Effects of Fiber and Phytate in Sorghum Flour on Iron and Zinc in Weanling Rats: A Pilot Study

H. I. ALI and B. F. HARLAND[1]

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ABSTRACT

The effects of dietary fiber and phytate (*myo*-inositol hexaphosphate) in sorghum flours on iron and zinc concentrations in selected tissues of weanling rats were investigated using whole and traditionally decorticated sorghum grains (white durra) grown in Somalia. Two methods were used: the Traditional and the Improved (research) method using fertilizers and pesticides. Total dietary fiber content in the Traditional and Improved whole flours did not differ much (12.84 and 12.58%, respectively). Phytate levels in the Traditional and the Improved whole sorghum flours were 4.03 and 7.26 mg/g, respectively. Iron levels were highest in the Traditional whole and lowest in the Traditional decorticated flour (52 and 39 ppm, respectively). Zinc levels ranged from 39 ppm in the Improved whole to 17 ppm in the Traditional decorticated flour. Results showed that the total weight gain and feed efficiency ratios of rats fed the Improved whole sorghum flour were significantly lower than those of the control rats. The mean tibia iron level of the rats in the control group (119.4 ± 10 ppm) was significantly higher ($P < 0.05$) than those of the rats fed diets containing Improved sorghum flours (76.8 ± 4 and 86.1 ± 5 ppm for whole and decorticated flours, respectively). Furthermore, the tibia zinc concentration of the control group was significantly higher ($P < 0.05$) than those of any of the four groups fed sorghum-containing diets. This study indicated that consumption of sorghum-containing diets by rats may result in lower mineral concentrations in the bone.

Sorghum is well adapted to the low rainfall prevalent in Somalia and other semiarid countries. It plays a major role in the diets of low-income Somali people and is a major source of calories and protein for the Somali nomads, especially during the dry season when milk is scarce (Abbas 1980). Both whole and decorticated forms of sorghum are consumed in Somalia, either alone or in combination with rice, corn, or beans. However, as with other cereal grains, sorghum contains phytate (*myo*-inositol hexaphosphate), which is of particular concern to nutritionists because of its possible adverse effects on mineral absorption. Phytate forms insoluble complexes with essential minerals such as calcium, copper, iron, and zinc at physiological pH levels (Graf 1986), and it may be partially responsible for the widespread mineral deficiencies observed in populations that subsist largely on sorghum and other cereals (Hulse et al 1980).

Although dietary fiber has been associated with a number of beneficial therapeutic effects (Anderson 1982, Kritchevsky 1982), certain components of dietary fiber may interfere with mineral and protein absorption and utilization (Cornu and Delputch 1981, Fernandez and Phillips 1982, Fleming and Lee 1983, Gillooly et al 1984). The adverse nutritional effects of fiber and phytate are of greater concern when the diets are already marginal in nutrients (Harland and Peterson 1978). Abbas (1980, 1982) reported dietary deficiencies of iron, zinc, and other essential

[1]Department of Human Nutrition and Food, School of Human Ecology, Howard University, Washington, DC 20059.

nutrients in diets commonly consumed in Somalia. Milling processes may result in partial losses of fiber and phytate from sorghum but are often accompanied by a loss of essential nutrients. In spite of the existence of commercial mills in large urban areas, most of the low-income households in Somalia still decorticate their sorghum at home, using the traditional method of hand-pounding the grains with a wooden mortar and pestle.

Agricultural trials conducted by the Bay Region Agricultural Development Program (BRADP, created by the joint efforts of the Somali government and international organizations) have shown that the yields of the Somali local durra varieties are increased by use of modern agricultural techniques such as fertilizers and pesticides (IDRC 1988). However, no information was obtained as to whether this higher-yielding sorghum is nutritionally different from that grown using traditional farming practices.

To investigate the effects of dietary fiber and phytate on the nutritive quality of sorghum commonly consumed in Somalia, a study was conducted with the following objectives: 1) to determine total dietary fiber, phytate, iron, and zinc levels in whole and decorticated flours obtained from Somali local white durra sorghum grown either by using traditional farming practices or under improved agronomic conditions (including the use of fertilizers and pesticides) and 2) to determine the effects of total dietary fiber and phytate in the sorghum flours on weight gain and concentrations of iron and zinc in selected tissues of weanling rats.

MATERIALS AND METHODS

Research Design

Fifty male weanling rats of the Long-Evans strain (Charles River, Wilmington, MA) were assigned by weight to five groups (10 rats each) to receive either a purified diet based on egg white (control) or one of four experimental diets, each containing 50% sorghum flour, for 21 days. The animals were housed individually in stainless steel cages located in a temperature-controlled room (24 ± 2°C) with a 12-hr light-dark cycle. Food and deionized water were available ad libitum for the duration of the study. The animals were weighed weekly, and food intake was recorded three times a week. After three weeks, the rats were sacrificed with an overdose of anesthesia (halothane). At the time of sacrifice, blood was drawn by cardiac puncture into heparinized tubes and centrifuged at 2,000 × *g* for 10 min; the plasma obtained was used for determination of zinc. In addition, the liver and the left hind leg were removed from each animal. These livers and the tibias were later analyzed for iron and zinc. The plasma, the livers, and the tibias were stored in a freezer at −4°C until analyzed.

All sorghum samples for the study were of the same Somali local durra variety with white pericarp. They were grown using either traditional farming practices (Traditional) or improved agronomic conditions, including the use of fertilizers and pesticides (Improved). The Traditional sorghum was grown by Somali farmers. It was purchased by one of the researchers from a marketplace in Baidoa, Somalia, and had been grown without the use of fertilizers and pesticides, as reported by the farmer who was selling the grains. The Improved sorghum was provided by the BRADP and had been grown at the Bonka Agricultural Research Station (Baidoa, Somalia) through the joint efforts of BRADP and the International Development Research Center (Montreal, Canada).

Both the Traditional and the Improved sorghum grains were grown under rain-fed (nonirrigated) conditions in the same geographic area (Bay Region) during the March–July 1988 growing season. Since little genetic variability exists among the white durra grains grown in the Bay Region (IDRC 1988), the effect of genetic factors on the chemical composition of the two types of sorghum used for the study was assumed to be minimal. Thus, any differences in the nutritional quality of the sorghum grains we used might be largely explained by the method of cultivation.

Flour Production from Sorghum Grains

The traditional Somali method of hand-pounding the grains in a wooden mortar and pestle to produce flour was employed. For the purpose of this study, deionized water instead of regular drinking water was used to minimize external mineral contamination. The grains were rinsed three times with deionized water to remove dirt and other external contaminants, then sun-dried for 10 min before being pounded. Pounding was performed by two Somali women under the supervision of the investigators and consisted of up and down strokes but also rotatory movement of the mortar to prevent uneven buildup around the walls of the mortar. To produce decorticated sorghum, about 4 kg of grain was first pounded gently with the pointed end of the pestle for 15 min. These grains were then sun-dried for 5 min, after which the bran was removed by using a winnowing basket. The decorticated sorghum was then returned to the mortar, where it was pounded vigorously with the flattened end of the pestle until reduced to flour (25 min). Whole sorghum flours were obtained by pounding the rinsed, sun-dried whole grains into flour (25 min). The flours were placed in labeled plastic bags, sealed, and then transported to the United States, where they were kept refrigerated (4°C) until used.

Animal Diets

The animal diets are presented in Tables I and II. The control diet was a modified AIN-76, casein-based diet (American Institute

TABLE I
Composition of Control Diet (Diet 1)

Ingredient	Amount (g)
Egg white (spray-dried)	20.00
DL-Methionine	0.30
Corn starch	40.00
Sucrose	26.00
Fiber[a]	5.00
Corn oil	4.00
Mineral mix[b]	3.50
Vitamin mix[c]	1.00
Choline bitartrate	0.20
Total	100.00
kcal	380.00

[a] Nonnutritive alphacel.
[b] American Institute of Nutrition mineral mixture, AIN-76.
[c] American Institute of Nutrition vitamin mixture, AIN-76.

TABLE II
Composition of Experimental Diets

	Amount			
Ingredient	Diet 2	Diet 3	Diet 4	Diet 5
Egg white,[a] g	12.03	11.94	11.36	11.65
Sorghum flour,[b] g	50.00	50.00	50.00	50.00
DL-Methionine, g	0.30	0.30	0.30	0.30
Sucrose, g	26.97	27.06	27.64	27.35
Corn oil, g	6.00	6.00	6.00	6.00
Mineral mix,[c] g	3.4909	3.4854	3.4908	3.4880
Vitamin mix,[d] g	1.00	1.00	1.00	1.00
Choline bitartrate, g	0.20	0.20	0.20	0.20
Ferric citrate,[e] mg	5.6890	9.7977	6.6372	8.5335
Zinc carbonate,[e] mg	3.4516	4.7939	2.5887	3.4516
Total	100.00	100.00	100.00	100.00
kcal	370.19	371.93	370.22	374.28

[a] Spray-dried.
[b] Diet 2 contained Traditional whole flour, Diet 3 Traditional decorticated flour, Diet 4 Improved whole flour, and Diet 5 Improved decorticated flour.
[c] American Institute of Nutrition mineral mixture, AIN-76, Fe- and Zn-free.
[d] American Institute of Nutrition vitamin mixture, AIN-76.
[e] Diets were supplemented with iron and zinc to equalize levels in all diets.

of Nutrition 1977), substituting egg white for casein. Each of the four experimental diets contained 50% sorghum flour. Diets 2 and 3 contained flours obtained from Traditional whole and Traditional decorticated sorghum grains, respectively. Diets 4 and 5 contained flours obtained from Improved whole and Improved decorticated grains, respectively. The control and the experimental diets were formulated to be isonitrogenous and as nearly isocaloric as possible. The kilocalories provided by each sorghum flour were calculated from the percent protein (N $\times$ 6.25), fat, and carbohydrate content. The control and the experimental diets were also formulated to contain the same levels of iron and zinc. The amounts of supplemental iron and zinc added to the experimental diets were reduced to accommodate for the iron and zinc provided by the sorghum flours.

The sorghum flours were the sole source of fiber and phytate in the four experimental diets. The ingredients of the control and experimental diets (except sorghum, iron, and zinc salts) were purchased from the United States Biochemical Corporation (Cleveland, OH). Reagent grade iron and zinc salts were purchased from Fisher (Columbia, MD).

Tissue Preparation for Mineral Analyses

Frozen livers were lyophilized by keeping them in a freeze-dryer for three days. Livers were then placed individually in sealed plastic bags and pulverized using a nonmetallic hammer, until a uniform product was obtained. In the case of the tibias, frozen legs were first defrosted; then the flesh and skin were cut away with a scalpel and scissors and the tibias removed. The tibias were placed in a 30-ml beaker containing 95% ethanol for 1–2 min to denature the tissue that adhered to the bone, and the bone was wiped clean with a cheesecloth. The bones were dried in a vacuum oven at 60°C for 24 hr before being weighed and ashed for atomic absorption spectrophotometry.

Analytical Methods

The four types of sorghum flour were analyzed for total dietary fiber according to the method of Prosky et al (1985) and for phytate content using the anion exchange method of Harland and Oberleas (1986). Iron and zinc levels in the flours and in rat tissues were determined by flame atomic absorption spectrophotometry (Perkin-Elmer, model 5000), after subjecting the sorghum flours, the livers, and the tibias to a combination of dry and wet ashing procedures (Hill et al 1986). Wheat flour (No. 1657) and bovine liver (No. 1577a) from the National Institute of Science and Technology (Gaithersburg, MD) were used as internal standards for the mineral analyses.

Statistical Analyses

The data were analyzed by computer using the Statistical Package for the Social Sciences (SPSS) (Nie et al 1975). One-way analysis of variance and Duncan's multiple range tests were used to determine significant differences in weight gain and tissue mineral concentrations among the five groups of rats. Analysis of covariance was used to adjust for differences in the caloric content of the five animal diets.

TABLE III
Total Dietary Fiber, Phytate, Iron, and Zinc in Sorghum Flours[a]

Flour Type	TDF[b] (%)	Phytate (mg/g)	Fe (ppm)	Zn (ppm)
Traditional				
Whole	12.84	4.03	52.0	24.0
Decorticated	10.59	3.46	39.0	17.0
Improved				
Whole	12.58	7.26	49.0	33.0
Decorticated	9.65	6.10	43.0	24.0

[a]Means of duplicate samples of fresh flour.
[b]Total dietary fiber.

TABLE IV
Weight Gain and Feed Efficiency Ratio (FER)[a]

Group	Diet	Weight Gain (g)	FER[b]
1	Control	94.0 ± 4.2 b	0.45 ± 0.02 b
2	Traditional whole	82.6 ± 3.5 ab	0.40 ± 0.02 ab
3	Traditional decorticated	87.9 ± 3.9 ab	0.41 ± 0.01 ab
4	Improved whole	77.0 ± 2.6 a	0.37 ± 0.01 a
5	Improved decorticated	88.4 ± 4.2 ab	0.42 ± 0.01 ab

[a]Values are means ± SEM. Values within a column bearing different letters differ significantly ($P < 0.05$).
[b]FER = grams of weight gain divided by grams of food intake.

RESULTS AND DISCUSSION

Composition of Sorghum Flours

Total dietary fiber, phytate, iron, and zinc levels found in duplicate samples of the four sorghum flours fed to rats are shown in Table III. The total dietary fiber levels of Traditional and Improved whole sorghum flours did not differ very much (12.84 and 12.58%, respectively). Although decortication resulted in reductions of total dietary fiber levels in the flours, the decrease was not as pronounced as levels reported for mechanically milled flours (Nyman et al 1984). Reichert and Young (1977) also showed that traditional decortication methods were less effective in the removal of crude fiber from sorghum than were mechanical methods.

Phytate levels ranged from 7.26 mg/g in the Improved whole to 3.46 mg/g in the Traditional decorticated. The two Improved sorghum flours contained higher phytate levels than the corresponding Traditional sorghum flours. The relatively high phytate content retained in the flours after decortication is not surprising since traditional decortication methods generally result in flours of higher extraction rates than those subjected to mechanical milling processes. Moreover, the highest phytate concentration in sorghum grains is found in the germ layer, which is often embedded in the sorghum endosperm and thus is not easily removed by hand-pounding the grains (Hulse et al 1980, Hoseney 1986).

Analysis of the minerals in the flours showed that the highest iron level was present in the Traditional whole and lowest in the Traditional decorticated (52 and 39 ppm, respectively). Relatively high iron levels in sorghum may be due to contamination from external sources (Hulse et al 1980, Pedersen and Eggum 1983).

The Improved sorghum was grown by a research project and thus was probably handled and stored under more controlled conditions than the Traditional sorghum before both sorghum samples were obtained for the study. This may explain in part the lower iron retention observed in the Traditional sorghum after decortication. Zinc levels in the flours ranged from 17 to 33 ppm (Traditional decorticated and Improved whole, respectively); the zinc levels in the Traditional whole and Improved decorticated were identical (24 ppm). In both Traditional and Improved sorghum, the decortication process resulted in losses of zinc.

Animal Experiment

This preliminary study evaluated the effects of dietary fiber and phytate in whole and traditionally decorticated white durra sorghum grown by the two methods on weight gain and iron and zinc concentrations in selected tissues of weanling rats fed nutritionally adequate diets. The study found no significant differences in the food intake of the control and experimental groups. In addition, the total weight gains of the rats fed the four sorghum-containing diets, with the exception of the group fed Improved whole sorghum flour, were not significantly lower than that of the control group (Table IV).

Feed efficiency ratios (FER) were calculated. FERs for the experimental groups were not different (Table IV). However, the FER of the control group was significantly higher than that of the rats fed the Improved whole sorghum flour ($P < 0.05$).

No significant differences were found in liver iron levels (Table

TABLE V
Iron Concentrations in Liver and Tibia[a]

Group	Diet	In Liver (ppm)	In Tibia (ppm)
1	Control	179.95 ± 11.3	119.36 ± 10.3 b
2	Traditional whole	174.78 ± 12.9	99.19 ± 9.7 ab
3	Traditional decorticated	178.21 ± 9.4	99.46 ± 14.7 ab
4	Improved whole	161.01 ± 11.7	76.79 ± 4.2 a
5	Improved decorticated	175.59 ± 10.9	86.16 ± 5.2 a

[a]Values are means ± SEM dry weight. Values within a column bearing different letters differ significantly ($P < 0.05$).

TABLE VI
Zinc Concentrations in Liver, Tibia, and Plasma[a]

Group	Diet	In Liver (ppm)	In Tibia (ppm)	In Plasma (μg/ml)
1	Control	75.97 ± 1.5	310.35 ± 14.9 b	2.26 ± 0.2
2	Traditional whole	71.93 ± 1.4	177.16 ± 3.0 a	1.95 ± 0.1
3	Traditional decorticated	75.92 ± 2.6	178.86 ± 8.3 a	2.04 ± 0.1
4	Improved whole	73.38 ± 5.6	152.55 ± 9.3 a	1.94 ± 0.1
5	Improved decorticated	73.45 ± 3.4	166.51 ± 6.7 a	1.97 ± 0.1

[a]Values are means ± SEM, based on liver and tibia dry weight (μg/ml). Values within a column bearing different letters differ significantly ($P < 0.05$).

V). The tibia iron concentration of the control group was higher ($P < 0.05$) than those of the two groups fed diets containing Improved sorghum flours (groups 4 and 5, Table V).

Zinc concentrations in the liver and plasma of the five groups did not differ (Table VI). Control group tibia zinc was higher ($P < 0.05$) than that in any of the four experimental groups. This difference found for tibia zinc concentration, in the absence of similar findings for liver and plasma, is in agreement with the findings of Deeming and Weber (1977), who reported that unlike bone zinc, liver and plasma were poor indicators of dietary zinc. The skeletal system has the slowest zinc turnover and accumulation rates in the body (Smith 1988). Moreover, femur zinc concentration in rats was found to be a better indicator of zinc bioavailability than weight gain (Momcilovic et al 1975, Morris and Ellis 1980, Forbes et al 1984).

Studies of the effects of fiber and phytate in sorghum on mineral bioavailability are not conclusive, and the discrepancies in the results are probably due to differences in experimental conditions of the various studies. Stuart et al (1987) found that iron and zinc were highly bioavailable from nutritionally balanced, sorghum-based foods fed to weanling rats previously marginally depleted of these minerals. Gillooly et al (1984) found that certain dietary fiber components and phytate in sorghum-based foods decreased iron absorption in human diets. Moreover, some disagreement exists among researchers as to whether the poor mineral bioavailability from whole grain cereals is due to fiber or to phytate, since phytate-containing foods generally contain fiber. Harland (1989) reviewed the effects of dietary fiber on mineral bioavailability and concluded that although fiber may impair mineral absorption, it is phytate in fiber-containing foods that is the major cause of poor mineral bioavailability. In the present study, small differences existed in the total dietary fiber content of the Traditional and Improved whole sorghum flours. On the other hand, the phytate content of the two Improved flours was much higher than the levels in the corresponding Traditional sorghum flours. This may have contributed to the relatively lower mineral concentrations observed in the tissues of the rats fed the Improved sorghum flours.

In this study we found that sorghum grown under improved conditions contained more zinc than the Traditional sorghum. Also, previous agricultural trials have shown that Improved sorghum has a higher yield than traditionally grown sorghum (IDRC 1988). However, efforts should be made to reduce the phytate content of the Improved sorghum by decreasing the level of phosphorus-based fertilizers used before this sorghum is grown on a large scale in the country. This decrease could be accomplished by using a combination of nitrogen- and phosphorus-based fertilizers. Fertilizer research conducted at the Bonka Agricultural Research Station has shown that plant height is significantly increased by the addition of both phosphorus- and nitrogen-based fertilizers but is not affected by nitrogen alone (IDRC 1988).

Although the white durra sorghums grown in the Bay Region of Somalia show little genetic variation (IDRC 1988), the ability to generalize the findings of this preliminary study on the nutritive quality of sorghum grown in Somalia is restricted by the fact that the Traditional sorghum samples used for the study were obtained from a single source within the Bay Region and thus cannot be considered a random sample of all the Traditional white durra sorghum grown in the Bay Region or throughout Somalia during the March–July 1988 growing season. In contrast, the Improved sorghum was randomly selected from the white durra grown at the Bonka Agricultural Research Station in the same period.

Finally, the diets used for the animal experiment were formulated to provide minerals and other nutrients that met the nutritional requirements of the weanling rat (National Research Council 1978). In contrast, the mineral intakes of populations who subsist on sorghum and other cereals are often low. Thus, caution should be applied to extrapolating the findings of the study to human subjects, especially in those areas where diets are marginal in nutrients and the major portion of daily caloric intake comes from foods of plant origin.

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