B-D glucans (24), which comprise the soluble fiber of oat and barley brans. They suggest the presence of a polyphenol-rich testa layer increases the amount of total dietary fiber in the sorghum due to an increase in lignin content.

Knudsen and Munck found that the fiber content increases after the sorghum is cooked due to the formation of enzyme-resistant starch. In high tannin sorghum, cooking also increased the amount of lignin present, due to an increase in polyphenol-protein complexes which are measured in the lignin portion of fiber (25). As a result, significantly more protein is associated with the total dietary fiber fractions of sorghum (38%) than those of barley, rye, wheat and maize (12-18%) (24). Therefore, protein digestibility decreases in the presence of dietary fiber. This is a major concern in areas where sorghum is a staple food source and protein is scarce. However this protein-fiber complex may significantly enhance the physicochemical properties of the fiber in the colon.

Previous Studies Utilizing Sorghum Fiber

Only a few studies on the effect of dietary sorghum fiber exist. Fedail et al compared the effect of sorghum bran with wheat bran on colonic functions (26). Ten healthy medical

students supplemented their diets with twenty grams of wheat and sorghum bran supplement. The sorghum and wheat brans both increased mean stool weight, decreased transit time, and increased frequency of evacuation compared to the normal diet. However, only the wheat bran results were statistically different because the sorghum bran was only 12.4% fiber compared to 39.5% fiber in the wheat bran; thus the actual fiber intakes were very different.

Klopfenstein et al (27) compared the effects of wheat, oats, pearl millet, and sorghum upon liver and serum cholesterol levels. Sorghum effectively lowered serum cholesterol and significantly lowered liver cholesterol ($p<0.05$) in comparison to the other diets. However, the dietary fiber levels in the diets were not reported and only three to five animals were used per diet group which is not enough to give statistically realiable data.

Reasons to Study Sorghum and Millet Fiber

There are three main reasons for studying sorghum and millet fiber. First, as previously examined, there is a lack of solid research in this area. Secondly, much of the initial work done by Burkitt and Trowell in the development of the dietary fiber hypothesis was done in Africa, where a great proportion of the diet is based on sorghum and millet (1-3). Third, if positive health benefits can be found, it may eventually lead to increased consumption and production of sorghum and millet. A prime example of this phemomena is oat bran. In 1987, there were only three products on the market

which contained oat bran. Two years later, after the cholesterol-lowering benefits of oat bran had been publicized, well over two-hundred products containing oat bran were being marketed (28).

Hypothesis

The goal of this research project is to determine the potential health benefits of sorghum and millet brans. As sorghum and millet fiber are insoluble, these fibers should not lower cholesterol but should have positive effects in the colon.

Objectives

1) To determine the effect of sorghum and millet bran upon lipid metabolism.
2) To determine the effects of sorghum and millet bran upon colon physiology.
3) To develope a standard blend of fibers that represents the fiber consumed daily in an average American's diet.

MATERIALS AND METHODS

Experimental Animals

One hundred and ten male Sprague-Dawley rats (Harlan Sprague Dawley, Houston, TX) weighing 200-220 grams upon arrival were randomly assigned to one of nine diet groups. Male rats were used to avoid the effect of the female estrous cycle on hormone levels and because maleness is an independent risk factor for cardiovascular disease. The Sprague-Dawley strain of rat was used because of the excellent literature available for comparison. In addition, they have a mild disposition and are easy to handle. Two hundred to two hundred-twenty gram rats were used because they were large enough to supply sufficient tissue yet young enough to test the effects of the diets on growth. Twelve or more rats were used per diet group because previous studies (29-31) have shown that at least ten rats per assay are needed to obtain statistically reliable results; however not every rat provides material for every assay.

Rats were housed individually on double grid wire floors in temperature and humidity-controlled animal facilities. They were maintained on a daily photoperiod of twelve hours light and twelve hours dark, the dark period being between 1800 and 0600 hours. Food and water were provided ad libitum.

Basal Fiber Free Diet

Following a three-day acclimitization period during which Rat Chow (Ralston-Purina, St. Louis, MO) was fed, animals were

provided with assigned intervention diets. The basal fiber-free diet is shown in Table 1. Diet ingredients were chosen for specific reasons. Sucrose was used as the carbohydrate source because it has been shown to induce higher cholesterol levels than other commonly used carbohydrate sources such as corn starch (32,33).

Casein, the protein source, is supplemented with its limited amino acid, L-methionine. An animal protein, it has been shown to be hypercholesterolemic in comparison to vegetable proteins (34). It has been used in similar studies and is well tolerated by the rat.

The American Blend Fat, a product of the Institute of Shortening and Edible Fats, contains a mixture of saturated and unsaturated fats designed to mimic the fat consumption pattern of an average American's diet. It was provided at a level of 15% by weight to closely approximate the 30% of calories from fat recommended by the American Heart Association (35).

Vitamin and salt mixes were standard AIN-76A (36,37). Choline bitartrate was provided because rats are unable to synthesize their own choline. Cholesterol and cholic acid were added to the diet to induce hypercholesterolemia (38). Diets were varied by the addition of different fiber sources.

Fiber Diets

The fiber supplemented diets (Table 2) were prepared by uniformly diluting the basal fiber-free diet with the addition of the fiber supplement to achieve 6% dietary fiber by weight.

Table 1

Percentage composition of the basal fiber-free control diet

Ingredient [1]	% by weight
Sucrose	51.4
Casein	23.6
L-methionine	0.4
American Blend Fat [2]	15.0
Salt Mix	6.0
Vitamin Mix	2.0
Choline Bitartrate	0.4
Cholesterol	1.0
Cholic Acid	0.2
Total	100.0

1 The source of each diet ingredient was as follows: sucrose(#3900, Bioserve Inc., Frenchtown, NJ); casein (#1100, Bioserve Inc., Frenchtown, NJ); L-methionine(#10860, Teklad Diets, Madison, WI); American Blend Fat(supplied by the Institute of Shortening and Edible Oils, gift from the Proctor and Gamble Company, Cincinnati, OH); Salt Mix(AIN-76 Mineral Mix #F8000, BioServe, Inc., Frenchtown, NJ); Choline bitatrate(#6090, Bioserve Inc, Frenchtown, NJ); Cholesterol(#80050, Teklad Diets, Madison, WI); cholic acid(#01383, ICN Biochemicals, Cleveland, OH).

2 The composition of the American Blend Fat is as follows: Tallow, 27%; butterfat, 15%; lard, 13%; partially hydrogenated soybean and palm oils, 27%; partially hydrogenated soybean oil, 9%; cottonseed oil, 3%; peanut oil, 5%; and corn oil, 1%.

Table 2

Percentage composition of the fiber diets

Diet 1	% Basal Diet	% Fiber Supplement
Fiber Free	100.0	0.0
Cellulose	94.0	6.0
Pectin	94.0	6.0
Wheat Bran	86.0	14.0
Oat Bran	59.2	40.8
American Blend	94.0	6.0
White Sorghum Bran	86.4	13.6
Brown Sorghum Bran	80.6	19.4
Millet Bran	82.2	17.8

1 The source of each fiber supplement is as follows: cellulose(Avicel microcrystalline cellulose pH 101, a gift from FMC Corp, Philadelphia, PA); pectin(citrus pectin, polygalacturonic acid methyl ester #19955, U.S. Biochemical Corp., Cleveland, OH); wheat bran(American Association of Cereal Chemists, St. Paul, MN); oat bran(Quaker Oat Bran, Quaker Oats, Chicago, IL); American Blend(see Table V); white sorghum bran, brown sorghum bran, and millet bran(Gift from Cereal Quality Lab, Texas A&M University).

Because the bran fiber supplements were not 100% pure fiber, they were added to the basal diet in greater amounts to obtain 6% fiber in the diet. Previous studies (39-41) have shown that when a basal diet is made less energy dense by the addition of a fiber supplement, rats eat more to compensate, thus maintaining equivalent nutrient intakes. In terms of human consumption, 6% dietary fiber in a rat diet corresponds to approximately thirty grams of fiber/day, assuming a person consumes .5 kg food/day on a dry weight basis. This is a very realistic amount and falls in the recommended twenty-five to thirty grams of fiber/day (15).

Standard fiber diets:

Cellulose, pectin, wheat bran, and oat bran were used as standard diets because the physiochemical properties of these fiber sources have been well researched. Cellulose and wheat bran are insoluble fibers which have been shown to be an excellent bulking agents (42) and generally protective against experimentally induced colon cancer (14). However, these insoluble fibers are ineffective in lowering serum cholesterol levels (43,44).

Pectin and oatbran are soluble fibers. Unlike cellulose and wheat bran, these fibers lower serum cholesterol in cholesterol-fed rats and in humans (44-53). In some studies, soluble fibers are promotive of experimentally induced colon cancer (14).

Experimental Fiber Diets:

The experimental diets consisted of a white sorghum bran, a brown sorghum bran and millet bran and the American blend fiber.

Grain Samples:

The white sorghum used was Malisor 7, a white food sorghum without tannins. The brown sorghum (ATX 623 X SC0103) was a typical bird resistant hybrid. The sorghums were grown at Texas A&M University. The pearl millet was grown at the University of Nebraska neas Meade, Nebraska. It was a composite of several varieties and breeding lines. It was a typical slate grey. The sorghum and millet grains were donated by the Cereal Quality Lab, Texas A&M University.

Milling of the Grains:

The pericarp of the kernel was removed by abrasive action using an IDRC Pilot Plant Mill (Nutana Machine Company, Saskatoon, Canada). Five kilogram batches of whole grains were ground for two minutes and the bran and decorticated grain was separated using a Clipper sifter. The pericarp was removed from the white and brown sorghums and millet at the 7.88%, 7.32% and 7.45% levels respectively.

Development of the American Blend Fiber: The American Blend fiber contained a variety of pure fibers formulated to mimic the fiber components in the average American's diet (Table 3) as defined by Anderson and Bridges (16). To create the simulated American diet, commonly consumed food items were

Table 3

Composition and source of the American blend fiber

Fiber Component	Source	%
Soluble Nonstarch Polysaccharides(SNSP)	Pectin/Guar Gum(1:1)	35.90
Insoluble Noncellulose Polysaccharides(INCP)	Best Bran 90 Corn Bran	30.15
Cellulose	Avicel pH 101 Cellulose	16.18
	Best Bran 90 Corn Bran	10.42
Lignin	Indulin AT Kraft Lignin	6.50
	Best Bran 90 Corn Bran	0.85
		100.00

1 Sources of ingredients are as follows: pectin(#19955, U.S. Biochemical Corp., Cleveland, OH); guar gum(#105009, ICN Biochemicals, Cleveland, OH); Best Bran 90 Corn Bran(G-ultrafine, AE Staley Manufacturing Company, Decatur, IL); cellulose(Avicel microcrystalline cellulose type pH101, FMC Corp., Philadelphia, PA); lignin(Indulin AT Kraft Lignin, Westvaco Polychemicals Department, Charleston, SC).

chosen to represent food groups as reported in the USDA's Household Nationwide Food Consumption Survey. From these items, total dietary fiber, soluble nonstarch polysaccharides (SNSP), insoluble noncellulose polysaccharides (INCP), cellulose, and lignin contents were calculated using previously published data (54). Cereal products contributed 42% of the dietary fiber intake, while legumes and vegetables provided 25% each. From previously published data, (54) the sugar residues from the INCP and SNSP could be deduced and their sources and ratios calculated (Table 4 and 5). The soluble nonstarch polysaccharides had approximately equivalent amounts of glucose and uronic acids while the amounts of arabinose and xylose were 1.4 times the amount of glucose. The 'other' category included rhamnose, fructose, mannose, and galactose and was present at 1.6 times the amount of glucose.

Therefore, a representative soluble fiber was sought that would reflect a similar sugar composition. A 1:1 ratio of guar gum to pectin was used in the American blend fiber. Pectins are esterified alpha 1-4 linked galacturonans or rhamnopyranan chains with sidechains of alpha L-rhamnopyranose residues. Other sidechain constituents include D-galactose, L-arabinose and D-xylose (55). Guar gum is a high molecular weight galactomannan with a mannose:galactose ratio of 2:1 (56).

In the insoluble noncellulose polysaccharides, glucose and uronic acid were present in equivalent amounts. The xylose content was 2.5 times that of glucose and arabinose content

Table 4. Soluble nonstarch polysaccharide sugar residues in the simulated American diet.

Source	Ara[1]	Xyl[2]	Glu3	Other[4]	Uronic[5]	Total
Cereals	15.42	20.24	9.64	5.78	0.73	51.81
legumes	2.65	0.24	0.96	8.92	4.82	17.59
vegetable	2.89	0.72	4.10	8.43	8.92	25.06
fruits	0.48	0.01	0.48	0.96	3.61	5.54
totals	21.44	21.21	15.18	24.09	18.08	100.00
ratio to glucose	1.41	1.40	1.00	1.59	1.19	

[1] arabinose [2] xylose [3] glucose
[4] rhamnose, fructose, mannose and galactose
[5] uronic acid

Table 5. Insoluble noncellulose polysaccharide sugar residues in the simulated American diet.

Source	Aral	Xyl[2]	Glu[3]	Other[4]	Uronic[5]	Total
cereals	13.58	19.65	9.25	4.62	2.02	49.12
legumes	3.47	2.31	0.58	9.83	5.49	21.68
vegetable	6.36	7.23	2.02	5.49	4.05	25.15
fruit	1.16	0.58	0.00	1.16	1.16	4.06
totals	24.57	29.77	11.85	21.10	12.72	100.00
sugar ratio to glucose	2.07	2.51	1.00	1.78	1.07	

[1] arabinose [2] xylose [3] glucose
[4] rhamnose, fructose, mannose and galactose
[5] uronic acid

was twice as much as glucose. Best Bran 90 corn bran, a refined by-product of the corn wet milling process, was used as the source of INCP. Since most of the starch, protein, and fat was removed during the corn wet milling, the bran is 92% insoluble dietary fiber. The main fiber constituent in corn bran is hemicellulose (60-70%) and minor components include cellulose (22-23%) and lignin (0.7-3.0%). The insoluble hemicellulose contains xylose:arabinose in a 1:1.5 ratio with small amounts of galactose and glucuronic acid. This composition fits the sugar profile of the simulated diet desirably, since 47% of the INCP is obtained from cereal products in which the main sugar components are xylose and arabinose.

The portion of the cellulose not provided by the corn bran was added in the form of pure microcrystalline avicel cellulose, pH 101. The portion of lignin not provided by the corn bran was provided by using Indulin AT Kraft lignin. Lignin is the only component of dietary fiber which is not a polysaccharide but a high molecular weight polymer of coumaryl, coniferyl and sinapyl alcohols. The combination of these pure fibers made up the American blend fiber, which was supplemented at the 6% TDF level.

Proximate and Dietary Fiber Analyses: The oat bran, wheat bran, white sorghum bran, brown sorghum bran, and millet bran were analyzed for proximates analysis (Table 6) and total, soluble and insoluble dietary fiber (Table 7). Crude protein

Table 6. Insoluble (IDF), soluble (SDF) and total dietary fiber (TDF) contents of brans on a dry weight basis.

Bran	%IDF	%SDF	%TDF
Wheat	44.66±0.27	3.06±0.02	47.72±0.29
Oat	9.69±0.01	6.74±0.05	16.43±0.06
White Sorghum	47.84±0.52	1.59±0.67	49.43±1.15
Brown Sorghum	35.09±3.34	1.04±0.45	36.13±3.79
Millet	37.91±1.73	1.05±0.14	38.96±1.87

Values are expressed as average ± standard deviation as determined by the method of Prosky et al (1988).

Table 7. Proximate analyses of brans on a dry weight basis.

Bran	Fat	Protein	Ash	CHO
	----------(g/100g)----------			
Oat	7.0	21.0	3.0	69.0
Wheat	6.9	18.0	6.6	68.5
White Sorghum	9.1	9.6	3.4	77.9
Brown Sorghum	7.0	11.1	3.5	78.4
Millet	12.8	16.9	4.4	65.9

Expressed as percent of dry weight basis.

(N X 6.25) was analyzed using the boric acid modification of the Kjeldahl Method (AACC 46-12).

Fat was analyzed by acid hydrolysis of a 2-3 gram sample and triple extractions with petroleum ether and ethyl ether in a Mojonnier extraction apparatus (AOAC 14.019). Moisture was determined by drying a 2-3 gram sample for two hours in a forced air oven at 135 C (AOAC 7.0007). Ash was determined by igniting a 3-5 gram sample in a preignited ashing dish for a period of six hours at 550 C. Total carbohydrates were determined by difference (100-(protein +fat + moisture + ash)).

Dietary fiber was determined according to the procedure of Prosky et al. (57). Duplicate samples were gelatinized with Termamyl, a heat stable alpha amylase, and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. The residue was filtered through coarse fritted crucibles and washed with water. The residue was washed with 95% ethanol and acetone, dried and weighed. One duplicate was analyzed for protein and the other was analyzed for ash. Insoluble dietary fiber (IDF) was the residue weight less weight of protein and ash. Soluble fiber was precipitated from the filtrate by the addition of four volumes of ethanol. The soluble fiber was filtered and washed with 78% ethanol, 95% ethanol, and acetone. The residue was dried, weighed, and one duplicate was analyzed for protein and the other for ash. Soluble dietary fiber (SDF) was the residue weight minus the

weight of protein and the weight of ash. Total dietary fiber was calculated by adding SDF and IDF. Each sample fiber was analyzed on two separate occasions and the results were averaged.

Rat Feeding Trials

Nine groups of twelve rats were provided with the basal fiber-free diet or the assigned fiber supplemented diet as described above. Diets were randomly assigned and provided ad libitum. To ensure freshness, diets were prepared weekly and stored at 5 C between feedings. Food cups were checked daily and filled as necessary. During the three week feeding period, the following data were obtained:

Food and Energy Intakes

At the beginning of week two and three, each rat's food cup was filled with diet and weighed. Exactly twenty four hours later, the food cups were reweighed and the amount of diet spilled was estimated. Food intake was calculated as the difference between beginning weight and ending weight and adjusted for spill. These data were multipied by the energy density of the specified diet (Table 8) to calculate energy intakes. Energy densities of the diets were calculated by multiplying the amount of total protein in the diet from both the bran and the basal diet by 4 Kcal/gram, the amount of fat from the bran and the basal diet by 9 Kcal/gram and the amount of total carbohydrates from the bran and the basal diet less the amount of total dietary fiber by 4 Kcal/gram.

Table 8. Energy densities of the intervention diets.

Diet	Kcal/gram
Fiber Free	4.37
Cellulose	4.10
Wheat Bran	4.03
Pectin	4.10
Oat Bran	3.90
American Blend	4.10
White Sorghum Bran	4.08
Brown Sorghum Bran	3.98
Millet Bran	4.07

Weight Gain

Each rat was weighed upon arrival, at the beginning of the feeding trial, at the beginning of weeks two and three and immediately prior to sacrifice. Weight gain was calculated as the difference between the final weight and the beginning weight. The intermediate weights were taken to monitor weight gain as an indicator of overall health of the animals.

Fecal Output

At the beginning of week two and week three, seventy-two hour fecal collections were taken. The rats were placed in clean cages in which the litter was covered with DACB board to facilitate collection. For three consecutive days, twenty four hours apart, feces were collected from each individual rat and placed in a pre-weighed glass scintillation vial. After the last collection was made, the vials were reweighed, dried at 100 C in a forced air oven to a constant weight, cooled and the dry weight was taken.

At the end of three weeks the animals were transported with food and water from the animal facility to the surgery room. All surgeries were carried out between 1200 and 1700 hours in order to minimize diurnal variations. All occurred within a five day period. At sacrifice, the rat was anesthetized with a mixture of ketamine (87mg/kg body weight) and rompun (13 mg/kg body weight). After a surgical plane was reached, a midline incision was made to expose the cecum and large intestine.

In vivo pH measurements, blood and tissue samples were taken according to the following protocols:

In vivo pH measurements

While the rat was anesthetized and after the midline incision was made, a tiny combination microelectrode (MI-710 tiny combination pH probe, Microelectrodes, Londonderry, NH) was inserted via a five millimeter incision into the cecum; in the proximal colon, one centimeter distal to the cecal/proximal colon junction; and in the distal colon at the pelvic ridge. Readings were recorded using a pH meter (Orion 811, Orion Research Inc, Cambridge, MA). The measurements were made within a minute while the animal was still alive.

Serum Cholesterol Determinations

Following the pH measurements, each animal was exsanguinated via intercardiac puncture using an 18 gauge needle and a 3-cc syringe. The blood was transferred to a test tube, allowed to clot for 15 minutes, and centrifuged at 900 X G for 10 minutes. The serum was removed using a

disposable glass pipet and bulb and was stored overnight at 5 C. Serum was then analyzed for total cholesterol using an enzymatic test based on the method of Flegg (58) and of Richmond (59), as modified by Allain (60) and Roeschlau (61). Serum triglycerides were determined by the method of Bucolo and David (62).

Liver Cholesterol and Lipids

Following the removal of blood, a bilateral incision of the diaphragm was made to kill the animal. The liver was resected, blotted dry and weighed on an analytical balance. It was then placed in a glass scintillation vial and stored at -20 C until futher analysis. At the time of analysis, the liver was allowed to defrost and a 1-1.5 gram sample was taken from the same lobe of each liver. Total lipids were determined by the procedure of Folch (63). Briefly, the liver sample was homogenized with a 2:1 chloroform-methanol solvent using a homogenizer (The Virtis Company, Cardiner, NY). After two chloroform extractions, the fat was dried under a steady stream of nitrogen and weighed. The lipids were then redissolved in chloroform to be used to determine liver cholesterol levels by a modification of the colorimetric method of Searcy and Bergquest (64). Each cholesterol determination was made in triplicate. The lipid was saponified using alcoholic potassium hydroxide and the cholesterol was extracted twice with hexane. An aliquot of the cholesterol extract was dried under nitrogen. An orange color was produced upon the addition of $FeSO_4$, glacial acetic acid,

and H_2SO_4 which was measured with a Coleman Spectrophotometer at a wavelength of 490.

Intestinal Morphology

The cecum and large intestine were resected and the length of the large intestine was recorded. The cecum was resected at the ileocecal valve and the cecal/proximal colon junction, opened along its greater curvature, rinsed clean with phosphate-buffered saline (NaH2PO4, 1.9mM; Na2HPO4, 8.4mM; NaCl, 145.4 mM, pH 7.4) and laid flat on dental wax. The perimeter was marked on the wax with a pencil and then transferred to tracing paper. The tracings were analyzed by Jandel Video Analysis Software for the area inside the perimeter.

Data analysis

One way analysis of variance was used to detect differences among diets. When differences were detected ($p<0.05$), means were separated using Duncan's new multiple range test (65) utilizing the Statistical Analysis System (66).

RESULTS

Effect of the Experimental Diets on Lipid Metabolism.

Validity of the Experiment:

The validity of the results were confirmed using food intakes, energy intakes, weight gains and final weights (Table 9 & 10). During the third week, the rats fed the American blend had a significantly lower food intake ($p<0.05$) which was reflected in the difference in energy intake. The depressed food intake of the American blend rats did not affect weight gain or final weight.

The animals gained weight at similar rates until the third week during which the brown sorghum bran group was significantly ($p<0.05$) heavier than the pectin group. The pectin group also gained the least amount of weight, which was significantly different from the brown sorghum bran, oat bran and the millet bran groups. The depressed weight gain and final weight of the pectin group was not surprising. Other studies (67-69) have found similar effects with pectin. Neither weight gain nor final weight were correlated with any of the indices measured and therefore had minimal effects upon the measurements.

Effect of Diet on Serum Cholesterol and Triglyceride Values

The soluble fibers resulted in the significantly lower serum cholesterol values (Table 11) than the insoluble fibers. The white sorghum bran and the brown sorghum bran cholesterol values were almost identical. They were intermediate to cellulose and wheat bran and not significantly different. The

Table 9. Effect of fiber on food and energy intake: weeks two and three.

Diet	Food Intake(2) (g/day)	Energy Intake(2) (Kcal/day)	Food Intake(3) (g/day)	Energy Intake(3) (Kcal/day)
Fiber Free	14.7 ±0.6 a	64.16 ±2.47 a	17.4 ±1.0 abc	76.07 ±4.26 a
Cellulose	16.0 ±0.8 a	65.61 ±3.43 a	17.5 ±0.6 abc	71.85 ±2.44 ab
Wheat Bran	17.6 ±0.7 a	70.91 ±2.82 a	18.5 ±1.0 abc	74.33 ±3.98 ab
Pectin	16.2 ±0.8 a	66.42 ±3.22 a	18.0 ±1.0 abc	73.67 ±3.99 ab
Oat Bran	17.4 ±1.0 a	67.61 ±3.97 a	20.0 ±0.8 a	77.81 ±3.22 a
American Blend	15.7 ±0.7 a	64.50 ±2.82 a	15.6 ±0.6 c	63.86 ±2.48 b
White Sor Bran	16.7 ±1.1 a	68.01 ±4.31 a	18.0 ±0.4 abc	73.34 ±1.71 ab
Brown Sor Bran	16.9 ±0.7 a	67.43 ±2.92 a	17.8 ±0.4 abc	70.92 ±1.52 ab
Millet Bran	15.4 ±1.3 a	62.86 ±5.40 a	16.8 ±1.3 bc	68.31 ±5.20 ab

Results are expressed as means ± the standard error of the mean of twelve rats. In each category, means not sharing the same letter are significantly different ($P<0.05$).

Table 10. The effect of fiber on final weight and weight gain.

Diet	Initial (g)	After 1 WK (g)	After 2 WKs (g)	Final (g)	Weight Gain(g)
Fiber Free	239.5 ±9.3 a	288.7 ±7.4 a	327.6 ±9.2 a	363.5 ±6.8 ab	124.0 ±5.9 ab
Cellulose	247.1 ±2.1 a	292.5 ±3.0 a	333.0 ±3.9 a	362.2 ±4.6 ab	115.2 ±3.6 ab
Wheat Bran	243.1 ±1.9 a	293.0 ±3.5 a	332.8 ±4.3 a	367.5 ±6.1 ab	124.5 ±5.0 ab
Pectin	215.4 ±2.7 a	277.0 ±4.9 a	316.5 ±6.5 a	348.8 ±9.0 b	105.9 ±6.8 b
Oat Bran	237.6 ±6.7 a	278.1 ±9.6 a	322.4 ±9.5 a	367.3 ±5.1 ab	129.7 ±8.2 a
American Blend	236.0 ±8.2 a	286.9 ±5.2 a	326.1 ±4.9 a	360.7 ±6.0 ab	124.7 ±7.6 ab
White Sor Bran	237.8 ±2.1 a	282.1 ±2.6 a	318.7 ±2.8 a	353.8 ±3.3 ab	116.1 ±2.9 ab
Brown Sor Bran	240.4 ±3.7 a	289.3 ±3.7 a	331.4 ±3.9 a	369.3 ±5.9 a	128.9 ±6.4 a
Millet Bran	229.8 ±8.5 a	276.0 ±9.2 a	321.0 ±6.1 a	360.4 ±4.4 ab	130.6 ±9.6 a

Results are expressed as means ± the standard error of the mean of twelve animals. In each category, means not sharing the same letter are significantly different ($P<0.05$).

Table 11. Effect of fiber serum cholesterol and triglyceride levels.

	Cholesterol mg/dl	Triglycerides mg/dl
Fiber Free	169 ±11 cde	133.64 ±22.05 a
Cellulose	199 ±17 bc	113.58 ±10.34 ab
Wheat Bran	261 ±17 a	109.92 ±15.34 ab
Pectin	151 ±10 de	91.17 ±10.93 ab
Oat Bran	144 ±9 e	96.42 ± 6.19 ab
American Blend	139 ±7 e	79.73 ± 5.92 b
White Sorghum Bran	232 ±16 ab	116.15 ± 9.55 ab
Brown Sorghum Bran	228 ±17 ab	108.36 ±22.64 ab
Millet Bran	188 ±10 cd	98.75 ±11.20 ab

Results are expressed as means ± the standard error of the mean on a sample size of eleven to thirteen rats per group. In each category, means not sharing the same letter are significantly different ($P<0.05$). Cholesterol determined by method of Flegg (58) and Richmond (59). Triglycerides determined by method of Bucolo and David (62).

millet bran was significantly lower than the wheat bran, white sorghum bran and brown sorghum bran. The American blend was not significantly different from the soluble fibers, pectin and oat bran.

Triglyceride values were similar between the diets. The exceptions were the rats fed the fiber free diet, which had elevated triglyceride levels and the American blend rats whose triglycerides were significantly lower.

Effect of Diet on Liver Lipid and Cholesterol Values

The soluble fiber groups (pectin, oat bran, and American blend) had significantly lower liver weights (Table 12) than the white sorghum bran, brown sorghum bran, millet bran and wheat bran; all insoluble fibers.

Brown sorghum bran had the highest lipid content (Table 12) while oat bran had the lowest lipid content. The millet bran and white sorghum bran contained lipids in the same range as the wheat bran. The American blend fiber liver lipid levels were similar to the soluble fiber liver lipid levels.

In liver cholesterol values (Table 12), there was again a difference between soluble and insoluble fibers; the soluble fibers having the lower liver cholesterol values.

Overall, the soluble fibers (pectin, oat bran, American blend) had significantly lower values in liver weight, lipids, and cholesterol than did the insoluble impure fibers (wheat bran, white sorghum bran, brown sorghum bran, millet bran). Serum cholesterol values and liver cholesterol values were

Table 12. Effect of fiber on liver cholesterol levels, liver lipids, and liver weight.

Diet	Cholesterol (mg/g liver)	Lipids (mg/g liver)	Weight (g)
Fiber free	38.42 ±2.21 c	151.4 ±8.1 c	17.5267 ±0.5051 ab
Cellulose	40.41 ±1.42 c	149.1 ±6.2 c	17.4301 ±0.3927 ab
Wheat Bran	49.08 ±1.09 ab	183.6 ±7.1 b	17.8549 ±0.4213 a
Pectin	35.58 +2.30 cd	144.4 ±8.3 c	15.3436 ±0.6357 c
Oat Bran	30.37 ±1.67 d	121.3 ±5.2 d	16.2066 ±0.3528 bc
American Blend	35.15 ±1.75 cd	156.6 ±5.7 c	15.9606 ±0.4257 c
White Sorghum Br	46.24 ±2.25 b	178.8 ±10.8 b	17.7030 ±0.2319 a
Brown Sorghum Br	54.00 ±1.60 a	209.3 ±7.8 a	18.0657 ±0.4896 a
Millet Bran	48.20 ±1.74 b	186.4 ±8.4 b	18.0211 ±0.6593 a

Results are expressed as means ± the standard error of the mean on a sample size of twelve rats per diet group. In each category, means not sharing the same letter are significantly different ($P<0.05$). Lipids extracted by Folch method (63) and cholesterol determined by method of Searcy and Bergquest (64).

positively correlated using Pearson's coefficient (R=.5448 $p<0.0001$).

Effect of Experimental Diets on Colon Physiology

Effect of Diet on 72 Hour Fecal Outputs

For both fecal collections, the fiber diets had significantly heavier dry fecal outputs than the fiber-free diet (Table 13). During the second week, the brown sorghum bran had the heaviest fecal dry weight. The millet bran and white sorghum bran were not significantly different from the cellulose or wheat bran. The American blend diet and oat bran had similar effects on bulking ability and were significantly lighter than the insoluble fibers. Pectin had the lowest fecal dry weight of all of the fiber-supplemented diets. During the third week, the results followed a similar pattern.

Effect of Diet on In Vivo pH

In the cecum, oat bran had the lowest pH and was significantly different from the other diets (Table 14). Cellulose had the highest pH. Brown sorghum bran and millet bran were significantly different from both cellulose and oat bran. In the proximal colon, oatbran again had the lowest pH while wheat bran had the highest pH. The remainder of the diets showed intermediate effects. In the distal colon, no significance was found in pH between diet groups.

Effect of Diet on Gut Length and Cecal Surface Area.

The gut lengths of rats fed millet bran, oat bran, wheat bran, and cellulose were all significantly longer than the fiber free gut length (Table 15). The rats fed brown sorghum

Table 13. Effect of fiber on 72-hour fecal collections (dry weight) for week two and three.

Diet	Dry feces (g) Week Two	Dry feces(g) Week Three	Dry feces(g) Average
Fiber Free	2.41 ±.17 f	2.44 ±.07 f	2.43 ±.09 e
Cellulose	5.38 ±.27 b	5.51 ±.18 b	5.44 ±.20 b
Wheat Bran	5.57 ±.17 ab	5.48 ±.26 b	5.52 ±.17 b
Pectin	3.12 ±.23 e	3.35 ±.17 e	3.23 ±.18 d
Oat Bran	3.90 ±.25 d	4.01 ±.24 d	3.96 ±.22 c
American Blend	4.40 ±.32 cd	4.21 ±.14 d	4.31 ±.21 c
White Sorghum Br	5.04 ±.20 bc	4.93 ±.13 c	4.99 ±.14 b
Brown Sorghum Br	6.11 ±.16 a	6.14 ±.16 a	6.12 ±.16 a
Millet Bran	5.65 ±.30 ab	5.08 ±.21 bc	5.36 ±.24 b

Results are expressed as means ± the standard error of the mean of twelve rats. In each category, means not sharing the same letter are significantly different ($P<0.05$).

Table 14. Effect of fiber on colonic pH.

Diet	pH Cecum	pH Proximal	pH Distal
Fiber Free	7.08 ± .11 ab	6.96 ±.05 ab	6.93 ±.24 a
Cellulose	7.23 ± .10 a	6.90 ±.11 ab	7.27 ±.15 a
Wheat Bran	7.00 ± .12 ab	7.04 ±.14 a	6.67 ±.16 a
Pectin	6.87 ± .19 ab	6.78 ±.12 abc	6.82 ±.22 a
Oatbran	6.24 ± .13 c	6.43 ±.16 c	6.77 ±.22 a
American Blend	7.09 ± .10 ab	6.92 ±.10 ab	6.94 ±.16 a
White Sorghum Br	7.00 ± .06 ab	6.75 ±.13 abc	6.87 ±.31 a
Brown Sorghum Br	6.80 ± .07 b	6.66 ±.21 abc	6.64 ±.21 a
Millet Bran	6.79 ± .11 b	6.58 ±.05 bc	6.63 ±.30 a

Results are expressed as means ± the standard error of the mean of nine rats. In each category, means not sharing the same letter are significantly different ($P<0.05$).

Table 15. Effect of fiber on gut length and cecal surface area.

Diet	Gut Length (cm)	Cecal Surface Area (mm^2)
Fiber Free	17.96 ±.29 c	820.59 ±31.98 c
Cellulose	19.34 ±.43 a	810.72 ±36.25 c
Wheat Bran	19.81 ±.64 a	860.30 ±39.93 c
Pectin	19.17 ±.43 abc	1135.29 ±118.20 a
Oat Bran	19.54 ±.39 ab	1136.14 ±56.18 a
American Blend	18.86 ±.34 abc	1030.99 ±32.62 ab
White Sorghum Bran	18.78 ±.34 abc	887.66 ±44.36 bc
Brown Sorghum Bran	18.42 ±.45 bc	803.06 ±22.90 c
Millet Bran	20.07 ±.37 a	955.56 ±46.20 bc

Results are expressed as means ± the standard error of the mean of twelve rats. In each category, means not sharing the same letter are significantly different ($P<0.05$).

bran, white sorghum bran and the American blend were intermediate to the millet bran and fiber free values and not significantly different.

The soluble fibers (oat bran, pectin, and the American blend) had significantly larger cecal surfaces areas than the fiber free diet. The insoluble fibers, cellulose and wheat bran, exhibited low cecal surface areas which were not significantly different from both sorghum brans and the fiber free diet.

DISCUSSION

Effect of Sorghum and Millet Brans on Lipid Metabolism

The effect of sorghum and millet brans on lipid metabolism were measured by the indices of serum and liver cholesterol levels. The Lipid Research Clinics Program found that for every 1% drop in serum cholesterol levels one may expect a 2% drop in the incidence of coronary heart disease (5). As confirmed by analysis, the majority of fiber in sorghum and millet bran is insoluble (96-97%) with a very small soluble portion (3-4%). In general, insoluble fibers do not lower cholesterol and therefore, sorghum and millet were not expected to reduce cholesterol levels in blood serum or the liver. The brown sorghum and white sorghum brans affected cholesterol values as expected, being intermediate to wheat bran and cellulose. The presence of tannins in the brown sorghum seemed to have no effect upon cholesterol levels as the brown sorghum and white sorghum brans exhibited comparable serum cholesterol values.

The millet bran result was somewhat surprising as it did not differ in fiber composition from the sorghum brans. This lowering effect in the serum cholesterol, which was intermediate to cellulose and pectin, was not seen in the liver. It could be due to the presence of some component of the bran other than the fiber. A few cereal brans such as

rice and barley, which contain mainly insoluble fiber, have been shown to exhibit hypocholesterolemic effects in both humans and animals (70-72). This effect maybe caused by tocotrienols, fat soluble components which inhibits HMG CoA reductase, the key enzyme regulating the biosynthesis of cholesterol (73). It is possible that millet contains tocotrienols as they are present in barley, wheat, oats and rye (73), although whole millet grain contained very little tocotrienols (74). However, the whole millet grain only contained between 3-4% total lipids (21). The millet bran in this study contained 11% fat so it is possible that the tocotrienols were concentrated in the bran. However, liver cholesterol levels did not decrease for the millet bran diet. Since the liver is the main site of de novo synthesis this prevents a positive conclusion. More experiments to clarify the effects of the millet bran on cholesterol levels are needed.

The results of the present study did not support the results of Klopfenstein et al (27) who found that sorghum significantly lowered liver cholesterol levels. However, the study was seriously flawed in two ways. The levels of dietary fiber in the diets was not controlled or reported. Secondly, only three to five animals were used per diet group, which gave great within diet variances and is not enough animals to obtain statistically reliable data. The present study kept the amount of fiber in the diet constant at 6%. Twelve

animals were used per diet group in order to ensure statistically reliable data. Therefore, it is felt that the results of the present study are more plausible and much better represents the effect of sorghum and millet upon lipid metabolism.

Effect of Sorghum and Millet on Colonic Physiology

Fecal Bulking:

Fecal bulking, cecal surface area, gut length and colonic pH were measured as indices of colonic physiology. Fecal bulking is the ability of the fiber to produce larger masses of feces, as measured after drying. Increased fecal bulking has been correlated with decreased transit time, the time it takes the food residues to travel through the large intestine (75,76).

This is important when considering the effect of the fiber upon colon cancer. Carcinogens and tumor promoters are always present in the colon: either introduced from the diet, such as long chain fatty acids; produced by the body, such as bile acids; or produced by colonic microflora, such as short chain fatty acids and ammonia. The less time these carcinogens and promoters are in contact with colonic mucosa, the less chance there is for them to promote cell proliferation. Adding more bulk to the fecal matter dilutes the carcinogens so there is less probability of contact with the colonic mucosa.

The observation that bulking ability is negatively related to the fermentability of the fiber is well illustrated by the present study. The soluble fibers, which are highly

fermentable, showed the least bulking ability while the insoluble fiber, which are not as fermentable, showed the greatest bulking ability. The sorghum and millet brans proved to be excellent bulking agents. The high bulking ability of the brown sorghum could be due to the tannin content, which may have resisted microbial degradation to a greater extent than the white sorghum or millet brans.

Colonic pH:

Decreasing pH and increasing cecal surface area have been correlated with increased cell proliferation and enhanced carcinogenesis (14). Fermentation of the fiber by colonic microflora produces gas and short chain fatty acids and gas which lower the pH.

In the present study, oat bran was the only fiber supplement that lowered colonic pH in both the cecum and proximal colon. The millet bran and brown sorghum bran pH values were intermediate between the cellulose and oat bran values in the cecum. Since these two fibers were very low in soluble fiber (2.70% and 2.88% respectively) the slight decrease in pH could be due to the fermentation of starch which escapes degradation in the upper GI tract. Resistant starch acts like soluble dietary fiber in the colon and is rapidly fermented by the colonic microflora (12). The brown sorghum and millet brans may have contained starch bound to the bran fraction which escaped digestion in the small intestine but was utilized by the colonic microflora.

Cecal Surface Area

Cecal surface area is an index of the growth of the cells in the colon. A previous study by Lupton et al. positively correlated increased cecal surface area with increased cell proliferation (69). As previously stated, increased cell proliferation and enhanced colonic tumorigenesis have been associated with one another (77-79). The brown sorghum had the smallest cecal surface area of any diet group which might be attributed to the tannin content. Since the brown sorghum, white sorghum or millet brans did not increase cecal surface area as compared to the fiber free control, wheat bran or cellulose, this may be seen as a positive influence in the colon. The present study confirms previous studies' (69,80) results that soluble fibers increase cecal surface area as seen by the significant increase in cecal surface area of the pectin and oat bran.

Gut Length

There is no clear cut explaination for the gut length measurements as some of the insoluble fibers increased gut lengths (wheat bran and millet bran) while another insoluble fiber did not increase gut length (brown sorghum bran). The soluble fibers, which were expected to increase gut length to a greater extend than the insoluble fibers, did not.

An increase in gut length could be due to 1) cell proliferation or 2) hypertrophy of the intestinal muscle coat due to increase bulk. Lupton and Meacher (80) found that

different fibers cause different pellet formation. For example, wheat bran caused large, oval pellet formation which may have stretched the muscle coat while a fiber free diet resulted in small, irregularly-shaped pellets which would not cause mechanical distension. Perhaps the increase in gut length was due to an increase in colon diameter instead of cell proliferation. Therefore it is uncertain in this study to what the lengthening of the gut was attributed. Future studies should include cell proliferation measurements.

The American Blend Fiber

This was the first test of the American blend fiber, a pure fiber mixture formulated to mimic the component fiber consumption in the American diet.

During the third week, American blend rats had a much lower food and energy intake than other rats. It appears that the American blend diet was not as well tolerated by the rats as the other diets because food intake did not increase during the third week as the other diets did. This decreased consumption could be due to the pure lignin portion of the fiber blend. A previous human study using purified lignin commented that the lignin was extremely unpalatable (81). However, the decrease in energy intake did not affect weight gain or final weight.

The fact that the American blend diet exhibited the lowest serum cholesterol was quite unexpected. The pure fiber supplement contained 35% soluble fiber and 65% insoluble

fiber. It was fed at 6% fiber by weight which corresponds to 30 grams of fiber/ day in human terms, well within the National Cancer Institute's recommended range of 25-35 gram/day. Americans are estimated to consume around 12 grams of fiber/day. If this diet is actually a true representation of the composition of fiber that an American consumes, the data seem to suggest that Americans are eating the right ratios of fibers, just not enough of it to have a positive effect.

In the American blend diet the mixture of fibers may have a synergistic effect. Both guar gum and pectin, the soluble fiber components, are hypocholesterolemic (44,82,83). A component of the insoluble fiber, lignin has been shown by Story and Kritchevsky (84) to lower cholesterol levels by binding bile acids and salts substantially more than bran, cellulose, and alfalfa. Even the corn bran used in the blend has been shown to be hypocholesterolemic when used in a large dose (85). Therefore, it seems that the combination of these fibers worked to lower cholesterol levels.

In the colon, the American blend fiber produced a bulking effect similar to oat bran, which is degraded to a large extent in the colon. Therefore, if the American blend fiber lowers cholesterol by binding bile acids and decreasing cholesterol absorption this could promote colon cancer because these tumor promoters are not diluted in the colon.

The American blend increased cecal surface area to the same extent that the oat bran did. It seems that the soluble portion of a fiber has a dominating effect, as the fiber in the American blend was only 36% soluble fiber but effectively lowered cholesterol levels and increased cecal surface area.

It is probably safe to say that this fiber mixture does not have the same physiochemical properties as naturally occurring fiber in foods. After all, this is a pure fiber mixture while the fiber taken in the normal diet of a person is chemically interacting with other food components which most likely affect its properties. However, the results seem to suggest that the amount of soluble fiber is important in the mixture and expresses its characteristics much more than the insoluble fiber does. The simulated diet upon which this mixture was based was compiled from the 1977-1978 USDA Nationwide Food Consumption Survey. Since then the type and quantity of fiber intake in the United States has changed drastically. Future areas of research should focus on updating the proportions in the American blend and reformulating according to changes in the American diet.

CONCLUSIONS

Sorghum and Millet Brans

The sorghum brans did not lower cholesterol levels in either the serum or liver. The millet bran showed a significant decrease in cholesterol levels in the serum but not in the liver, possibly due to the presence of tocotrienols.

In the colon, the brown sorghum bran, white sorghum bran and millet bran showed positive health benefits by having excellent fecal bulking abilities, not increasing cecal surface area, or decreasing pH in relation to the fiber-free control. Therefore, these brans should not increase cell proliferation and thus should be protective against colon cancer.

The presence of tannins seemed to have a positive influence on the colon in that the brown sorghum bran exhibited the best fecal bulking ability, lowest cecal surface area and shortest gut length of all the fiber supplemented diets.

From this study it may be concluded that sorghum brans have potential to be used as bulking agents in the diet; however, they have little value as hypocholesterolemic agents as hypothesized. Millet bran seemed to have the potential to have a cholesterol lowering effect while being a good bulking agent. However, confirmation of these results is necessary.

Future studies in the area of sorghum and millet brans should focus on the effects of sorghum and millet bran on

colonic functions such as transit time and cell proliferation instead of their effect on lipid metabolism.

The American Blend Fiber.

The American blend fiber was added to this study to see the interactive effects of a pure mixed fiber source in the rat model. The final fiber mixture consisted of corn bran, lignin, cellulose, guar gum and pectin. This blend had excellent cholesterol lowering abilities but did not exhibit positive effects in the colon and therefore may promote cancer.

CURRENT AND FUTURE PLANS FOR THIS RESEARCH:

1). A poster session entitled "Possible Health Benefits of Sorghum and Millet Brans" will be presented at the 75th Annual Meeting of the American Association of Cereal Chemists in Dallas, Texas in October, 1990.

2) An abstract was submitted for the National Institute of Food Technologists Undergraduate Research Competition. The research findings will be presented at the IFT National Meeting in Anaheim, California in June, 1990.

3) A paper will be submitted to the Journal of Food Science for publication.

LITERATURE CITED

1. Burkitt, D.P. (1971) Epidemiology of cancer of the colon and rectum. Cancer 28:3-13.
2. Burkitt D.R., and Trowell, H.C. (1975) Refined carbohydrates and disease. Some implications of dietary fibre. London: Academic Press.
3. Trowell, H.C. (1972) Ischaemic heart disease and fiber. Am J Clin Nutr 25:926-932.
4. American Heart Association (1986) Heart facts. Dallas, Texas: American Heart Association.
5. Lipids Research Clinics Program. (1984) The lipid research clinics coronary primary prevention trial results: the relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 251:351-374.
6. American Cancer Society (1987) American Cancer Society: cancer facts and figures. New York: American Cancer Society, Inc.
7. Greenwald, P., Lanza, E., and Eddy, G.A. (1987) Dietary fiber in the reduction of colon cancer risk. J Amer Diet Assoc 87(9): 1178-1188.
8. Klurfeld, D.M. (1987) The role of dietary fiber in gastrointestinal disease. J Amer Diet Assoc 87(9): 1172-1177.
9. Keys, A.,ed. (1970) Coronary heart disease in seven countries. Circulation 41: suppl 1.
10. Kritchevsky, D. (1986) Dietary fiber and atherosclerosis. In: Vahouny, G.V. and Kritchevsky, D.,eds. Dietary fiber: basic and clinical aspects. New York: Plenum Press: 265-274.
11. Trowell, H., Southgate, D.A.T., Wolever, T.M.S., Leeds, A.R.I., Gussell, M.A., and Jenkins, D.A. (1976) Dietary fiber redefined. Lancet 1:967.

12. Cummings, J.H. (1983) Fermentation in the human large intestine: evidence and implications for health. Lancet 1:1206-1208.
13. Chen, W.L.J., and Anderson, J.W. (1986) Hypocholesterolemic effects of soluble fibers. In Vahouny, G.V., and Kritchevsky, D., eds. New York: Plenum Press: 275-286.
14. Jacob, L.R., and Lupton, J.R. (1986) Relationship between colonic luminal pH, cell proliferation and colon carcinogenesis in 1,2 dimethylhydrazine treated rats fed high fiber diets. Cancer Res 46:1727-1754.
15. Butrum, R.R., Clifford, C.K., and Lanza, E. (1988) Dietary guidelines: rationale. AM J Clin Nutr 48: 888-895.
16. Anderson, J.W., and Bridges, S.R., Tietyan, J., and Gustafson, N.J. (1989) Dietary fiber content of a simulated American diet and selected research diets. Am J Clin Nutr 49:352-357.
17. Hibbler, M., and Stanley, R. (1981) Prospecting for research gold. IDRC reports 10(1): 18-19.
18. Rooney, L.W., and Serna-Saldivar, S.O. (in press) Sorghum. In: Larenz, K., and Kulp, K. Handbook of cereal science and technology. New York: Mercel Dekker.
19. Food Agriculture Organization. (1986) Production yearbook. FAO statistics series: 48(37) Rome, Italy.
20. Stoskopf, N.C. (1985) Sorghum in cereal grain crops. Reston, Virginia: Reston Publishing Co, Inc: 369-385.
21. Hulse, J.H., Laing, E.M., and Pearson, O.E. (1980) Sorghum and millets: their composition and nutritive value. New York: Academic Press.
22. Jambunathan, R., and Mertz, E.T. (1973) Relationship between tannin levels, rat growth, and distribution of proteins in sorghum. J Agric. Food Chem. 21:692-696.
23. Rooney, L.W., and Plugfelder, R.L. (1986) Factors affecting starch digestibility with special emphasis on sorghum and corn. J Anim Sci 63:1607-1623.

24. Bach Knudsen, K.E., and Munck, L. (1985) Dietary fibre contents and compositions of sorghum and sorghum-based foods. J Cereal Sci 3:153-164.
25. Bach Knudsen, K.E., Kirleis, A.W., Eggum, B.O., and Munck, L. (1988) Carbohydrate composition and nutritional quality for rats of sorghum to prepared from decorticated white and whole grain red flour. J Nutr 118:588-597.
26 Fedail, S.S., Badi, S.E.M., and Musa, A.R.M. (1984) The effects of sorghum and wheat bran on the colonic functions of healthy Sudanese subjects. Am J Clin Nutr 40:776-779.
27. Klopfenstein, C.F., Varriano-Marston, E., and Hoseney, R.C. (1981) Cholesterol-lowering effect of sorghum diet in guinea pigs. Nutr Rept Int 24(3): 621-627.
28. Quaker Oat front, Food Business: March 12, 1990: 12-13.
29. Chen, W.J.L. and Anderson, J.W. (1986) Hypocholesterolemic effects of soluble fibers. In: Kritchevsky, D. and Vahouny, G.V., eds. Dietary fiber: basic and clinical aspects. New York; Plenum Press: 275-286.
30. Lupton, J.R., Coder, D.M., and Jacobs, L.R. (1985) Influence of luminal pH on large bowel epithelial kinetics. Am J Physiol 249: G382-G388.
31. Lupton, J.R., and Ferrell, R.G. (1986) Using density rather than mass to express the concentration of gastrointestinal tract constituents. J Nutr 116:164-168.
32. Chang, M.L.W., and Johnson, M.A. (1976) Influence of fat level and type of carbohydrate on the capacity of pectin in lowering serum and liver lipids in young rats. J Nutr 106: 1562-1568.
33. Taylor, D.D., Conway, E.S., Shuster, E.M., and Adams, M. (1967) Influence of dietary carbohydrates on liver content and on serum lipids in relation to age and strain of the rat. J Nutr 91:275-282.
34. Forsythe, W.A., Green, M.S., and Anderson, J.J.B. (1986) Dietary protein effects on cholesterol and lipoprotein concentrations: a review. J Amer College Nutr 5:533-49.

35. Grundy, S.M., Bilheimer, D., Blackburn, H., Brown, W.V., Kwilerovich, P.O., Mattson, F., Shonfeld, G., and Wiedman, W.H. (1982) Rationale of the diet heart statement of the American Heart Association. Circulation 65:839A-854A.
36. American Institute of Nutrition Ad Hoc Committee (1977) Report of the American Institute of Nutrition Ad Hoc Committee on standard for nutritional studies. J Nutr 107:1340-1348.
37. American Institute of Nutrition Ad Hoc Committee (1980) Second report of the American Institute of Nutrition Ad Hoc Committee on standard for nutritional studies. J Nutr 110:1185-1188.
38. Story, J.A., Tepper, S.A., Kritchevsky, D. (1974) Influence of synthetic conjugates of cholic acid on cholesterolemia in rats. J Nutr 104:1185-1188.
39. Jacobs, L.R., and White, F.A. (1983) Modulation of mucosal cell proliferation in the intestine of rats fed a wheat bran diet. Am J Clin Nutr 37:945-953.
40. Jacobs, L.R., and Lupton, J.R. (1984) Effect of dietary fibers on rat large bowel mucosal growth and cell proliferation. Am J Physiol 346:G378-G385.
41. Jacobs, L.R. (1983) Enhancement of rat colon carcinogenesis by wheat bran consumption during the stage of 1,2 dimethylhydrazine administration. Cancer Res 43:4057-4061.
42. Gazzaniga, J.M., and Lupton, J.R. (1986) Dilution effect of dietary fiber sources: an in vivo study in the rat. Nutr Res 7:1261-1268.
43. Kiriyama, S., Okazaki, Y., and Yoshida, A. (1969) Hypocholesterolemic effect of polysaccharides and polysaccaride-rich foodstuffs in cholesterol-fed rats. J Nutr 97:383-388.
44. Tsai, A.C., Elias, J., Keeley, J.J., Lin, R.S.C., and Rolson, J.R.K. (1976) Influence of certain dietary fibers on serum and tissue cholesterol levels in rats. J Nutr 106:118-123.

45. Wells, A.F., and Ershoff, B.H. (1961) Beneficial effects of pectin in prevention of hypercholesterolemia and increase in liver cholesterol in cholesterol-free rats. J Nutr 74:87-92.
46. Chang, M.L.W. and Johnson, M.A. (1976) Influence of fat level and type of carbohydrate on the capacity of pectin in lowering serum and liver lipids in young rats. J Nutr 106:1562-1568.
47. Leveille, G.A., and Sauberlich, H.E. (1966) Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat. J Nutr 88:209-214.
48. Judd, P.A., and Truswell, A.S. (1982) Comparison of the effects of high and low methoxyl pectins on blood and faecal lipids in man. Br J Nutr 48:451-458.
49. Anderson, J.W., and Tietyen-Clark. (1986) Dietary fiber: hyperlipidemia, hypertension and coronary heart disease. Am J Gastro 81:907-919.
50. Shinnick, F.L., Longacre, M.J., Ink, S.F., and Marlett, J.A. (1988) Oat fiber: composition versus physiological functions in rats. J Nutr 118:114-151.
51. Klurfeld, D.M. (1987) The role of dietary fiber in gastrointestinal disease. J Am Diet Assoc 87:1172-1177.
52. Anderson, J.W., Story, L., Sieling B., Chen W.J.L., Petro, M.S., and Story J. (1981) Hypocholesterolemic effects of oat bran or bean intake for hypercholesterolemic men. Am J Clin Nutr 40:1146-1155.
53. Kirby, R.W., Anderson, J.W., Seiling G.B.H., Rees, E.D., Chen, W.J.L., Miller, R.E., and Key, R.M. (1981) Oat bran intake selectively lowers serum low density lipoprotein concentrations of hypercholesterolemic men. Am J Clin Nutr 40:1146-1155.
54. Anderson, J.W., and Bridges, S.R. (1988) Dietary fiber content of selected foods. Am J Clin Nutr 47:440-447.

55. Aspinall, G.O. (1980) The biochemistry of plants. In: Chemistry of cell wall polysaccharides, vol 3., New York: Academic Press: 473-500.
56. Dreher, M.L. (1987) Conventional and unconventional fiber components. In: Handbook of dietary fiber: an applied approach. New York: Marcell Dekker, Inc.: 22-38.
57. Prosky, L., Asp, N.G., Schweizer, T.F., DeVries, J.W., and Furda, I. (1988) Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. JAOAC 71(5):1017-1023.
58. Flegg, H.M. (1973) An investigation of the determination of serum cholesterol by an enzymatic method. Ann Clin Chem Biochem 10:79-84.
59. Richmond, W. (1973) Preparation and properties of a cholesterol oxidase from Norcardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 19(12):1350-1356.
60. Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., and Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20(4):470-475.
61. Roeschlau, P., Bernt, E., and Gruber, W. (1974) Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem 12(5):266.
62. Bucolo, G., and David, H. (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19(5): 476-482.
63. Folch, J., Less, M., and Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 266:497-509.
64. Searcy, R.L., and Bergquest, L.M. (1960) A new color reaction for the quantification of serum cholesterol. Clin Chem Acta 5:192-199.
65. Duncan, D.B. (1955) Multiple range and multiple f tests. Biometrics 11:1-42.

66. Nu, N.H., Hull, C.H., Jenkins, J.G., Steinbrener, K., and Bent, O.H. (1975) Statistical package for the social sciences, second edition. New York: McGraw-Hill.
67. Farness, P.L., and Schneeman, B.O. (1982) Effects of dietary cellulose, pectin and oatbran on the small intestine in the rat. J Nutr 112:1315-1319.
68. Vahouny, G.V., Khalafi, R., Satchithanandam, S., Watkins, D.W., Story, J.A., Cassidy, M.M., and Kritchevsky, D. (1987) Dietary fiber supplementation and fecal bile acids, neutral steriods and divalent cations in rats. J Nutr 117: 2009-2015.
69. Lupton, J.R., Coder, D.M., and Jacobs, L.R. (1988) Long-term effects of fermentable fibers on rat colonic pH and epithelial cell cycle. J Nutr 118: 840-845.
70. Newman, R.K., Newman C.W., Hofer, P.J., and Goering, K.J. (1989) Effect of a barley beta-glucan fraction and barley oil on chick serum lipids. American Association of Cereal Chemists 74th Annual Meeting Abstracts:109.
71. Kahlon, T.S., Saunders, R.M., Chow, F.I., Chiu, M.M., and Betschart, A.A. (1989) Effect of rice bran and oat bran on plasma cholesterol in hamsters. American Association of Cereal Chemists 74th Annual Meeting Abstracts:111.
72. Robinson, M.C., and Lupton, J.R. (1990) The effects of barley flour and barley oil in hypercholesterolemic men and women. J Amer Diet Assoc: In press.
73. Qureshi, A.A., Burger, W.C., Peterson, D.M., and Elson, C.E. (1986) The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem 261(23):10544-10550.
74. Piironen, V., Syvaoja, E.L., Varo, P., Salminen, K., and Koivistionen, P. (1986) Tocopherols and tocotrienols in cereal products from Finland. Cereal Chem 63(2):78-81.
75. Burkitt, D.P., Walker, A.R.P., and Painter, N.S. (1972) Effect of dietary fibre on stools and transit times, and its role in the causation of disease. Lancet 2:1409-1412.

76. Read, N.W., Miles, C.A., Fisher, D., Holgate, Kime, N.D., Mitchell, M.A., Reeve, A.M., Roche, T.B., and Walker, M. (1980) Transit of a meal through the stomach, small intestine and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 79:1276-1282.
77. Farber, E. (1984) Cellular biochemistry of the stepwise development of cancer with chemicals: G.H.A. Clowes memorial lecture. Cancer Res 44:5463-5474.
78. Oscarson, J.E.A., Veen, H.F., Ross, J.S., and Malt, R.A. (1979) Ileal resection potentiates 1,2 dimethylhydrazine-induced colonic carcinogenesis. Ann Surg 189:503-508.
79. Williamson, R.C.N., Bauer, F.L.R., Ross J.S., Watkins, J.B., and Malt, R.A. (1979) Enhanced colonic cacinogenesis with azoxymethane in rats after pancreaticobilary division to mid small bowel. Gastroenterology 76:1386-1392.
80. Lupton, J.R., and Meacher, M.M. (1988) Radiographic analysis of the effect of dietary fibers on rat colonic transit time. Am J Physiol 225:G633-G639.
81. Hillman, L.C., Peters, S.G., Fischer, C.A., and Pomare, E.W. (1985) The effects of fiber components pectin, cellulose, and lignin on serum cholesterol levels. Amer J Clin Nutr 42:207-213.
82. Aro, A., Uusitupa, M., Voutilainen, E., and Korhonen, T. (1984) Effects of guar gum in male subjects with hypercholesterolemia. Am J Clin Nutr 39: 911-916.
83. Miettinen, T.A., and Tarpila S. (1977) Effect of pectin on serum cholesterol, fecal bile acids, and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin Chim Acta 79:471-480.
84. Story, J.A., and Kritchevsky, D. (1976) Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J Nutr 106:1291-1294.
85. Earll, J.M. (1986) Feasibility and matabolic effects of a precisely measurable dietary food supplement. Unpublished.