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The University of North Carolina at Greensboro PH.D. 1983

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EFFECTS OF IRON AND ZINC SUPPLEMENTS ON BIOAVAILABILITY OF IRON, COPPER, AND ZINC IN YOUNG RATS FED HIGH FIBER DIETS

by

Lovie King Booker

A Dissertation submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
1983

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March 23, 1983
Date of Acceptance by Committee

March 23, 1983
Date of Final Oral Examination

BOOKER, LOVIÉ KING. Effects of Iron and Zinc Supplements on Bioavailability of Iron, Copper, and Zinc in Young Rats Fed High Fiber Diets. (1983)
Directed by: Dr. Aden C. Magee. Pp. 59

The effects of iron and zinc supplements on growth and on trace mineral parameters of young rats fed high levels of fiber were studied. The sources for fiber were Kellogg's all-bran, Quaker corn bran, Kellogg's Most, and Ener-G rice bran. Animals fed low and high levels of all-bran and corn bran diets not supplemented with iron or zinc had weight gains at the end of four weeks which were essentially the same as the weight gains of animals fed a non-cereal fiber control diet. Animals consuming diets containing Kellogg's Most exhibited the greatest weight gains, while animals consuming the rice bran cereal diet showed the least weight gains. Hemoglobin concentrations and concentrations of copper, iron, and zinc in the livers were essentially the same in all animals, regardless of the source of fiber. Iron or zinc supplements had no significant effects on hemoglobin or liver mineral deposition in animals fed all-bran cereal. In the absence of extra iron or zinc, animals consuming all-bran, corn bran, and rice bran diets were in positive copper balance and negative iron balance. Animals fed Kellogg's Most were in negative copper balance and positive iron balance. All animals exhibited positive zinc balance, regardless of the source of fiber. An increase in all-bran fiber level was associated with a decrease in copper retention. Iron or zinc supplements appeared to improve copper retention in

animals fed a high level of all-bran fiber. Iron and zinc supplements had no significant effects on iron or zinc retention in animals fed high or low all-bran fiber diets.

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to Dr. Aden Magee, for his guidance, advising, and patience throughout the direction of this study. Gratitude is also expressed to the members of the advisory committee, Dr. Mildred Johnson, Dr. William Powers, and Dr. Elizabeth Schiller for their helpful comments and to Mrs. Shih-min L. Wu for her technical assistance and encouraging advice.

Special thanks is extended to Dr. C. W. Seo (A & T State University) for the use of the Atomic Absorption Spectrophotometer and Mrs. Sarah Williamson for her technical assistance. Special mention should be made of the author's husband, Evans Booker, and her daughter, Evette, for their patience and understanding throughout my graduate study.

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CHAPTER I

INTRODUCTION

The main components of dietary fiber are cellulose, hemicellulose, pectic substances, and lignin. Dietary fiber may be defined as the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of species of nonruminant animals. Recently, the beneficial effects of dietary fiber have received a great deal of attention. The U.S. Dietary Goals recommend an increase of dietary fiber in American diets. Dietary fiber preparations and fiber-enriched foods are available, and their consumption by the health-conscious public is increasing. In many areas of the world, cereal grains contribute the major sources of dietary proteins, and in developing countries wheat and rice grains contribute significantly to the total daily energy intake.

Whole grains and legumes contain high concentrations of minerals and are found to be excellent sources of copper, iron, and zinc. The ability of natural sources of fiber, such as that found in bran, to chelate mineral ions is well known. Iron from plant foods is not well absorbed, and its availability depends on the influence of other food in the diet more than does the availability of heme iron from animal sources.

The significance of trace elements in living systems was noted about a century ago when iron, copper, and zinc were found to be essential to the growth of plants and microorganisms and to be constituents of certain respiratory and blood pigments in snails and mollusk. Iron, which is an important transition ion found in the body is essential for hemoglobin formation and many enzyme activities. Copper is notable for its function as cofactor in a number of enzyme systems and its relationship to hemoglobin formation, cellular resporation, phospholipid formation, collagen synthesis, and melanin production. Zinc is required for the activity of many biological enzymes and is involved in a wide range of cellular activities including RNA, DNA, and protein synthesis (Underwood, 1977).

It is difficult to determine the relative roles of phytic acid and dietary fiber in altering mineral absorption, but current evidence suggests that fiber may have an independent role. This study was directed toward investigating growth patterns of young rats fed different types of cereal fiber and the bioavailability of copper, iron, and zinc in relation to the levels of minerals in the diet.

CHAPTER II

REVIEW OF LITERATURE

Current interest regarding the role of dietary fiber was generated initially from research findings of some British investigators who observed that the incidence of specific types of cancers was lower in certain African localities than in Western countries (Burkitt, 1973; Trowell, 1972). These investigators surmised that some environmental component present in the African settings and apparently not present in Western settings was responsible for the differences noted in connection with cancer development. The particular factor postulated as being involved with the prevention of cancer was dietary fiber. The fiber content of many of the African diets that were evaluated were much higher than the levels of fiber intakes in many typical Western dietary regimens. Burkett, Walker, and Painter (1972) reported that an increase in dietary fiber was associated with a decrease in transit time of food passing through the gastrointestinal tract.

Although fiber may be beneficial in the prevention of certain types of cancers, there is evidence that dietary sources which provide fiber may contain other substances, as well as fiber, which may interfere with the absorption and/or utilization of nutrients. Reinhold et al. (1973) reported that diets high in whole wheat products resulted in negative zinc balance and initially suggested that this was due to the phytic acid content of these foods. In later studies, Reinhold and coworkers (1973, 1975), showed that zinc binds quite strongly to dephytinized fiber in vitro. Preliminary evidence on the properties of other fiber preparations has yielded conflicting results (Breshgetoor, Kies, & Fox, 1977; Sandstead, Klevay, Munoz, Jacob, Peck, Tucker, Logan, Eilkema, Inglett, Dintzis, & Shuey, 1977). Since dietary fibers vary in their chemical and physical properties, they probably do not behave identically physiologically (Eastwood, 1973). Frantz, Kennedy, & Fellers (1980a) determined the relative bioavailability of zinc (RBAZ) from corn, rice, wheat, and legumes and reported that the phytic acid content of the foods was inversely related to RBAZ. The relationship between phytic acid and RBAZ, however, was more noticeable in cereals than in legumes. Davies, Hristic, and Flett (1977) reported that phytate rather than fiber was a major determinant in zinc availability. It was found that extracted bran fiber resulted in rat growth rates equal to those of zinc-adequate controls but that growth was reduced with diets containing phytic acid and bran. Shah, Giroux, and Belonje (1979) reported that an infant cereal containing soy protein was a better source of zinc than infant cereal of barley or rice. Ismail-Beigi, Faraji, and Reinhold (1977) reported that zinc binding in vitro to a wheat whole-meal (Tanok), dephytinized Tanok, and cellulose was highly pH-dependent, reaching a maximum at pH 6.5 to 7.5. Removal of phytate from Tanok did not reduce its binding capability.

In a study on the absorption of calcium, phosphorus, magnesium, copper, and zinc by sheep fed one of two diets based on varying proportions of dried grass-meal, ground straw, and barley, Stevenson and Unsworth (1978) found significant differences between diets for calcium only. The diets were fed to the animals at a level slightly above maintenance. This was achieved by feeding 653 g dry matter (DM)/day of diet 1 and 535 g DM/day of diet 2. Intakes of calcium by sheep fed on diet 1 were significantly higher ($p \leq 0.05$) than those for sheep fed diet 2. Marked increases were observed in the amount of copper, zinc, and phosphorus reaching the proximal duodenum relative to that ingested in the sheep fed the high dry matter diet.

Iron absorption is known to vary considerably depending on the type and composition of the diet being consumed, and iron availability from whole wheat products is particularly poor (Bjorn-Rasmussen, 1974). Other studies have shown that iron absorption was impaired from diets containing whole meal bread or rice and vegetables (Hallberg, Garby, Suwanik, & Bjorn-Ramussen, 1974; Widdowson & McCance, 1942). Other investigators found decreased serum iron levels after three or five weeks of diets containing bran and whole wheat products (Jenkins, Hill, & Cummings, 1975). Recently, Kelsay and associates (1979) reported decreases in the absorption of calcium, magnesium, silicon, zinc, and copper in individuals fed diets high in vegetable and fruit fibers. The absorption of iron or phosphorus, however, did not appear to be affected by these same diets.

Fairweather-Tait (1982) reported that amounts of wheat bran fiber equivalent to levels available in commercially available bread had no effect on the absorption of dietary iron. Fernandez and Phillips (1982), studying the effects of soluble and insoluble components of fiber on iron absorption, reported that anemic dogs absorbed iron more efficiently than did healthy animals. It was found that lignin and psyllium mucilage were potent inhibitors of iron absorption. Pectin inhibited iron absorption to a lesser degree, and the presence of cellulose had no apparent effect on iron absorption. Reinhold, Garcia, and Garzon (1981) reported that iron was firmly bound by neutral detergent fiber (NDF) prepared from wheat or maize and that the NDF accounted for nearly all of the iron binding capability of these cereals. The amount of bound iron depended upon iron concentration, pH, quantity of fiber, and the presence or absence of quantities of binding inhibitors.

Harmuth-Hoene and Schelenz (1980) studied the effect of "indigestible" polysaccharides fed at the 10% level in a semi-synthetic diet on the absorption of calcium, iron, zinc, copper, chromium, and cobalt; on weight gain, and on fecal dry matter excretion in wealing male rats. Absorption of all minerals were reduced in rats fed either carrageenan (C) or agar-agar (AA) while the presence of sodium-alginate (NA-A) was associated with decreases in iron, chromium, and cobalt absorption. Carob bean gum (CBG) and gum guar (GG) interfered with the absorption of zinc, chromium, copper, and cobalt. In a second study the long-term effects of 10% dietary levels of gum guar and agar-agar on mineral absorption were investigated (Harmuth-Hoene & Schelenz, 1980). Mineral contents of rat carcasses, assayed at the termination of the experimental period, did not reveal any significant differences between the control groups and animals fed either polysaccharide, suggesting that the rat is able to compensate for increased fecal losses, presumably by reduced urinary losses. The long term ingestion of AA, C, or NA-A resulted in a marked increase of fecal dry matter, indicating that these substances were not degraded to any degree. Considerable portions of GG and CBG were metabolized, however, and this was probably due to the action of intestinal bacteria. Spiller, Chernoff, and Gates (1980) fed pig-tailed monkeys graded levels of dietary fiber (3, 6, and 9 g/day/monkey) from soft white wheat bran (WB), semi-purified corn bran (CB), purified cellulose (PC), and purified pectin (PE). As the levels of the purified dietary fibers (CB, PC, and PE) were increased, the fecal excretion of Ca, Mg, Fe, Zn, and Cu did not increase; as the level of non-purified WB was increased, losses of magnesium (Mg), zinc (Zn), and copper (Cu) did not change. However, fecal losses of calcium and iron increased significantly even after adjustment for the mineral contribution of the WB to the diet was made. These losses would seem to be due to components of WB other than the fibrous polymers since no losses were apparent with the purified dietary fibers tested.

Ganapathy, Booker, Craven, and Edwards (1981) reported the effects of an all-plant diet on the copper, iron, molybdenum, selenium, zinc, amino acid, and plasma protein levels of adult men. The addition of pinto beans, white rice, or peanut butter to the control diet did not alter excretion patterns of copper significantly. However, significantly less iron was retained when subjects consumed the pinto bean and peanut butter diets. Supplementation of the white bread control diet with pinto beans resulted in a decrease in the absorption of dietary iron from 16 to 7%, and a marked inhibitory effect was observed (from 16 to 1%) when peanut butter was fed.

CHAPTER III

EXPERIMENTAL PROCEDURES

The purposes of this study were to investigate the growth patterns of young rats fed different types of cereal fiber and to evaluate the effect of fiber level of the diet on the bioavailability of copper, iron, and zinc. Criteria used for evaluation included weight gains; hemoglobins; liver concentrations of copper, iron, and zinc; and the apparent retention of copper, iron, and zinc in young rats.

The study was divided into two experiments. In experiment 1 Kellogg's all-bran, Quaker corn bran, Kellogg's Most, and Ener-G rice bran cereals[1] were used for the formulation of seven test diets. Two all-bran and two corn bran diets were formulated to provide 5 and 10 percent levels of fiber, respectively. The amount of fiber provided by Kellogg's Most or by Ener-G rice bran was 5 percent. Information provided by the manufacturers regarding the fiber content of each cereal was used in determining the amounts of each cereal that would be required to provide either 5 or 10 percent dietary fiber levels (Appendix A, Table 3). A non-cereal

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[1]Kellogg's all-bran and Kellogg's Most are products of the Kellogg Company, Battle Creek, Michigan. Quaker corn bran is a product of the Quaker Oats Company, Chicago, Illinois. Ener-G rice bran is a product of Ener-G Foods, Inc., Seattle, Washington. All cereals were purchased from local food markets.

control diet was also included for comparative purposes. This control diet contained 2 percent cellulose[2], a level of fiber recommended for basal rat diets. The type of fiber provided by all-bran was wheat, while the fiber provided by Kellogg's Most was a mixture of wheat germ and wheat bran. Compositions of the test and control diets are given in Appendix A, Tables 1 and 2. Experiment 2 was designed to study the growth and development of young rats fed 5 and 10 percent levels of fiber from all-bran with and without iron and zinc supplements. The extra amount of iron, provided by ferrous sulfate, used for some of the diets was 75 ppm, a physiological level which has been used in similar studies. A level of 50 ppm of zinc provided by zinc carbonate was used as a supplement in some of the diets. This level of zinc has been shown to affect animal performance in previous studies involving other types of fiber.

In addition to cereal fiber or cellulose, the test and control diets contained 10 percent protein[3], 20 percent vegetable shortening[4], 4 percent mineral mix[5], and approximately 52-60 percent corn starch[6] (depending upon the level of cereal

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[2]Alphacel, ICN Nutritional Biochemicals, Cleveland, Ohio.

[3]Vitamin-test casein, ICN Nutritional Biochemicals, Cleveland, Ohio.

[4]Crisco, Proctor and Gamble Company, Cincinnati, Ohio.

[5]Salt Mixture W, ICN Nutritional Biochemicals, Cleveland, Ohio.

[6]Powdered corn starch 105-A, Clinton Corn Processing Company, Clinton, Iowa.

in the diet). Vitamins A and D were provided by oleum percomorphum.[7] Compositions of the vitamin mixture and mineral mixture are given in Appendix A, Tables 4 and 5, respectively. Trace mineral analyses of the diets used in the experiments are given in Appendix A, Tables 6 and 7.

Aliquots of food samples for each diet were collected when diets were first prepared and periodically during the four-week feeding period, compounded into a total composite for each diet, and kept in a refrigerator until analyzed.

Fifty-six weanling male rats[8], averaging 45-48 grams in weight, were used for experiment 1. In experiment 2, 64 weanling male rats, averaging 59 grams, were used. The rats were kept in individual stainless steel cages under standard conditions (22°C room temperature, 60% relative humidity, 12-hour light-dark cycle) for 30 days. The animals were randomized into replications according to initial body weight, and were allowed free access to distilled water and food. The distilled water was changed once a week or sooner if it became turbid. Individual food records were kept, and the total food consumption per animal for the experimental period was determined. The animals were weighed at the end of each week, and the total weight gain per animal was determined.

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[7]Mead Johnson and Company, Evansville, Indiana. The composition of this product is listed as 1250 USP units of Vitamin A and 180 USP units of Vitamin D per drop.

[8]Sprague-Dawley rats purchased from Holtzman Company, Madison, Wisconsin.

At the end of three weeks, during experiment 1, randomly selected replications were fasted overnight and placed in metabolic cages, and 24-hour collections of urine and feces were made for a 5-day period. While each experiment was in progress, animals from randomly selected replications were fasted overnight and placed in metabolic cages. Feces and urine were collected from each animal in a metabolic cage for a 5-day period. During experiment 2, animals from three randomly selected replications were fasted overnight and transferred to metabolic cages. Twenty-four-hour collections of urine and feces were made for a 5-day period. Daily urine volumes were recorded, and the total 5-day composites were kept in a refrigerator until analyzed. Fecal composites were weighed, dried at 60°C to a constant weight, and ground into a fine powder before analyzing. Blood samples were taken from the tails of all animals at the end of each experimental period, and hemoglobin levels were determined by the method of Shenk, Hall, and King (1934).

At the end of each experimental period, animals from four randomly selected replications were sacrificed, and the livers from the animals were removed and weighed. The dry weight of each liver was obtained from a 0.5 - 1.0 gram sample dried in an oven at 60°C. The remainder of each liver was ashed, with hot nitric and perchloric acids, and the ashed residues were dissolved in 3 ml of 0.6N HCL and diluted to 25 ml with redistilled water.

An appropriate aliquot of the total composite urine samples was ashed with hot nitric and perchloric acids, and the ashed residues were dissolved in 3 ml of 0.6N HCL and diluted to 50 ml with redistilled water. Approximately 0.5 gram of each compsite fecal sample was ashed with hot nitric and perchloric acids, and the ashed residues were dissolved in 3 ml of 0.6N HCL and diluted to 25 ml with redistilled water.

Copper, iron, and zinc determinations were made on the food, liver, urine, and fecal samples by means of an atomic absorption spectrophotometer.[9]

All data were analyzed by standard analysis of variance methods (Snedecor, 1962). Significant differences between means were determined with the Duncan's Multiple Test (Agricultural Research Service, USDA, 1957).

[9]Model 551, Instrumentation Laboratories, Wilmington, Massachusetts.

CHAPTER IV

RESULTS AND DISCUSSION

In this study two experiments were designed to test the effects of dietary cereal fibers on growth, hemoglobin, liver mineral deposition, and the copper, iron, and zinc balances of young male rats in the presence and absence of additional iron and zinc supplements. Means of animals' weight gains, hemoglobin levels, and liver mineral constitutents for experiment 1 are shown in Table 1, and in Table 3 for experiment 2. Data on the intake, excretion, and retention of copper, iron, and zinc in experiments 1 and 2 are shown in Tables 2 and 4, respectively. Detailed data obtained from this study are presented in Appendix B. Statistical analyses of all data are given in Appendix C.

Experiment 1

Growth

In this experiment all animals showed increased weight gains during the experimental period, regardless of the source of dietary fiber (Table 1). Animals consuming diets containing Kellogg's Most exhibited the greatest weight gains, while animals consuming the rice cereal diet showed the least weight gains. With the commercial cereals used in this study, it was possible to formulate diets containing 10 percent fiber with only the all-bran or corn bran cereal. With the other

Table 1

Responses of Young Rats Fed Different Sources of Fiber

(Experiment 1)[1]

Source of Fiber	Percentage of Fiber	Weight Gain[2]	Hemoglobin[2]	Liver Mineral Constituents[3] Cu	Fe	Zn
		gm/4 weeks	gm/100 ml	mcg/gm dry weight		
0	0	$154^{ab} \pm 30$	$13.0^{a} \pm 0.5$	$16.0^{a} \pm 1.7$	$397.8^{a} \pm 69.8$	$73.8^{a} \pm 5.0$
All-bran	5	$171^{bc} \pm 33$	$13.1^{a} \pm 1.1$	$17.6^{a} \pm 2.4$	$266.9^{bc} \pm 24.1$	$83.4^{a} \pm 8.6$
All-bran	10	$171^{bc} \pm 27$	$13.0^{a} \pm 1.4$	$15.2^{a} \pm 0.9$	$239.2^{c} \pm 12.5$	$85.8^{a} \pm 11.0$
Corn bran	5	$172^{bc} \pm 35$	$12.8^{a} \pm 0.9$	$16.4^{a} \pm 2.0$	$261.4^{bc} \pm 38.5$	$79.3^{a} \pm 7.9$
Corn bran	10	$162^{ab} \pm 12$	$13.5^{a} \pm 0.7$	$16.3^{a} \pm 1.9$	$389.7^{a} \pm 57.6$	$97.6^{a} \pm 8.4$
Kellogg's Most	5	$192^{c} \pm 26$	$12.9^{a} \pm 0.5$	$16.3^{a} \pm 2.0$	$332.8^{ab} \pm 43.4$	$85.2^{a} \pm 7.8$
Rice bran	5	$108^{a} \pm 18$	$14.0^{a} \pm 0.7$	$17.9^{a} \pm 1.7$	$237.5^{c} \pm 15.6$	$85.3^{a} \pm 6.6$

[1]Means not sharing a common superscript are significantly different ($p \leq 0.05$).

[2]Each value is the mean of eight (8) animals $\pm$ standard error.

[3]Each value is the mean of four (4) animals $\pm$ standard error.

two cereals, approximately 50-60 percent of the formulated diets were composed of Kellogg's Most or rice bran, respectively, to give a 5 percent fiber level in the diet. These results would suggest that rice bran contains some dietary component which may interfere with growth to some extent. However, in this study, the growth of animals fed rice bran were not significantly different than the growth of rats fed the control diet. A level of 10 percent fiber of all-bran or corn bran had no apparent adverse effect on growth.

Hemoglobin

There were no significant differences between hemoglobin levels of young rats fed the various kinds and amounts of fiber (Table 1). The type and level of fiber apparently had no adverse effect on the availability of the hemopoietic factors, particularly copper and iron, necessary for adequate hemoglobin formation.

Liver Mineral Constituents

The amounts of copper and zinc found in the livers of animals fed the various fiber diets were not significantly different (Table 1; Appendix C, Table 1). These data would suggest that the types or levels of fiber used for the experiment had no effect on the availability of either copper or zinc.

The liver iron levels of animals consuming the rice bran, 5 percent corn bran, and 10 percent all-bran fibers were significantly lower ($p \leq 0.05$) than the liver iron levels of animals fed no source of fiber (Table 1; Appendix C, Table 1). Liver iron levels of rats fed Kellogg's Most, 5 percent all-bran, and 10 percent corn bran, however, were not statistically different from iron levels of rats fed no fiber source. These data would suggest that different types and levels of fiber do have effects on the availability of iron in the diet.

Mineral Balance

All animals, except those fed diets containing Kellogg's Most, were in apparent positive copper balance (Table 2). Analysis of the data (Appendix C, Table 2), however, indicated that apparent copper retention in rats fed Kellogg's Most was not significantly different from rats fed corn bran. At a 5 percent level, all-bran appeared to improve copper retention, while the presence of the other fiber sources resulted in copper retention which was lower than that found in animals fed no fiber.

All animals, except those fed Kellogg's Most, were in negative iron balance (Table 2). Analyses of the diets used in experiment 1 (Appendix A, Table 6) revealed that the Kellogg's Most diet contained approximately 250 ppm of iron, whereas the iron content of the other diets ranged between 59-183 ppm. Thus, it is possible that all diets, with the exception of the Kellogg's Most diet, did not contain sufficient iron to maintain a positive balance in growth of rats.

All animals receiving the different fiber sources and levels were found to be in positive zinc balance (Table 2).

Table 2

Effects of Different Sources of Fiber on the

Mineral Balance of Young Rats

(Experiment 1)[1]

Source of Fiber	Percentage of Fiber	Intake (mg/day)	Excretion (mg/day) Fecal	Excretion (mg/day) Urine	Apparent[3] Retention (mg/day)
		Copper			
0[2]	0	.505	.253	.016	+.236[b]
All-bran	5	.817	.356	.017	+.444[a]
All-bran	10	.684	.466	.024	+.158[b]
Corn bran	5	.393	.362	.025	+.006[c]
Corn bran	10	.482	.324	.016	+.142[bc]
Kellogg's Most	5	.370	.417	.023	-.070[bc]
Rice bran	5	.598	.426	.019	+.153[b]
		Iron			
0[2]	0	3.537	4.313	.003	- .779[b]
All-bran	5	5.672	6.097	.006	- .431[b]
All-bran	10	7.982	9.822	.007	-1.847[b]
Corn bran	5	10.585	10.770	.010	- .195[b]
Corn bran	10	12.576	15.194	.044	-2.662[b]
Kellogg's Most	5	16.855	13.097	.007	+3.751[a]
Rice bran	5	5.421	7.307	.003	-1.889[b]
		Zinc			
0[2]	0	.165	.063	.015	+.088[a]
All-bran	5	2.256	1.263	.028	+.965[d]
All-bran	10	3.542	2.932	.035	+.702[cd]
Corn bran	5	2.192	1.546	.036	+.610[bc]
Corn bran	10	3.871	3.008	.033	+.829[cd]
Kellogg's Most	5	1.356	.955	.026	+.374[ab]
Rice bran	5	1.795	1.457	.011	+.327[ab]

[1]Each value is the mean of six (6) animals.
[2]Basal control diet with no extra fiber.
[3]Means not sharing a common superscript are significantly different ($p \leq 0.05$).

The amounts of zinc retained by animals fed either Kellogg's Most, or rice bran were approximately half the amounts of zinc retained in rats fed either all-bran or corn bran. The total amounts of zinc consumed by animals fed all-bran or corn bran was greater than the amounts consumed by rats fed Kellogg's Most and rice bran.

Experiment 2

Growth

The addition of iron or zinc supplements to the all-bran diets used in this experiment had no apparent effect on growth since the means of the animals fed the various diets were not statistically significant (Table 3).

Hemoglobin

The hemoglobin levels of rats fed 5 or 10 percent all-bran supplemented with and without iron or zinc were essentially the same (Table 3). Thus, it would appear that increasing the fiber content or the iron and zinc levels of the diet had no influence on hemoglobin formation in this study.

Liver Mineral Constituents

There were no significant differences in the copper, iron, or zinc deposition in the livers of animals fed either level of fiber in the presence or absence of added iron and zinc (Table 3).

Mineral Balance

Increasing the dietary fiber or zinc level was associated with decreases in the apparent retention of copper in young

Table 3

Effects of Iron and Zinc Supplements on Young Rats

Fed Two Levels of All Bran Cereal

(Experiment 2)

Mineral Supplement Iron	Mineral Supplement Zinc	Percentage of Fiber	Weight Gain[2]	Hemoglobin[2]	Liver Mineral Constituents[3] Cu	Liver Mineral Constituents[3] Fe	Liver Mineral Constituents[3] Zn
ppm	ppm		gm/4 weeks	gm/100 ml	mcg/gm dry weight		
0	0	5	140^{a} ± 22	12.5^{a} ± 0.4	13.7^{a} ± 2.1	181.4^{a} ± 28.1	66.0^{a} ± 10.8
75	0	5	141^{a} ± 44	12.6^{a} ± 0.9	14.5^{a} ± 1.8	201.9^{a} ± 32.0	63.8^{a} ± 5.8
0	50	5	154^{a} ± 25	12.3^{a} ± 0.7	15.6^{a} ± 2.4	178.8^{a} ± 48.7	69.9^{a} ± 5.0
75	50	5	153^{a} ± 26	13.0^{a} ± 1.0	15.7^{a} ± 0.9	170.2^{a} ± 15.0	63.4^{a} ± 8.4
0	0	10	164^{a} ± 12	12.8^{a} ± 0.9	14.1^{a} ± 0.8	182.0^{a} ± 44.9	63.3^{a} ± 1.2
75	0	10	164^{a} ± 18	12.8^{a} ± 1.0	14.2^{a} ± 1.7	198.5^{a} ± 17.7	71.0^{a} ± 4.0
0	50	10	168^{a} ± 20	12.4^{a} ± 0.6	13.7^{a} ± 1.6	175.8^{a} ± 21.8	65.5^{a} ± 3.9
75	50	10	176^{a} ± 18	12.8^{a} ± 0.6	13.2^{a} ± 2.4	181.7^{a} ± 33.9	66.6^{a} ± 11.9

[1]Kellogg's all-bran cereal was the source of fiber for all diets.
[2]Each value is the mean of eight (8) animals ± standard error.
[3]Each value is the mean of four (4) animals ± standard error.

rats (Table 4). The addition of iron supplements to the diets was associated with increases in copper retention. Zinc supplementation appeared to result in negative copper balances in animals fed a 5 percent level of fiber, but in the presence of 10 percent fiber zinc supplements were associated with positive copper balances.

All animals were in positive iron balance, regardless of fiber level or the presence or absence of iron and zinc supplements (Table 4). The positive iron retention in animals fed all-bran in this experiment was in contrast to the negative iron balance fed all-bran in experiment 1. One explanation for this difference is the fact that the animals in experiment 2 consumed approximately twice the amount of iron as did the animals in experiment 1. Increasing dietary iron levels were associated with some increase in iron retention.

All animals in experiment 2 were in positive zinc balance (Table 4). Increases in zinc retention were generally observed in rats fed the 10 percent level of fiber, and there was evidence that a 50 ppm supplement of zinc resulted in highly significant increases ($p \leq 0.01$) in zinc retention.

General Discussion

The concentration and availability of trace minerals in plant food sources are of considerable interest to nutritionists and clinicians because of the apparent relationship to fiber and mineral availability. The possibility that dietary fiber may play a part in altering the absorption of trace minerals is well documented (Breshgetoor et al., 1977;

Table 4

Effects of Iron and Zinc Supplements on the Mineral Balance of Young Rats Fed Two Levels of All Bran Cereal (Experiment 2)[1]

Mineral Supplement: Iron (ppm)	Mineral Supplement: Zinc (ppm)	Percentage of Fiber[2]	Intake (mg/day)	Excretion (mg/day): Fecal	Excretion (mg/day): Urine	Apparent[3] Retention (mg/day)
			Copper			
0	0	5	2.069	.863	.005	+ 1.200[c]
75	0	5	1.337	.809	.004	+ .525[bc]
0	50	5	.728	.842	.002	- .117[ab]
75	50	5	.723	.774	.004	- .054[ab]
0	0	10	1.143	1.566	.005	- .428[a]
75	0	10	1.190	1.037	.002	+ .151[ab]
0	50	10	1.467	1.174	.003	+ .290[ab]
75	50	10	2.261	1.047	.004	+ 1.210[c]
			Iron			
0	0	5	13.111	11.356	.003	+ 1.759[a]
75	0	5	27.055	17.806	.005	+ 9.245[a]
0	50	5	21.420	11.262	.003	+10.155[a]
75	50	5	18.438	16.001	.005	+ 7.532[a]
0	0	10	19.633	12.099	.010	+ 7.523[a]
75	0	10	23.224	17.804	.005	+ 5.415[a]
0	50	10	20.087	13.747	.004	+ 6.336[a]
75	50	10	32.896	18.547	.007	+14.342[a]
			Zinc			
0	0	5	4.097	2.639	.008	+ 1.450[a]
75	0	5	5.306	3.628	.006	+ 1.673[a]
0	50	5	11.882	7.913	.005	+ 3.964[c]
75	50	5	9.616	7.520	.004	+ 2.093[ab]
0	0	10	9.198	5.569	.005	+ 3.624[c]
75	0	10	8.945	5.826	.007	+ 3.112[bc]
0	50	10	21.261	10.362	.009	+10.890[d]
75	50	10	20.440	9.494	.007	+10.939[d]

[1]Each value is the mean of three (3) animals.
[2]Kellogg's all-bran cereal was the source of fiber for all diets.
[3]Means not sharing a common superscript are significantly different ($p \leq 0.05$).

Cummings, 1978; Harmuth-Hoene & Schelenz, 1980; Reinhold, Ismail-Beigi, & Paradji, 1975; Sandstead, Munoz, Jacob, Klevay, Reck, Logan, Dintzis, Inglett, & Shuey, 1978). Reinhold et al. (1975) contended that the fiber content of foodstuffs largely determined the availability of minerals such as copper, iron, and zinc. The results of this study suggest that some types of fiber have little effect on copper availability, while other fiber sources may interfere with copper availability. Decreases in copper availability were also observed in young rats fed diets containing 10 percent fiber.

Results of this study indicate that the level of dietary iron provided by a cereal source may be a more important factor in iron availability than the type or level of fiber in the diet. Other dietary components provided by different cereal sources, such as phytate, may also have a more important influence on iron availability than fiber per se. Previous studies (Bjorn-Rasmussen, 1974; Reinhold et al., 1973) have indicated that phytate in cereals has a significant effect on iron availability.

Results of this study indicate that fiber per se has little effect on zinc availability to young rats since zinc parameters observed in the study did not change significantly in animals fed the different sources and levels of fiber. These results are similar to those observed by Spiller et al. (1980) in monkeys.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

Young rats were fed diets containing Kellogg's all-bran (wheat bran), Quaker corn bran, Kellogg's Most (a mixture of wheat bran and wheat germ), and Ener-G rice bran cereals for the purpose of studying the effects of a high level of various dietary cereal fibers on growth, and the copper, iron, and zinc balances of young male rats in the presence and absence of iron and zinc supplements. Two experiments were designed to test these effects. Criteria used to evaluate the effects of the various fiber sources with and without iron and zinc supplementation were weight gains; hemoglobin levels; liver copper, iron, and zinc deposition, and mineral balance of young rats.

Animals maintained on a 5 percent level of Kellogg's Most cereal showed a highly significant increase ($\underline{p} \leq 0.01$) in weight gain, whereas animals maintained on a 5 percent rice bran diet had a highly significant decrease in weight gain. In experiment 2 when the above two levels of all-bran cereal were supplemented with iron and zinc supplements there was no significant difference in weight gains of animals. In experiment 1 the different sources and levels of fiber did not significantly affect liver copper and zinc levels. However, except for 10 percent corn bran and 5 percent Kellogg's

Most diets, there was a significant depression in liver iron content. When the rats were fed two levels of all-bran cereal with iron and zinc supplements there was not a significant difference in the liver content of copper, iron, and zinc.

Recommendations

Recent studies have shown that fiber of different composition and from contrasting sources produces different physiological effects. Dietary fiber is largely digested in the colon by microflora and so influences colonic function, fecal weight, and composition (Southgate, 1973). The significance of the changes in fat, nitrogen, and energy output remains to be evaluated. In addition to the increasingly well documented effects fiber has on colonic function, it is now clear that fiber is a nutrient of importance in its own right. The amount and type of fiber included in the diet may have significant nutritional implications. Additional information on the interaction of other minerals and dietary constituents with dietary fiber needs to be investigated. It is also important that long-term research studies be done to establish the effect of long-term use of high fiber foods on nutrient metabolism. It is important to know whether long-term adaptive effects occur with high fiber diets. Whether a long-term requirement exists for fiber in man remains to be answered.

There is also a need to investigate the possible effect of fiber on vitamin metabolism and absorption in the gut.

With the known capacity of fiber to absorb organic compounds and the general association of malnutrition and vitamin deficiency with stable diets containing large amounts of fiber, this seems a reasonable area for research.

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APPENDIX A

Table 1

Composition of Diets Used in Experiment 1

	Diets						
Constituents	1	2	3	4	5	6	7
	grams per 2 kilograms						
Caesin	220	220	220	220	220	220	220
Corn starch	1220	907	594	664	108	506	20
Cellulose	40	40	40	40	40	40	40
Fat	400	400	400	400	400	400	400
Vitamin-mix	40	40	40	40	40	40	40
Mineral-mix	80	80	80	80	80	80	80
Fiber food[1]	-	313	626	556	1112	714	1200
Oleum percomorphum	48 drops per 2 kilograms						

[1]Diets: Source of fiber

2,	Kellogg's all-bran	4,	Quaker's corn bran	6,	Kellogg's Most
3,	Kellogg's all-bran	5,	Quaker's corn bran	7,	Ener-G rice bran

Table 2

Composition of Diets Used in Experiment 2

Constituents	Diets 8	9	10	11	12	13	14	15
	grams per 2 kilograms							
Caesin	220	220	220	220	220	220	220	220
Corn starch	907	906	907	906	594	594	594	593
Cellulose	40	40	40	40	40	40	40	40
Fat	400	400	400	400	400	400	400	400
Vitamin-mix	40	40	40	40	40	40	40	40
Mineral-mix	80	80	80	80	80	80	80	80
Fiber food[1]	313	313	313	313	626	626	626	626
$FeSO_4 \cdot 7H_2O$	-	7467	-	7467	-	7467	-	7467
$ZnCO_3$	-	-	192	192	-	-	192	192
Oleum percomorphum	48 drops per 2 kilograms							

[1]Fiber sources: Kellogg's all-bran cereal was the source of fiber for all diets.

Table 3

Dietary Composition of Selected Cereals**

Constituents	Sources of Fiber			
	All-bran	Corn bran	Kellogg's Most	Rice bran
	Nutrition Information/100 gm			
Protein	14	7	14	20
Carbohydrate	74	48	78	54
Fat	4	4	0	3
	Carbohydrate Information/100 gm			
Starch and Related Carbohydrates	25	46	42	0
Sucrose and Other Sugars	18	21	21	0
Dietary Fiber	32	18	15	8
	Percentage of U.S. Recommended Daily Allowances			
Protein	6	2	6	20
Vitamin A	25	*	100	*
Vitamin C	25	*	100	*
Thiamine	25	20	100	40
Riboflavin	25	15	100	4
Niacin	25	25	100	40
Calcium	2	2	*	4
Iron	25	25	100	60
Vitamin E	0	0	100	0
Vitamin B_6	25	25	100	0
Folic Acid	25	25	100	0
Vitamin B_{12}	0	15	100	0
Phosphorus	25	2	15	0
Magnesium	30	2	15	0
Zinc	25	15	10	0
Copper	15	0	10	0
Pantothenic Acid	0	20	0	0

*Contains less than 2% of the U.S. RDA of this nutrient.
**Information from package label.

Table 4

Composition of Vitamin Mixture

Constituents[1]	gm/2Kg Mix
Biotin	0.020
Folic Acid	0.100
Thiamin HCl	0.500
Pyridoxine HCL	0.500
Menadione (2-methyl-naphthoquinone)	1.000
Riboflavin	1.000
Nicotinic Acid	1.000
Ca pantothenate	3.000
p-aminobenzoic acid	10.000
0.1% vitamin B_{12} (mannitol trituration)	2.000
Inositol	100.000
Choline Chloride	150.000
Corn Starch[2]	1732.000

[1]All vitamins were from ICN Biochemicals, Cleveland, Ohio.

[2]Powdered corn starch 105-A, Clinton Corn Processing Company, Clinton, Iowa.

Table 5

Composition of Salt Mixture-W[1]

Constituents	Percent
Calcium Carbonate	21.000
Copper Sulfate ($5H_2O$)	0.039
Ferric Phosphate	1.470
Manganous Sulfate (anhyd.)	0.020
Magnesium Sulfate (anhyd.)	9.000
Potassium Aluminum Sulfate	0.009
Potassium Chloride	12.000
Potassium Dihydrogen Phosphate	31.000
Potassium Iodide	0.005
Sodium Chloride	10.500
Sodium Fluoride	0.057
Tricalcium Phosphate	14.900

[1]Product of ICN Biochemicals, Cleveland, Ohio.

Table 6

Mineral Analysis of Diets Used in Experiment 1

Diet	Source of Fiber	Percentage of Fiber	Mineral Constituents[2] Cu	Fe	Zn
			mcg/gm dry weight		
1[1]	0	0	8.5	59.4	2.7
2	All-bran	5	12.5	66.8	34.5
3	All-bran	10	9.8	120.0	53.3
4	Corn bran	5	5.2	141.1	29.2
5	Corn bran	10	7.0	182.7	56.2
6	Kellogg's Most	5	6.5	250.9	20.2
7	Rice bran	5	12.5	112.9	37.4

[1]Basal control diet with no extra fiber.

[2]Each value is the mean of triplicate determinations.

Table 7

Mineral Analysis of Diets Used in Experiment 2

Diet	Mineral Supplement: Iron (ppm)	Mineral Supplement: Zinc (ppm)	Percentage of Fiber[1]	Mineral Constituents: Cu (mcg/gm dry weight)	Mineral Constituents: Fe (mcg/gm dry weight)	Mineral Constituents: Zn (mcg/gm dry weight)
8	0	0	5	13.3	84.2	26.3
9	75	0	5	8.3	167.4	32.8
10	0	50	5	4.5	133.0	73.8
11	75	50	5	5.0	127.4	66.5
12	0	0	10	7.0	119.7	57.0
13	75	0	10	7.2	141.0	54.3
14	0	50	10	8.8	120.8	127.8
15	75	50	10	13.4	214.4	120.9

[1]Kellogg's all-bran cereal was the source of fiber for all diets.

APPENDIX B

Table 1

Growth of Young Rats Fed Different Sources of Fiber

(Experiment 1)

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	5	6	7	8	Mean
		weight gain at 4 weeks (gm)								
0	0	168	142	217	167	133	106	152	150	154
All-bran	5	158	178	232	158	137	144	218	146	171
All-bran	10	169	144	192	158	190	140	225	153	171
Corn bran	5	191	223	197	149	179	170	97	168	172
Corn bran	10	145	173	181	153	173	158	151	162	162
Kellogg's Most	5	168	199	243	191	190	156	172	213	192
Rice bran	5	97	147	114	112	110	104	80	99	108

Table 2

Hemoglobin Levels of Young Rats Fed

Different Sources of Fiber

(Experiment 1)

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	5	6	7	8	Mean
		gm/100 ml blood								
0	0	12.4	13.6	13.2	12.7	13.3	12.1	13.6	12.7	13.0
All-bran	5	13.1	14.2	12.8	14.3	11.7	11.2	12.9	14.3	13.1
All-bran	10	11.5	15.7	13.4	11.9	14.4	12.6	13.1	11.5	13.0
Corn bran	5	13.9	13.7	12.1	11.9	12.7	13.3	13.5	11.3	12.8
Corn bran	10	13.9	12.5	14.7	13.8	14.1	12.6	13.3	13.1	13.5
Kellogg's Most	5	12.2	12.8	13.2	12.0	13.1	12.8	13.6	13.5	12.9
Rice bran	5	15.1	13.8	13.5	13.1	13.3	14.9	14.4	13.9	14.0

Table 3

Liver Copper Levels of Young Rats Fed

Different Sources of Fiber

(Experiment 1)

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	Mean
		mcg/gm dry weight				
0	0	13.87	17.61	14.71	17.75	15.98
All-bran	5	19.03	15.99	20.62	14.62	17.57
All-bran	10	14.36	15.18	14.47	16.72	15.18
Corn bran	5	17.62	17.08	12.73	17.13	16.14
Corn bran	10	14.31	18.69	17.53	14.51	16.26
Kellogg's Most	5	12.83	17.43	18.00	16.93	16.30
Rice bran	5	19.46	19.67	15.89	16.44	17.86

Table 4

Liver Iron Levels of Young Rats Fed

Different Sources of Fiber

(Experiment 1)

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	Mean
		mcg/gm dry weight				
0	0	400.95	380.08	307.61	502.71	397.84
All-bran	5	289.94	227.14	281.52	269.22	266.95
All-bran	10	223.28	257.95	240.60	239.09	239.24
Corn bran	5	308.36	261.60	201.68	273.81	261.36
Corn bran	10	306.70	376.39	465.80	409.86	389.69
Kellogg's Most	5	552.12	461.33	253.97	363.87	332.82
Rice bran	5	225.38	242.34	260.94	221.36	237.51

Table 5

Liver Zinc Levels of Young Rats Fed

Different Sources of Fiber

(Experiment 1)

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	Mean
		mcg/gm dry weight				
0	0	76.58	74.35	65.63	78,73	73.82
All-bran	5	88.33	81.24	93.50	70.61	83.42
All-bran	10	104.37	77.63	77.80	83.24	85.76
Corn bran	5	90.66	76.92	68.71	80.93	79.31
Corn bran	10	109.01	101.38	93.05	86.82	97.57
Kellogg's Most	5	73.82	92.68	82.02	92.08	85.15
Rice bran	5	83.42	93.58	88.38	75.73	85.28

Table 6

Food Consumption Data for Experiment 1

Source of Fiber	Percentage of Fiber	Replications 1	2	3	4	5	6	Total	Mean
		gm/4 weeks							
0	0	429	329	431	326	240	350	2168	361
All-bran	5	460	421	408	427	320	330	2366	394
All-bran	10	416	355	411	420	273	330	2205	366
Corn bran	5	490	508	462	400	420	380	2660	443
Corn bran	10	373	409	421	380	330	360	2273	379
Kellogg's Most	5	423	456	493	390	330	420	2512	419
Rice bran	5	297	362	346	233	270	310	1818	303

Table 7

Effects of Iron and Zinc Supplementation on the Growth of Young Rats Fed Two Levels of All-bran Cereal

(Experiment 2)

Mineral Supplement: Iron (ppm)	Mineral Supplement: Zinc (ppm)	Percentage of Fiber	Replications: 1	2	3	4	5	6	7	8	Mean
			weight gain at 4 weeks (gm)								
0	0	5	133	107	153	186	126	124	135	152	140
75	0	5	140	158	36	173	194	140	140	150	141
0	50	5	148	186	186	123	129	182	142	133	154
75	50	5	133	171	190	130	184	123	128	163	153
0	0	10	153	156	172	175	188	155	164	152	164
75	0	10	194	166	158	160	195	146	147	150	164
0	50	10	172	165	157	203	188	162	167	129	168
75	50	10	159	166	212	197	168	176	161	167	176

Table 8

Effects of Iron and Zinc Supplements on the Hemoglobin Levels

of Young Rats Fed Two Levels of All-bran Cereal

(Experiment 2)

Mineral Supplement			Replications								
Iron	Zinc	Percentage of Fiber	1	2	3	4	5	6	7	8	Mean
(ppm)			gm/100 ml blood								
0	0	5	12.8	12.8	12.8	12.4	13.0	11.6	(13.3)[1]	12.3	12.6
75	0	5	13.1	12.3	12.2	14.5	12.9	12.3	12.1	11.5	12.6
0	50	5	12.9	13.0	13.1	12.3	11.3	12.3	11.4	11.7	12.2
75	50	5	13.8	13.9	12.9	12.5	14.5	12.2	11.4	12.7	13.0
0	0	10	12.1	12.5	12.4	11.8	13.0	14.6	12.1	13.9	12.8
75	0	10	13.3	11.6	12.7	11.5	13.6	12.2	13.4	14.5	12.8
0	50	10	12.5	13.1	12.5	13.2	12.1	12.9	11.9	11.4	12.4
75	50	10	13.5	12.9	13.2	12.3	12.7	12.7	13.5	11.4	12.8

[1]Calculated missing plot.

Table 9

Effects of Iron and Zinc Supplements on the Liver Copper of Young Rats Fed Two Levels of All-bran Cereal

(Experiment 2)

Mineral Supplement			Replications				
Iron	Zinc	Percentage of Fiber	1	2	3	4	Mean
(ppm)			mcg/gm dry weight				
0	0	5	15.67	15.49	10.55	13.24	13.75
75	0	5	12.56	15.71	16.80	13.10	14.54
0	50	5	16.34	18.89	12.15	14.98	15.59
75	50	5	15.56	14.37	16.88	15.85	15.66
0	0	10	15.42	13.97	13.09	13.87	14.09
75	0	10	15.73	12.38	12.57	16.10	14.20
0	50	10	11.10	15.24	13.97	14.44	13.69
75	50	10	13.44	16.44	9.73	13.26	13.22

Table 10

Effects of Iron and Zinc Supplements on the Liver Iron of Young Rats Fed Two Levels of All-bran Cereal

(Experiment 2)

Mineral Supplement Iron (ppm)	Zinc (ppm)	Percentage of Fiber	Replications 1	2	3	4	Mean
			mcg/gm dry weight				
0	0	5	181.18	217.77	139.05	187.44	181.36
75	0	5	233.03	192.15	153.78	228.58	201.88
0	50	5	261.51	166.24	140.66	146.56	178.74
75	50	5	151.14	183.84	160.08	185.94	170.25
0	0	10	151.05	201.67	129.93	245.17	181.96
75	0	10	226.44	200.95	181.21	185.42	198.50
0	50	10	207.73	182.37	163.67	149.45	175.78
75	50	10	222.15	184.23	128.48	192.02	181.72

Table 11

Effects of Iron and Zinc Supplements on the Liver Zinc of Young Rats Fed Two Levels of All-bran Cereal

(Experiment 2)

Mineral Supplement			Replications				
Iron	Zinc	Percentage of Fiber	1	2	3	4	Mean
(ppm)			mcg/gm dry weight				
0	0	5	69.63	69.26	48.10	77.08	66.02
75	0	5	60.66	73.53	62.70	58.40	63.82
0	50	5	64.92	66.64	82.22	65.96	69.94
75	50	5	53.07	75.39	66.17	58.68	63.33
0	0	10	64.13	64.47	61.37	63.28	63.31
75	0	10	73.06	75.49	64.64	70.88	71.02
0	50	10	67.58	67.94	58.74	67.93	65.55
75	50	10	81.79	63.83	49.14	71.47	66.56

Table 12

Food Consumption Data for Experiment 2

Mineral Supplement: Iron (ppm)	Zinc (ppm)	Percentage of Fiber	Replications 1 (gm/4 weeks)	2	3	Total	Mean
0	0	5	375	457	380	1212	404
75	0	5	438	437	440	1315	438
0	50	5	492	369	427	1288	429
75	50	5	438	380	361	1179	393
0	0	10	433	447	412	1292	431
75	0	10	479	435	390	1304	435
0	50	10	446	471	442	1359	453
75	50	10	472	452	468	1392	464

APPENDIX C

Table 1

Analysis of Variance of Data from the Responses of Young Rats Fed Different Sources of Fiber (Experiment 1)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
	Weight Gain		
Total	55	73850.00	
Replications	7	13626.00	1946.64
Treatments	6	33014.00	5502.32**
Error	42	27210.00	647.84
	Hemoglobin		
Total	55	51.38	
Replications	7	5.55	.79
Treatments	6	8.68	1.45
Error	42	37.69	.90
	Liver Copper		
Total	27	117.34	
Replications	3	8.31	2.77
Treatments	6	20.90	3.48
Error	18	88.14	4.90

Table 1 (cont'd.)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
	Liver Iron		
Total	27	187932.80	
Replications	3	8028.52	2676.18
Treatments	6	115136.21	19189.37**
Error	18	64768.07	3598.23
	Liver Zinc		
Total	27	3088.73	
Replications	3	328.01	109.34
Treatments	6	1259.48	209.91
Error	18	1501.24	83.40

**Highly significant ($p \leq 0.01$)

Table 2

Analysis of Variance of Data from the Effects of Different Sources of Fiber on the Mineral Balance of Young Rats (Experiment 1)

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares
		Copper	
Total	41	1.53	
Replications	5	.43	.08
Treatments	6	.64	.11**
Error	30	.46	.02
		Iron	
Total	41	69483700.00	
Replications	5	50226719.00	10045344.00
Treatments	6	430133421.00	71688904.00**
Error	30	214477560.00	7149252.00
		Zinc	
Total	41	7433916.17	
Replications	5	2387368.52	477473.70
Treatments	6	3424832.34	570805.39**
Error	30	1621715.30	54057.18

**Highly significant ($p \leq 0.01$)

Table 3

Analysis of Variance of Data from the Effects of Iron and Zinc Supplements on Young Rats Fed Two Levels of All Bran Cereal

(Experiment 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
	Weight Gain		
Total	63	48503.93	
Replications	7	4214.19	602.00
Treatments	7	9268.44	1324.00
Error	49	35021.30	715.00
	Hemoglobin		
Total	63	43.11	
Replications	7	3.12	.45
Treatments	7	3.44	.49
Error	49	36.55	.75
	Liver Copper		
Total	31	125.62	
Replications	3	17.78	5.92
Treatments	7	21.98	3.14
Error	21	85.87	4.09

Table 3 (cont'd.)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
		Liver Iron	
Total	31	36748.14	
Replications	3	13439.07	4479.69
Treatments	7	3325.95	475.14
Error	21	19983.12	951.58
		Liver Zinc	
Total	31	2025.72	
Replications	3	263.61	87.87
Treatments	7	239.18	34.17
Error	21	1522.93	72.52

Table 4

Analysis of Variance of Data from the Effects of Iron and Zinc Supplements on the Mineral Balance of Young Rats Fed Two Levels of All Bran Cereal (Experiment 2)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares
		Copper	
Total	23	10112049	
Replications	2	69132	30966
Treatments	7	7572140	1081734**
Error	14	2477977	176998
		Iron	
Total	23	582431586	
Replications	2	2612912	1306456
Treatments	7	284735633	40676519
Error	14	295083040	21077360
		Zinc	
Total	23	329068771	
Replications	2	193480	96740
Treatments	7	323947683	46278240**
Error	14	4927608	351972

**Highly significant ($p \leq 0.01$)