THE BIOAVAILABILITY OF ZINC IN WHOLE WHEAT

A Thesis

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by

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INTRODUCTION

Diets high in whole wheat have caused a decreased zinc absorption in Iran and Egypt resulting in retarded growth and hypogonadism (Prasad, 1961). This decreased absorption has been attributed to high phytic acid and high dietary fiber content of wheat. The relative influence of phytic acid and dietary fiber in decreasing zinc absorption is controversial. Some researchers support phytic acid as the dominant factor while others support dietary fiber. The purpose of this study was to determine the relative value of both phytic acid and dietary fiber on the bioavailability of zinc.

LITERATURE REVIEW

History of Zinc in Human Nutrition

Zinc was first isolated from human liver in 1879 (Vallee et al., 1959). By the 1900's zinc was found in human feces, and urine. Birkman (1919) found zinc in the white ash of milk, eggs and other foods.

By the 1930's studies were begun to determine the biological role of zinc. At this time zinc was found to be a part of crystalline insulin and was a necessary part for the crystalization to occur (Scott, 1934). Zinc was reported to be high in the pancreas (Kumar and Jata Rao, 1974) and was observed to be an essential part of carbonic anhydrase (Keilin and Mann, 1939). Other roles of zinc in humans were determined by Prasad et al., (1961) working in the middle east where they noticed the frequent occurrence of dwarfism and hypogonadism in adolescent boys. An analysis of the diets of the dwarfed boys showed them to be high in phytic acid. Phytic acid can bind zinc and prevent its absorption, thus causing deficiency symptoms (Oberleas et al., 1966). Zinc supplementation alleviated the dwarfism and hypogonadism (Prasad et al., 1969).

Phytic Acid

Phytic acid is chemically designated as myoinsitol 1,2,3,4,5,6-hexakis (dihydrogen phosphate), (Figure 1). It is a strong acid and forms a wide variety of salts with several heavy metals. The pH of the medium and presence of secondary cations influences the complexing action of phytic acid. For example zinc and calcium have a synergistic effect which increases phytic acid precipitation at pH 6, the pH of the small intestine (Oberleas, 1973).

Phytic acid occurs in large amounts in cereals, nuts and legumes; in lesser amounts in potatoes, sweet potatoes, and artichokes. Traces can be found in green beans, carrots, and broccoli. In cereals the highest concentration is in the germ and bran. As the cereal flour is refined, phytic acid concentration decreases.

In the human intestinal tract, zinc can also complex with phytic acid to prevent its absorption. Prasad (1973) correlated the hypogonadism and dwarfism in adolescent boys in the middle east with diets consisting mainly of unleavened whole wheat bread which was high in phytic acid. Zinc supplementation resulted in sexual maturation and increased growth.

Phytic acid has been found to reduce zinc availability from the diet. One of the present hypotheses is that zinc in the gut complexes with phytic acid and calcium and is rendered insoluble at an intestinal pH of 6 and thus becomes unavailable for absorption (Oberleas, 1973).

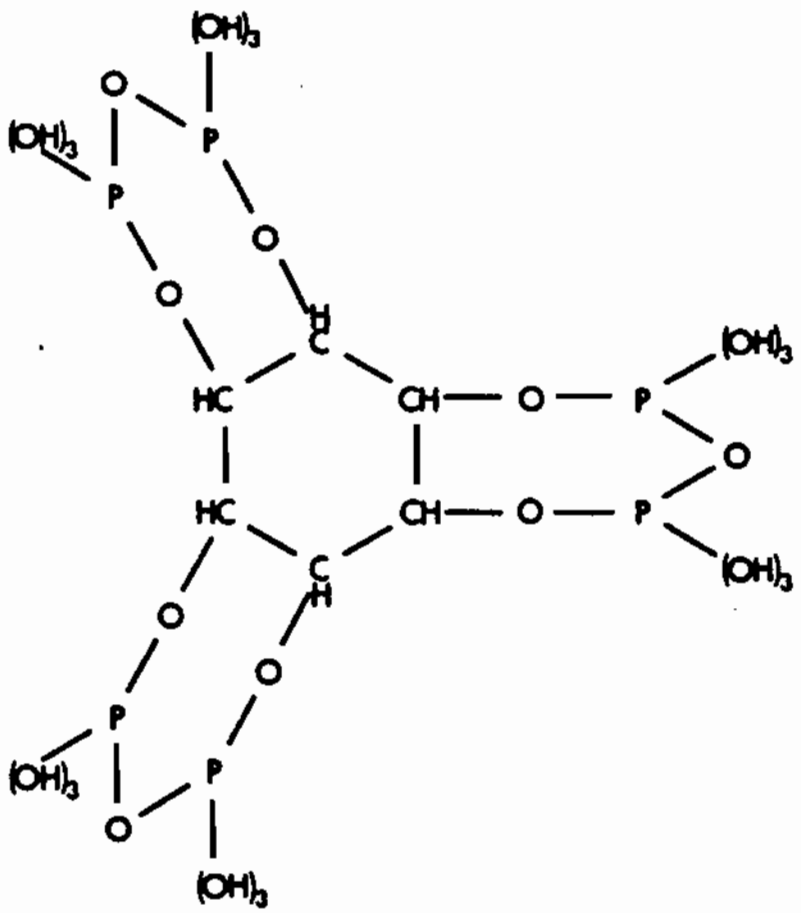

Figure 1. Phytic acid.

Davis and Nightingale (1975) found that phytic acid in a purified diet containing zinc significantly reduced growth rate and food intake in rats. Rats on this diet excreted more zinc in their feces than control or pair fed rats. They also found that the dietary phytic acid reduced the accumulation and whole body retention of zinc.

Phytic Acid:Zinc Molar Ratio

The ratio of the concentration of phytic acid to zinc in food has an influence on zinc availability. Prasad and Oberleas (1973) found zinc became a limiting factor in Iran when tanok (unleavened flat bread) was the predominant item in the diet. The phytic acid:zinc molar ratio for this bread was approximately 22.6. Prasad and Oberleas (1973) reported the ratio should not be greater than 5.0 for optimum growth in rats. A high ratio would be detrimental to zinc absorption. Conversely, in foods with a low ratio there is less phytic acid to bind the zinc and more is available for absorption. Morris and Ellis (1980) found that with a phytic acid:zinc molar ratio less than 12 there was no decrease in growth or bone zinc content.

Fiber

An increased amount of fiber in the diet has lead to a concern about the possible binding of fiber with minerals and thus a reduction in mineral bioavailability.

Tompson and Weber (1979) evaluated the influence of pH on the ability of fiber to bind minerals. At pH 0.65

little mineral remained bound, while at pH 6.8 most of the minerals remained bound to the fiber. Ismail-Beigi et al. (1977a) determined the zinc binding of tanok (Iranian flat bread), dephytinized tanok, and cellulose over a pH range of 5.0 to 7.5. They also found zinc binding was highly pH dependent and reached its maximum at pH 6.5 to 7.5, a pH similar to the small intestine of monogastric animals.

Ismail-Beigi et al. (1977b), while studying the effects of low and high fiber diets on zinc balance in men, found that men went into a negative zinc balance and their plasma zinc levels decreased on both diets. They concluded that under conditions present in the small intestine decreased absorption of zinc resulted from the ability of fiber to bind the zinc firmly. Others have also found dietary fiber can reduce zinc absorption in man (Kies et al., 1976, 1979, and Drews et al., 1979). Davies et al. (1977) studied fiber and phytates as possible determinates of zinc availability in rats, and found that phytates reduced availability, not fiber. Rats consuming extracted bran fiber had growth rates and feed intakes similar to control rats consuming adequate zinc with no fiber or phytic acid. It appears that both phytic acid and fiber may reduce zinc absorption in man. Whether fiber or phytic acid has greater influence is unclear.

Crude Fiber

Dietary fiber was first determined by the crude fiber method (Van Soest and McQueen, 1973). This

method ccnsists of extracting the sample with petroleum ether, boiling in acid, boiling in dilute alkali, and finally washing with acid, alcohol, and ether. The residue is then weighed and ashed. The crude fiber is the final residue weight minus the ash weight (Horwitz, 1970). This method does present problems (Cummings, 1976).

1) It does not provide an accurate measure of the nutritional value of feeds.

2) The determination is method-dependent and at low levels it is insensitive.

3) It only measures a variable part of the total cell-wall constituents.

Therefore, crude fiber is not a good method for dietary fiber determination.

Neutral Detergent Fiber

Goering and Van Soest (1971) developed a method that is thought to give an accurate measure of the constituents in the cell-wall of vegetable foodstuffs. This method determines neutral detergent fiber, which is the fiber that is considered to be important in human nutrition. It consists of lignin, cellulose, and hemicellulose (Van soest and McQueen, 1973). In this method nonfiber components in food are digested with a neutral detergent solution and the remaining fiber is isolated.

Zinc Bioavailability

Results of zinc bioavailability can be expressed as the absolute percentage of dietary zinc availability or as a percentage availability relative to a zinc standard (Franz, 1978). Evans and Johnson (1977) used the absolute percentage in their zinc bioavailability study in which they used ^{65}Zn in intrinsic and extrinsic labeled corn. Methods to determine relative bioavailability of zinc have included log-dose response of growth (O'Dell et al., 1972), slope-ratio assay using total femur zinc (Momcilovic et al., 1975), and curvilinear assays of growth (Franz et al., 1980). The most recent methods reported are the curvilinear assays. Here sigmoidal curves are mathematically determined and fitted to data points using a non-linear, least squares curve fitting computer program (Mercer et al., 1977). Information derived from these curves can be used to determine the bioavailability of zinc.

EXPERIMENTAL PROCEDURE

Experimental Design

Human zinc deficiency was not recognized before it was found to exist in the middle east. In Iran the cause was found to be a diet high in unleavened whole wheat bread (Prasad, 1966). This bread was high in both fiber and phytic acid. It is possible that zinc status may be compromised in individuals with high intakes of unleavened wheat products.

The purpose of this study was to analyze seed wheats for phytic acid and zinc in order to determine their phytic acid:zinc molar ratios. Bioavailability of zinc from wheats with low and high phytic acid:zinc molar ratios was determined using rat growth and femur zinc. Feed intake and erythrocyte zinc levels were also determined.

Wheats

Twenty five samples of seed wheat were collected from five areas of the midwest and western United States (Table 1) and stored in plastic containers at room temperature until analyzed. For analysis the wheats were ground and then frozen. All wheats were analyzed for phytic acid, moisture, and zinc and selected wheats for neutral detergent fiber.

TABLE 1

WHEAT CULTIVARS AND THEIR SOURCES

Source	Cultivar
K.S. Corp Impr Assoc. 205 Call Hall KSU Manhattan, Kansas	1. Centurk 2. Triump 64 3. Newton 4. Eagle 5. Larned
Dr. W.G. Dewey - Plant Science Utah State University Logan, Utah	1. Delmar 2. Hansel 3. Bridger 4. Cache 5. Komar
Institute of Agriculture and Natural Resources Lincoln, Nebraska	1. Sage 2. Scout 66 3. Lancer 4. Centurk 5. Lancota
Minn Corp Improvement Assoc. 1900 Hendon Ave. St. Paul, Minn	1. Kitt 2. Roughrider 3. Erad 4. Olaf 5. Butte
Business Office Montana State University Bozeman, Montana	1. Winalta-Maccasin 2. Centurk-Maccasin 3. Cheyenne-Macassin 4. Warrior-Maccasin
Montana State University Montana Cooperative Extension Extension Agronomist Arthur F. Shaw Bozeman, Montana	1. Unlabeled bag

Phytic Acid

Ground wheat samples were analyzed in triplicate for phytic acid by a modification of the method of de Lange et al., (1961). This method measured phytic acid indirectly by determining the amount of iron needed to precipitate phytic acid as ferric phytate. Three moles of iron will precipitate one mole of phytic acid. Phytic acid was extracted from ground wheat using 5% trichloroacetic acid. Excess ferric chloride was then added causing a precipitation of ferric phytate. Sodium hydroxide was added to the washed precipitate to form ferric hydroxide and sodium phytate. Ferric hydroxide was collected on a filter paper, washed and then acidified with hydrochloric acid. The resultant ferric chloride was analyzed for iron colorimetrically and the phytic acid content of the wheat was calculated (Appendix A).

Moisture

Triplicate samples, weighing 1.000 gm were weighed into aluminum cups and dried at 108°C for 24 hours. Samples were cooled in a desiccator, weighed and moisture calculated.

Zinc

Samples of wheat (about 0.5 gm) were weighed into 100 ml beakers, then 2.5 ml of concentrated H_2SO_4 and a few drops of 30% H_2O_2 were added. Samples would turn black, as heating continued drops of 30% H_2O_2 were added until the contents became clear and colorless. The clear samples

were transfered to acid washed (Appendix B) 25 ml volumetric flasks and brought to volume with deionized water. Samples were transfered to polypropylene test tubes[1] and stored at room temperature until analyzed for zinc.

Analysis of Zinc

Zinc was determined by flame atomic absorption spectrophotometry[2] using standards containing zinc in concentrations from 0.2 mg/ml to 1.5 mg/ml that had been diluted from a 500 mg/ml stock standard. The standards and samples were read in absorption units at a wave length of 213.5 nm. Calculation of zinc concentration was determined from the standards using regression lines.

Neutral Detergent Fiber

Neutral detergent fiber in the wheat samples was determined by a modification of the method used by Goering and Van Soest (1971) (Appendix C). Samples were boiled in a neutral detergent solution for one hour and filtered by suction through a fritted glass crucible. The samples were washed with hot water to remove the solution and nonfiber materials and finally rinsed with acetone. The crucibles were then placed in a drying oven at 100°C for 2 days. Neutral detergent fiber weight was determined and the percentage calculated.

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[1]Test Tubes-polypropylene with caps, 17 x 100 cm, Falcon, Oxnard, CA 93030.

[2]Perkin-Elmer. Model 306, fitted with a single slot burner head, Perkin-Elmer Corp., Norwalk, CT.

The modification used in this procedure was the addition of a crude bacterial, ∝-amylase[1], which contains both amylase and proteases. This helps to digest the starches and proteins which clog the filter. Van Soest and Robertson (1976) developed the enzyme modification to overcome this problem. They added amylase solution to the boiling mixture, and later during filtering. This enzyme was derived from Bacillus subtilis, and has an optimum temperature of 70°C but can function even at boiling temperatures for a short while. The detergent used in the solution destroys the activity of the enzyme so it was added to the crucible in the washing steps.

Animal Study

Twelve groups of animals were used in this study and fed different diets. Five rats were in each group for a total of 60 rats. Diets used in the study were developed to have various levels of zinc with high and low phytic acid:zinc molar ratios.

Zinc Contamination

Since zinc contamination can be a serious problem, procedures were developed to minimize exogenous zinc. In diet preparation stainless steel mixing bowls and paddles were used to mix the premixes and diets. All containers used in this preparation were rinsed in deionized water

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[1] ∝-amylase: microbial amylase from Bacillus subtilis, Sigma Chemical Corp., St. Louis, MI.

before coming in contact with any of the diet ingredients. The rats were housed in stainless steel cages in a room with no other animals or galvanized metals. Food cups and new plastic water bottles were rinsed in 1.0 N HCl and deionized water. Stainless steel guards prevented rats from gnawing on the rubber stoppers of the water bottles.

Diet Composition

Diets were based on a semi-purified diet (Table 2) containing spray dried egg white, corn oil, dextrose and added vitamins and minerals, prepared from premixes. Added zinc was provided from a zinc premix or from wheat.

Premixes

B Vitamins plus K Premix. Vitamins and sucrose (Table 3) were weighed and mixed[1] for 20 minutes. After mixing, the premix was transferred to a plastic bag and stored in a brown bottle in the refrigerator.

Fat soluble Vitamin Premix. Vitamins and corn oil (Table 4) were weighed and then added to a brown bottle. Vitamins were dispersed in the oil by shaking and then stored in the refrigerator.

Choline Bitartrate Premix. Choline and sucrose (Table 5) were weighed and mixed for 20 minutes. The premix was stored in plastic bags in the refrigerator.

Macromineral Premix. Minerals (Table 6) were weighed and placed in a 5 quart mixing bowl and mixed for

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[1]Kitchen Aid Mixer, Hobart Mfg. Co., Troy, OH.

TABLE 2

COMPOSITION OF SEMI-PURIFIED RAT DIET

Item	Percentage in Diet
Egg white[a]	20
Corn oil[b]	5
Dextrose[c]	67.4
Macromineral premix[d]	3.5
Micromineral premix[e]	0.1
B vitamins plus vitamin K premix[f]	2
Vitamins A, D, & E premix[g]	1
Choline bitartrate premix[h]	1

[a]Marshall Foods, Marshall, MN.

[b]Mazola Corn Oil, CPC International, Englewood Cliffs, NJ.

[c]Dextrose Staleydex 333, AE Staley Mfg. Co., Decatur, IL.

[d]Table 6

[e]Table 7

[f]Table 3

[g]Table 4

[h]Table 5

TABLE 3

COMPOSITION OF THE B VITAMINS
PLUS VITAMIN K PREMIX

Vitamin	mg/500 gm[a] premix	ug/100 gm of diet containing 2% premix
Thiamin·HCl[b]	250	1000
Riboflavin[b]	500	2000
Niacinamide[c]	3000	12000
Ca d-pantothenate[c]	1500	6000
Pyridoxine·HCl[b]	480	1920
Folic acid[c]	100	400
Biotin[c]	50	200
B-12[c]	1[d]	4
K(Menadione)[c]	20	80

[a]Vitamins total 7.0 gm; sucrose was added to bring total to 500 gm.

[b]INC Pharmaceuticals Inc., Cleveland, OH.

[c]United States Biochemical Corp., Cleveland, OH.

[d]Vitamin B-12 was added as a 1% triturate in mannitol; 1 mg of vitamin B-12 is equal to 1000 mg of the 0.1% triturate in mannitol.

TABLE 4

COMPOSITION OF THE VITAMINS A, D, & E PREMIX

Vitamin	IU/500 gm[a]	IU/100 gm of diet containing 1% of premix
Vitamin A[b]	500,000	1000
Vitamin D[c]	50,000	100
Vitamin E[b]	11,000	22

[a]Vitamins total 45.05 gm; corn oil was added to bring total to 500 gm.

[b]INC Pharmaceuticals Inc., Cleveland, OH.

[c]United States Biochemicals Inc., Cleveland, OH.

TABLE 5

COMPOSITION OF THE CHOLINE BITARTRATE PREMIX

Item	gm/1000 gm[a] premix	mg/100 gm of diet containing 1% of premix
Choline bitartrate[b]	180	180

[a]180 gm choline bitartrate was added to 820 gm sucrose to bring up to 1000 gm premix.

[b]United States Biochemical Corp., Cleveland, OH.

TABLE 6

COMPOSITION OF THE MACROMINERAL PREMIX

Salt	gm/3513 gm premix	Element	mg/100 gm of diet containing 3.5% premix
KIO_3	0.1	I	0.06
$CuSO_4$	1.3	Cu	0.52
$Fe(C_2H_3O_2)_3 \cdot H_2O$	30.2	Fe	5.0
$MnSO_4 \cdot H_2O$	15.4	Mn	5
$MgSO_4$	230.0	Mg	46
KCl	730.0	Cl	347
Na_2HPO_4	651.0	K	382
$CaHPO_4$	1130.0	Na	211
$CaCO_3$	725.0	P	400
Total	3513	Ca	622

30 minutes. The premix was stored at room temperature in a plastic bag.

Micromineral Premix. Microminerals and sucrose (Table 7) were weighed, placed in a mixing bowl, and mixed for 20 minutes. The premix was then stored at room temperature in a plastic bag inside a brown bottle.

Zinc Premix. Premix was made by first making a 11 mg/gm using heptahydrate zinc sulfate by weighing 24.2125 gm zinc sulfate and 475.7875 gm sucrose. This premix was diluted 1:10 with sucrose resulting in a zinc concentration of 1.1 mg/gm premix. This second premix was further diluted 1:10 with sucrose to give a zinc concentration of 110 ug/gm premix. Each premix was mixed for 20 minutes. The zinc content of the premix was determined by the same method used for wheat.

Diet Preparation

Ingredients were weighed and placed in a large bowl and mixed[1] for 20 minutes. The mixed diets were placed in labeled, large plastic bags, then placed in plastic buckets, and stored in the refrigerator.

Procedure

Cages contained an eight ounce glass food cup and a four ounce glass jar. The smaller jar provided a

[1]The amount of premix or wheat required to provide the necessary zinc in the diets was calculated from the zinc concentration of the premix and selected wheats (Table 8).

TABLE 7

COMPOSITION OF MICROMINERAL PREMIX

Formula	gm/500 gm[a] premix	Element	mg/100 gm of diet containing 0.1% premix
NaF[b]	0.95	F	84
$V\ SO_4 \cdot 2H_2O$[c]	2.0	V	100
$Cr(C_2H_3O_2)_3 \cdot H_2O$[d]	2.5	Cr	100
$Co(C_2H_3O_2)_2 \cdot 4H_2O$[b]	2.1	Co	100
$Ni(C_2H_3O_2)_2 \cdot 4H_2O$[b]	2.1	Ni	100
$Na_2MoO_4 \cdot 2H_2O$[b]	1.25	Mo	100
$NaBr$[b]	0.65	Br	100
Na_2SeO_3[e]	0.15	Se	10
$Na_2B_4O_7 \cdot 10H_2O$[b]	11.55	B	250
$Sn(C_4H_4O_6)$[f]	1.1	Sn	100

[a]The minerals totaled 24.35 gm. Sucrose was added to the minerals to bring the total to 500 gm.

[b]BYU Chemistry Stores, Provo, UT.

[c]BHD Chemicals Ltd., Poole, England.

[d]Matheson, Coleman & Bell Manufacturing Chemist, Norwood, OH.

[e]INC Pharmaceuticals Inc., Cleveland, OH.

[f]Sigma Chemical Corp., St. Lewis, MO.

TABLE 8

SOURCES OF ZINC IN CONTROL AND EXPERIMENTAL DIETS

Diet	Calculated dietary zinc ppm	Dextrose gm	Wheat gm	Zinc premix gm
Control				
	0 ppm	1101	---	---
	3 ppm	955.3	---	55.70
	6 ppm	899.61	---	111.39
	12 ppm	788.22	---	222.78
Low Phytic Acid[a]				
	3 ppm	876.07	134.93	---
	6 ppm	741.14	269.86	---
	9 ppm	606.21	404.79	---
	12 ppm	471.28	539.72	---

TABLE 8 - Continued

Diet	Calculated dietary zinc ppm	Dextrose gm	Wheat gm	Zinc Premix gm
High Phytic Acid[b]				
	3 ppm	775.4	235.6	---
	6 ppm	539.8	471.2	---
	9 ppm	304.2	706.8	---
	12 ppm	68.6	942.4	---

[a]Triump Wheat K.S. Corp. Improvement Assoc., Manhattan, KS.

[b]Kittspring Wheat Minn. Corp. Improvement Assoc., St. Paul, MN.

sleeping space for the weanling rats so they would not sleep in their food cups. Newspapers were placed on trays under the cages, then covered with paper towels to collect spilled diet. Deionized water was provided.

Weanling rats[1] were assigned to their cages in a randomized block design so that each group of rats had comparable mean weights. The Sprague Dawley derived males weighed 43-56 gm. Rats were weighed three times a week. Weekly diet consumption was measured by determining the difference in weight of the food cup and estimating spillage. After three weeks the rats were decapitated and blood was collected in heparinized[2] polypropylene tubes. The left and right femurs were removed, placed in plastic bags and frozen.

Dietary Zinc

At the end of the animal study a small sample of each diet was stored in polypropylene tubes and frozen until analyzed. After bringing frozen samples to room temperature, about 0.5 gm of the diets were weighed into 100 ml beakers and digested with concentrated H_2SO_4 and 30% H_2O_2 as described for the wheat samples. After appropriate dilutions, zinc concentration was determined

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[1]Simonsen Laboratories, Inc., Gilroy, CA.

[2]Heparin Sodium 1,000 unites/ml from beef lung, The Upjohn Co., Kalamazoo, MI.

by flame atomic absorption spectrophotometry as previously described.

Zinc Premix

When the final zinc premix was made, 20 samples were taken in a random manner. About 0.25 gm of the samples were placed in 100 ml beakers and digested, diluted and read as with the wheats (Table 9).

Femurs

Femurs, removed from the animals after sacrifice, were thawed and cleaned of the muscle and other extraneous tissue. Cleaned femurs were placed in aluminum weighing cups and dried at 130°C for 1.5 hours. Cups and femurs were cooled in a desiccator, then weighed. Femurs were placed in 50 ml beakers for digestion using a modification of the method of Turnlund and Margen (1979). A few drops of water were added to each beaker, then 2.0 ml of concentrated HNO_3 was added. Beakers were covered with watch glasses and left to sit over night. The next day the beakers were heated slowly while a few drops of 30% H_2O_2 were added to help oxidize the organic materials until the samples were clear. At this point the samples were heated to dryness, one milliliter of HNO_3 was added to the dried samples and the above procedure was repeated. After the samples were heated to dryness the second time, the beakers were allowed to cool, then 10.0 ml of 1 N HCl were added to

TABLE 9

ZINC CONCENTRATION IN ZINC PREMIX

Sample	Zinc concentration ppm
1	74.5[a]
2	71.0
3	79.2
4	78.7
5	84.8
6	99.0
7	----[b]
8	88.2
9	86.0
10	75.0
12	69.7
13	87.4
14	79.8
15	89.7
16	80.7
17	79.8
18	95.0
19	62.0
20	84.8
Total ± S.D.	80.2 ± 9.8

[a]Wet weight.

[b]Sample 7 was lost during digestion.

the beakers to dissolve the ash and the contents were transferred to polypropylene tubes. After appropriate dilutions, zinc concentration was determined.

Erythrocytes

After sacrifice, blood was collected and washed three times with 0.9% saline solution. The washed erythrocytes were diluted with saline, their hematocrits were recorded, and samples were frozen until analyzed. After thawing, the lysed erythrocytes were diluted 1:10 and analyzed for zinc.

Statistics

Weight gain, feed intake, and femur zinc were analyzed by Mercer (1980) using a nonlinear, least squares curve fitting computer program (Mercer et al., 1977). From these results relative bioavailability of zinc was calculated.

RESULTS

Wheats

Zinc concentration in wheat, dry weight, varied from 14.6 to 59.1 with a mean of 32.1±12.1 ug/gm (Table 10). Phytic acid concentrations, dry weight, varied from 544 to 1346 mg/100 gm with a mean of 883±185 mg/100 gm. Phytic acid:zinc molar ratios varied from 14.5 to 46.4 with a mean of 29.8±8.6. Triump (ratio 19.3) and Kitt (ratio 46.4) were the wheats selected to represent the low and high phytic acid:zinc molar ratios. The neutral detergent fiber concentration, dry weight, of Triump was 11.14±0.24% and of Kitt, 9.38±.10%.

Dietary Zinc

The analyzed levels of zinc in the diets varied from the calculated values (Table 11). The basal diet, with no added zinc, contained 0.43 ppm zinc. Diets with added zinc would be expected to have the zinc added plus the basal levels. All diets except the 12 ppm control diet were higher than expected. The 12 ppm control diet was lower than expected; 10.57 ppm. The higher values might have been due to possible contamination at mixing although all precautions possible were made to prevent this. Why the 12 ppm control diet was lower is not known.

TABLE 10

MOISTURE, PHYTIC ACID, ZINC AND PHYTIC ACID:ZINC MOLAR RATIO OF WHEATS

Area	Cultivar	Moisture %	Phytic acid mg/100 gm	Zinc ug/gm	Phytic acid:zinc molar ratio
Kansas					
	Centurk	9.36	777±54[a]	39.9±0.16[a]	19.3
	Triump	9.68	722±11	37.0±1.07	19.3[b]
	Newton	8.99	999±137	44.5±1.2	21.7
	Eagle	9.21	846±10	33.8±0.57	24.7
	Larned	8.93	865±36	28.4±0.65	30.1
		9.23±0.27[c]	841±94[c]	36.7±5.4[c]	23.0±4.1[c]
Utah					
	Delmar	8.77	997±22	32.0±0.18	30.9
	Hansel	8.15	614±10	27.8±2.10	21.9
	Bridger	9.14	879±10	28.7±0.83	30.3
	Cache	9.12	951±11	22.0±1.25	42.8
	Komar	11.06	863±11	59.1±1.88	14.5
		9.25±0.97	861±133	32.12±13.7	20.1±9.5
Nebraska					
	Sage	10.69	1346±73	56.0±2.65	23.8
	Scout 66	10.73	1019±42	35.1±2.03	28.8

TABLE 10 - Continued

Area	Cultivar	Moisture %	Phytic acid mg/100 gm	Zinc ug/gm	Phytic acid:zinc molar ratio
Nebraska (cont.)					
	Centurk	9.95	963±43	27.5±1.83	34.6
	Lancota	9.84	1245±42	43.9±1.04	28.1
	Lancer	10.05	789±22	19.7±1.30	35.7
		10.34±0.37	1072±200	36.9±12.0	30.2±4.4
Montana					
	Unlabeled	7.16	719±21	21.8±2.34	35.7
	Winalta	9.76	848±57	18.6±0.78	45.1
	Centurk	10.48	570±11	14.6±2.37	38.3
	Cheyenne	10.11	647±11	17.5±1.32	36.7
	Warrior	10.00	544±18	20.2±0.22	26.7
		9.5±1.19	666±110	18.54±2.5	36.5±5.9
Minnesota					
	Kitt	9.70	990±10	21.1±0.55	46.4[b]
	Roughrider	10.28	984±10	35.3±0.80	27.6

TABLE 10 - Continued

Area	Cultivar	Moisture %	Phytic acid mg/100 gm	Zinc ug/gm	Phytic acid:zinc molar ratio
Minnesota (cont.)					
	Era	9.67	979±26	27.9±0.44	34.7
	Olaf	9.88	1013±34	32.3±1.03	31.1
	Butte	9.91	916±0.1	56.4±0.89	16.1
		9.89±.22	976±32	34.6±11.9	31.2±9.8

[a]dry weight

[b]Wheat samples used for the animal study

[c]Mean ± S.D.

TABLE 11

ANALYZED ZINC CONCENTRATION OF RAT DIETS

Diet	Calculated zinc ppm	Analyzed zinc[a] ppm
Zinc sulfate	0	0.43±0.62
	3	3.98±1.37
	6	7.02±1.45
	12	10.57±1.53
Low phytic acid wheat	3	3.98±0.40
	6	7.48±0.35
	9	10.84±2.30
	12	13.49±1.31
High phytic acid wheat	3	5.11±0.26
	6	7.60±1.68
	9	12.81±1.93
	12	14.38±0.89

[a]Four replications, wet weight

Weight Gain

Animal weight gain showed a positive pattern in relationship to zinc concentration and a negative pattern to molar ratios of the diets (Table 12). Control animals showed the most growth with increasing dietary zinc (Figure 2). Diets with low phytic acid:zinc molar ratios resulted in less growth than controls with increasing dietary zinc concentration. Those animals in the high phytic acid:zinc molar ratio group showed the lowest growth of all. In the 6 ppm control group a higher weight gain occurred then was expected. This may have been due to the contamination level being high enough to provide enough zinc for normal growth. The weight gain for the zinc free group was higher in this study than in other similar studies (Franz et al., 1980). The basal diet in this study contained 0.43 ppm zinc which was not enough to support the growth observed; it is therefore probable that the increased growth was stimulated by environmental zinc contamination from the air. Air may have been the vector for carrying environmental zinc. There is a steel mill within 7 miles of the site of the study, but no data are available concerning the amount of zinc carried to the animals from this source.

Femur Zinc

Femur zinc levels followed a pattern similar to weight gain (Table 13). The decrease in femur zinc was

TABLE 12

WEIGHT GAIN AND FEED EFFICIENCY OF RATS CONSUMING DIETS WITH ZINC SULFATE OR WHEATS

Diet	Calculated dietary zinc ppm	Weight gain gm	Feed efficiency[a]
Zinc sulfate	0	31±5.3	5.4
	3	76±6.8	2.6
	6	130±10.7	2.0
	12	138±14.4	1.9
Low phytic acid wheat	3	58±7.3	3.3
	6	81±7.3	2.6
	9	112±17.36	2.3
	12	128±8.9	1.9
High phytic acid wheat	3	53±3.1	2.8
	6	71±12.0	2.6
	9	80±6.2	2.4
	12	88±12.1	2.2

[a]Feed efficiency = $\frac{\text{grams food eaten}}{\text{grams weight gain}}$

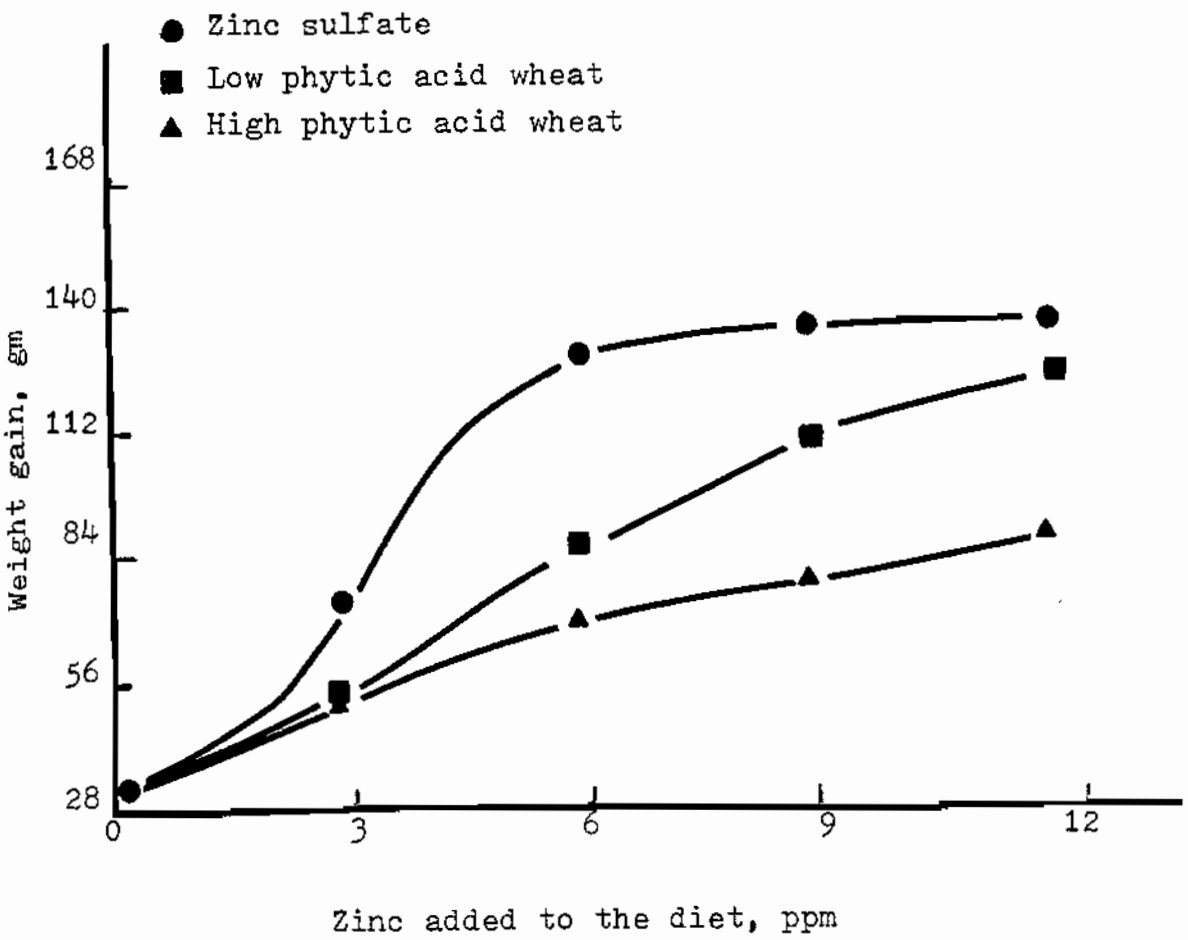


Figure 2. Three week growth response of rats consuming diets containing zinc sulfate and wheats with low and high phytic acid:zinc molar ratios.

TABLE 13

WEIGHT AND ZINC CONTENT OF FEMUR FROM RATS CONSUMING DIETS WITH ZINC SULFATE OR WHEATS

Diet	Calculated dietary zinc ppm	Femur weight[a] mg	Femur zinc[a] ug/femur	Femur zinc[a] ug/gm
Zinc sulfate	0	180±13.8	5.1±1.0	28.4±4.7
	3	245±21.0	9.3±1.7	38.0±5.7
	6	305±17.1	18.0±2.3	58.96±6.3
	12	281±20.1	52.6±9.3	186.2±21.0
Low phytic acid wheat	3	218±14.7	11.9±1.0	54.3±1.3
	6	234±17.4	14.3±0.9	61.4±4.8
	9	274±30.2	18.1±0.3	66.9±8.3
	12	274±12.2	22.0±2.4	80.8±12.2
High phytic acid wheat	3	197±6.9	8.3±1.4	43.0±6.5
	6	219±10.6	9.4±1.6	42.7±6.2
	9	249±12.6	13.8±2.6	55.3±10.1
	12	249±18.9	13.6±2.3	54.4±7.3

[a]Dry weight

correlated with an increase in the phytic acid:zinc molar ratio (Figure 3).

Erythrocyte Zinc

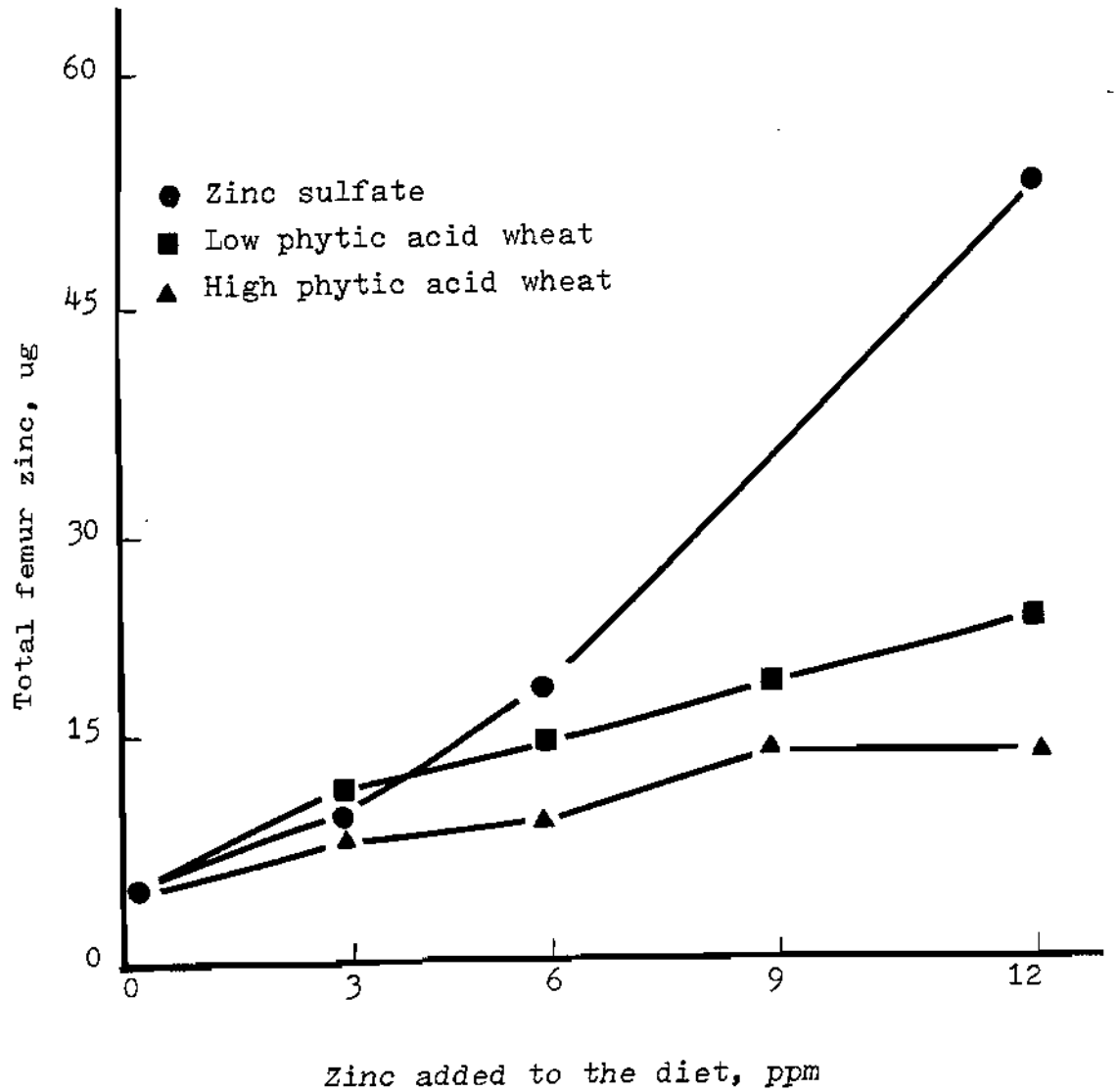
There were no statistically significant differences in zinc concentration in the erythrocyte levels due to large variations. The means, however, suggest that zinc concentrations in erythrocytes are nearly inverse to growth rate (Table 14, Figure 4). As the rats grew their blood volume would increase, but the zinc available for erythrocytes did not increase enough to keep the zinc levels constant. The rats on the low or zinc free diets did not grow enough to increase the blood volume so the erythrocyte zinc levels stayed about the same.

Zinc Bioavailability

Relative bioavailability of zinc was determined using relative net zinc utilization (RNZU) (Franz et al., 1980), curvilinear assays and log of the total femur zinc.

Weight Gain

RNZU is the ratio of the net weight gain of the experimental animals to the control animals at 6 ppm of added zinc. Net weight gain is the weight gain of the animals minus the weight gain of the animals consuming the basal diet with no added zinc. Values were 0.50 for the low phytic acid wheat and 0.40 for the high phytic acid wheat (Table 15).



[illegible] of rats consuming diets containing zinc sulfate, and wheats with low and high phytic acid:zinc molar ratios.

TABLE 14

ZINC CONCENTRATION OF ERYTHROCYTES FROM RATS CONSUMING DIETS WITH ZINC SULFATE OR WHEATS

Diet	Calculated dietary zinc ppm	Zinc ug/dl PCV[a]
Zinc sulfate	0	800±67
	3	532±292
	6	642±175
	12	641±122
Low phytic acid wheat	3	807±357
	6	638±156
	9	547±205
	12	462±55
High phytic acid wheat	3	808±224
	6	704±142
	9	573±117
	12	539±51

[a]Packed cell volume

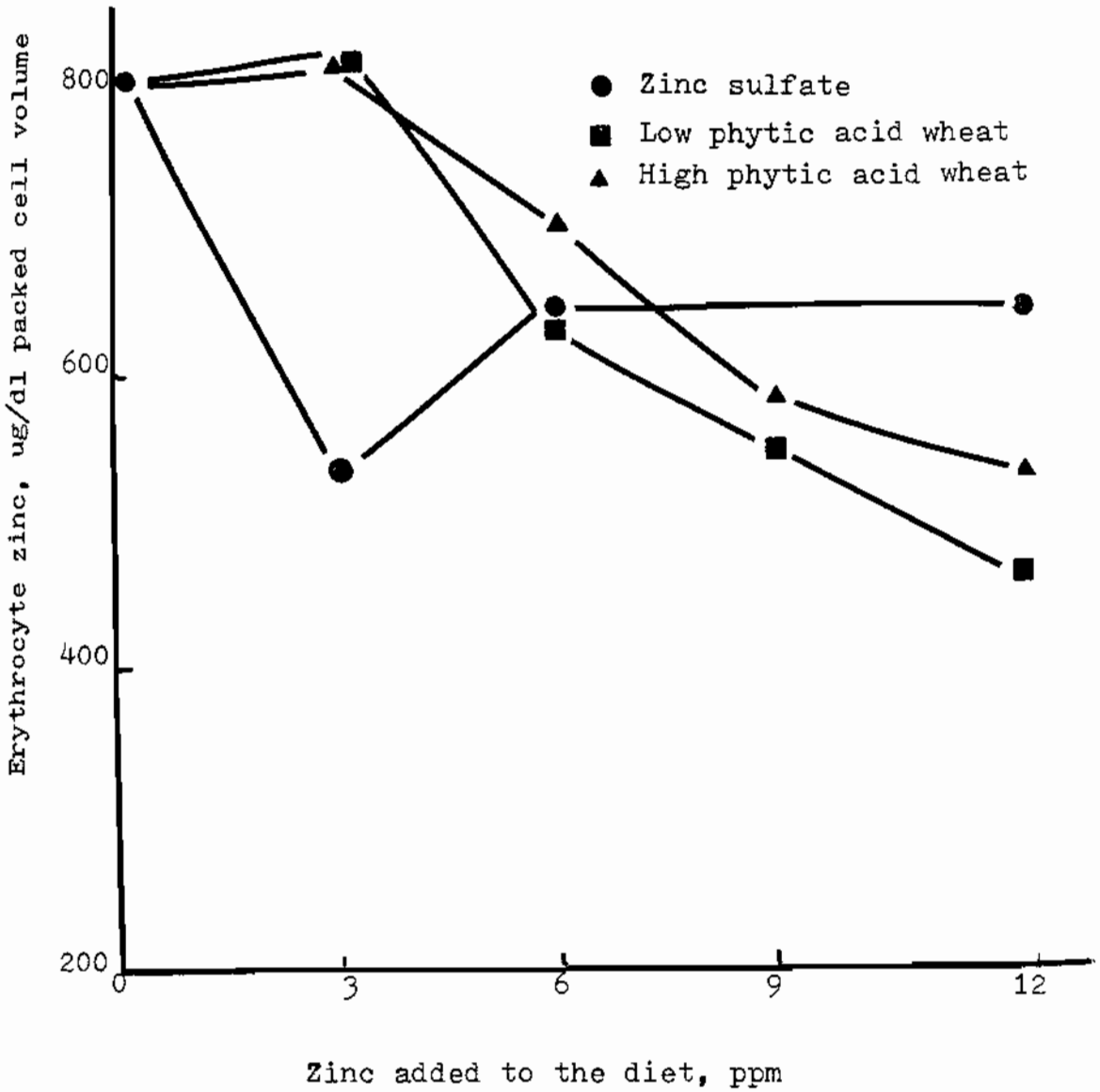


Figure 4. Erythrocyte zinc of rats consuming diets containing zinc sulfate, and wheats with low and high phytic acid:zinc molar ratios.

Curvilinear Assay

Sigmoidal curves to fit the weight gain were calculated using a curvilinear computer program (Figure 2). Parameters for these curves are found on Table 16. By using these parameters, a value for relative bioavailability of zinc can be determined using the ratio of $(R_{max}-b)/K_{0.5}$ of the experimental and control animals. For the low phytic acid group the value was 0.57 and for the high phytic acid group 0.35.

Log Total Femur Zinc

The ratios of the slopes of the log femur zinc (Table 17, Figure 5) of the experimental diet animals to the control animals were used to determine a value for relative zinc bioavailability. Results were 0.57 for the low phytic acid group and 0.42 for the high phytic acid group.

Phytic Acid vs. Fiber

The amount of wheat per 100 gm of diet at 4, 6, and 8 ppm of added zinc was calculated. From this the amount of phytic acid and neutral detergent fiber per 100 gm diet was also calculated (Table 18).

This Study

The correlations between the weight gain and neutral detergent fiber, weight gain and phytic acid, and weight gain and food, although high, were not statistically

TABLE 15

RELATIVE BIOAVAILABILITY OF ZINC OF LOW AND HIGH PHYTIC ACID WHEATS

Group	Weight gain		Log femur zinc Slope-ratio
	RNZU[a]	$(R_{max}-b)/K_{0.5}$[b]	
Zinc sulfate	1.0	1.0	1.0
Low phytic acid wheat	0.50	0.57	0.57
High phytic acid wheat	0.40	0.35	0.42

[a]Relative net zinc utilization =

$$\frac{\text{net weight gain at 6 ppm (experimental group)}}{\text{net weight gain at 6 ppm (zinc sulfate group)}}$$

[b]Ratio of experimental to control animals of the value derived from calculated maximum weight gain minus the calculated weight gain of animals consuming no added zinc divided by the amount of zinc (ppm) required for one-half the maximum response.

TABLE 16

PARAMETERS AND R^2 OF CURVES FITTED TO WEIGHT GAIN[a]

Diet	R^2	R_{max}	$K_{0.5}$	n	b	K_I
Zinc sulfate	94.98	138.8	3.36	3.98	31.10	124.40
Low phytic acid wheat	99.34	162.56[b]	7.21	1.76	32.02	32.36
High phytic acid wheat	99.98	124.73	8.22	1.15	31.09	11.27

[a]Individual points on the curve can be calculated by useing the following formula: $r=(bK_I + R_{max}I^n)/(I^n + K_I)$ where r = observed response of the animals, I = nutrient intake, K_I = nutrient constant, R_{max} = asymptotic response at high levels of nutrient intake, b = calculated ordinate intercept, n = apparent kinetic order of the response with respect to I as I^n becomes negligible compared to K_I, $K_{0.5}$ = I which produces $(R_{max} + b)/2 = K_I^{1/n}$.

[b]Values do not represent a true R_{max} or $K_{0.5}$ due to insufficient data at the upper end of the curve.

TABLE 17

LOG TOTAL FEMUR ZINC OF RATS CONSUMING DIETS WITH ZINC SULFATE OR WHEATS

Diet	Calculated dietary zinc ppm	Slope	Log femur zinc
Zinc sulfate	0	0.0844	0.71
	3		0.97
	6		1.26
	12		1.72
Low phytic acid wheat	3	0.0480	1.08
	6		1.16
	9		1.26
	12		1.34
High phytic acid wheat	3	0.0353	0.92
	6		0.97
	9		1.14
	12		1.13

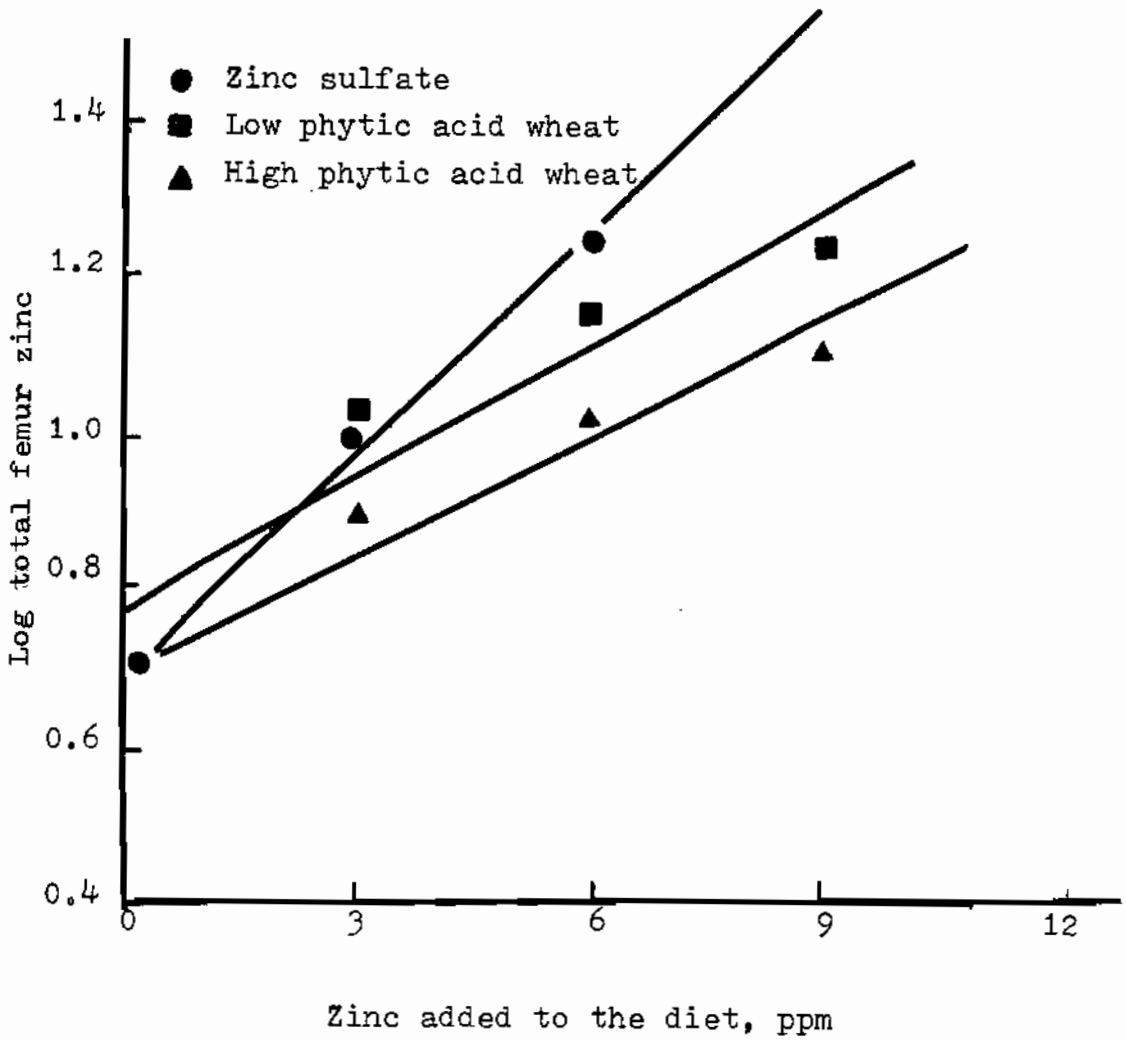


Figure 5. Log of the total femur zinc of rats consuming diets containing zinc sulfate, and wheats with low and high phytic acid:zinc molar ratios.

TABLE 18

WHEAT, NEUTRAL DETERGENT FIBER, AND PHYTIC ACID CONTENT OF 100 GRAMS OF DIET AT VARYING ZINC CONCENTRATIONS

Zinc Source	Calculated dietary zinc	Net weight gain	Diet component/100 gm diet		
			Wheat	NDF[a]	Phytic acid
	ppm	gm	gm	gm	mg
			This Study		
Zinc sulfate	4	73	0	0	0
	6	84	0	0	0
	8	98	0	0	0
Low phytic acid wheat	4	36	12.0	1.3	78
	6	51	18.0	2.0	117
	8	73	24.0	2.7	156
High phytic acid wheat	4	30	20.9	2.0	187
	6	41	31.4	3.0	281
	8	47	41.9	4.0	375
			Expanded Study		
Unleavened whole wheat bread	4	37	15.4	1.2	65
	6	54	23.1	1.8	98
	8	70	30.8	2.5	131
Leavened whole wheat bread	4	47	14.6	1.1	19
	6	76	21.7	1.7	29
	8	97	29.0	2.3	39
Whole wheat flour	4	27	16.3	1.1	101
	6	46	24.4	1.7	152
	8	67	32.5	2.3	203

TABLE 18 - Continued

Zinc Source	Calculated dietary zinc	Net weight gain	Diet component/100 gm diet		
			Wheat	NDF[a]	Phytic acid
	ppm	gm	gm	gm	mg
			Expanded Study (cont.)		
Unleavened white bread	4	49	44.0	0.6	0
Leavened white bread	4	50	42.6	0.4	0
White flour	4	40	48.2	0.2	19

[a]Neutral detergent fiber.

significant due to only one degree of freedom, even when the control animals were used (Table 19, Figures 6, 7, and 8).

Expanded Study

When the two wheats used in this study were added to the six wheats of Franz et al. (1980) (Table 18), significant correlations could be determined (Table 19). With the wheats of both studies added together five to eight samples were possible giving more degrees of freedom. From pooling these two studies the correlation of phytic acid was significantly related to weight gain at the three levels of dietary zinc while the food and neutral detergent fiber were not (Figures 9, 10, and 11).

TABLE 19

CORRELATIONS OF WEIGHT GAIN OF RATS WITH WHEAT, NEUTRAL DETERGENT FIBER AND PHYTIC ACID CONTENT OF 100 GRAMS OF DIET AT VARYING ZINC CONCENTRATIONS

Dietary omponent	Calculated dietary zinc, ppm 4	6	8
		This Study	
Number of samples	3	3	3
Wheat	-0.953	-0.977	-0.709
NDF[a]	-0.975	-0.994[b]	-0.978
Phytic acid	-0.882	-0.923	-0.996[b]
		Expanded Study	
Number of samples	8	5	5
Food	+0.569	-0.504	-0.709
NDF	-0.648[b]	-0.599	-0.787
Phytic acid	-0.861[c]	-0.866[c]	-0.953[d]

[a]Neutral detergent fiber

[b]$p < 0.10$

[c]$p < 0.05$

[d]$p < 0.01$

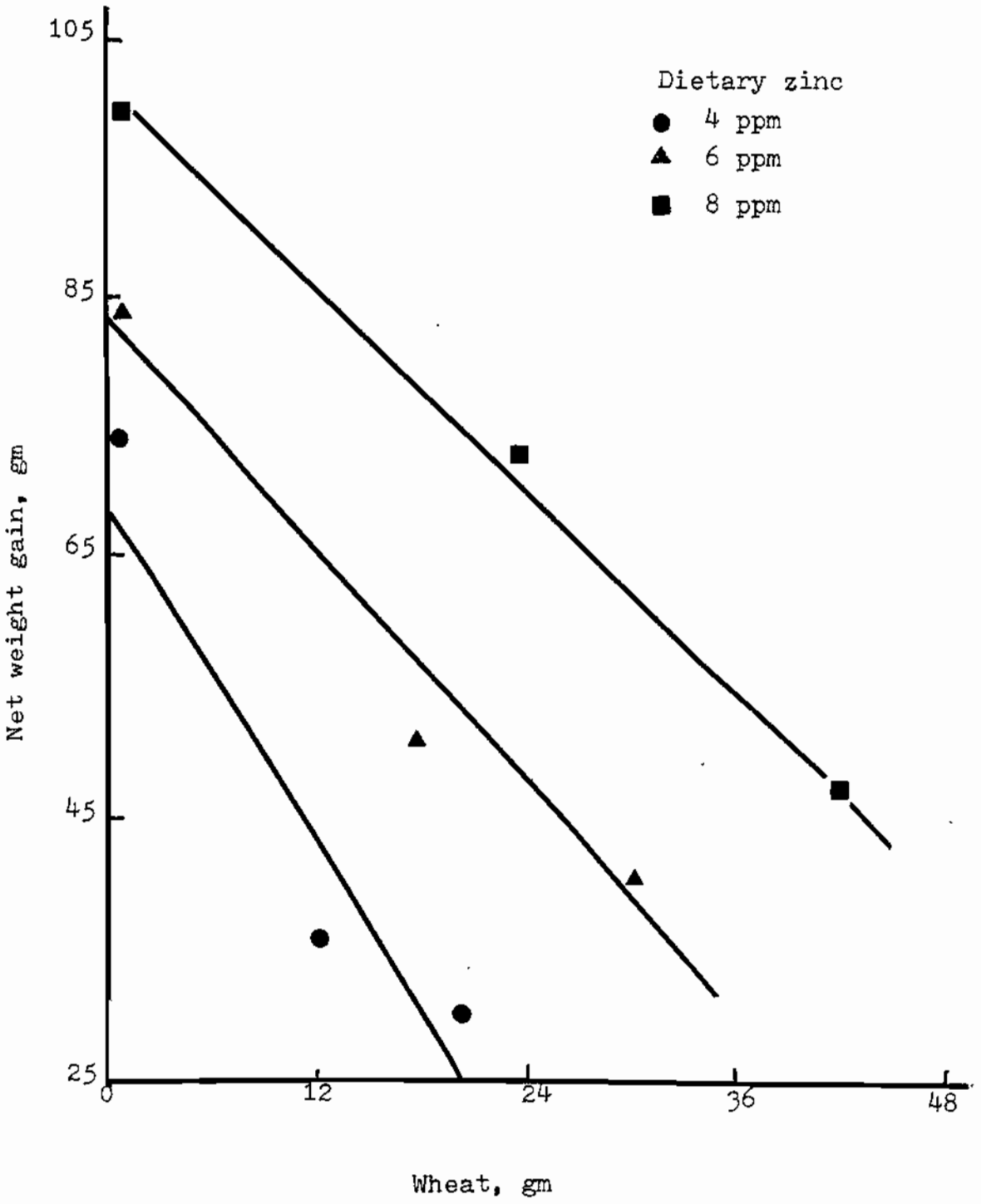


Figure 6. Graph of the relationship of the net weight gain of rats and grams of added wheat per 100 grams of diet in this study.

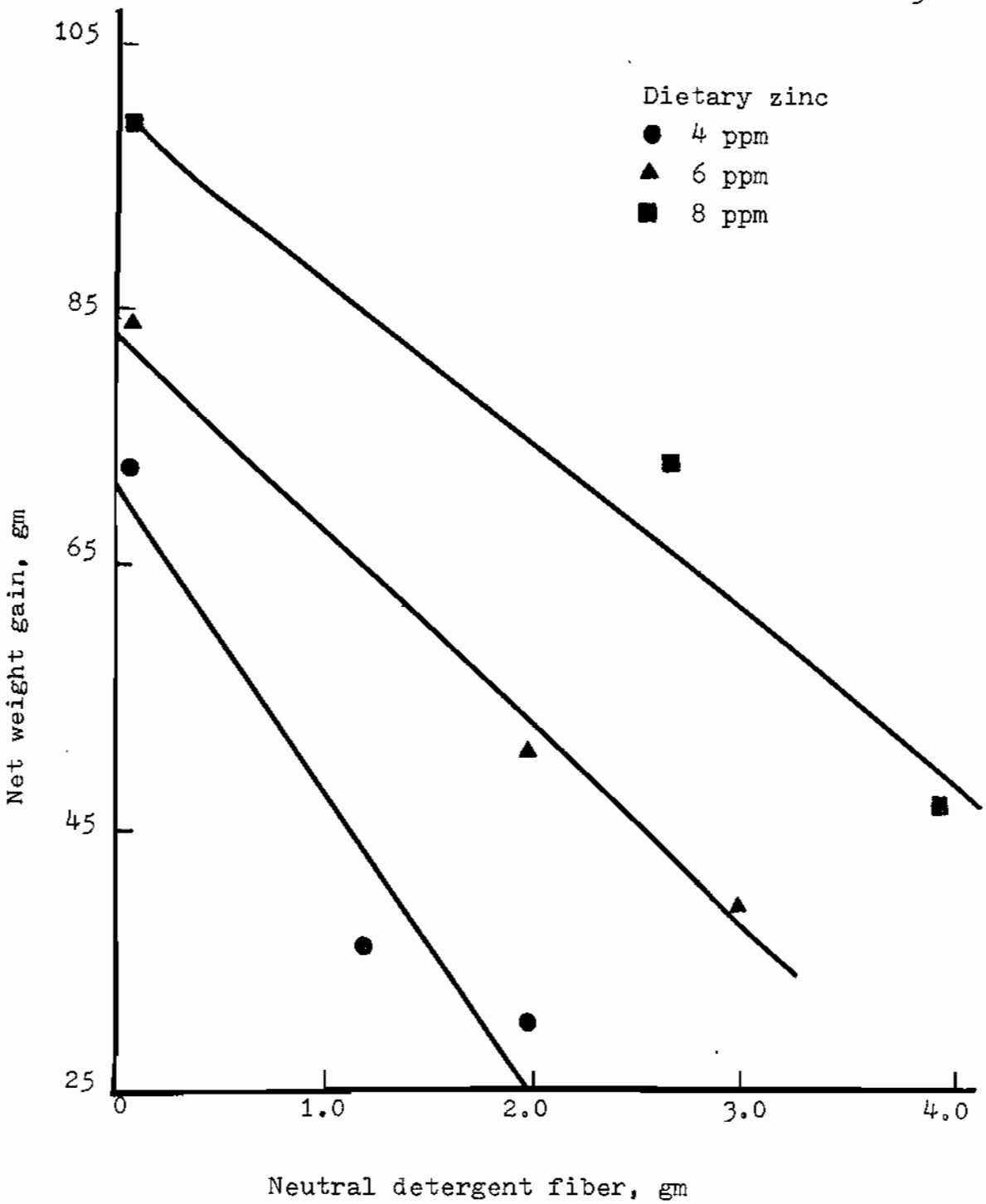


Figure 7. Graph of the relationship of the net weight gain of rats and grams of added neutral detergent fiber per 100 grams of diet in this study.

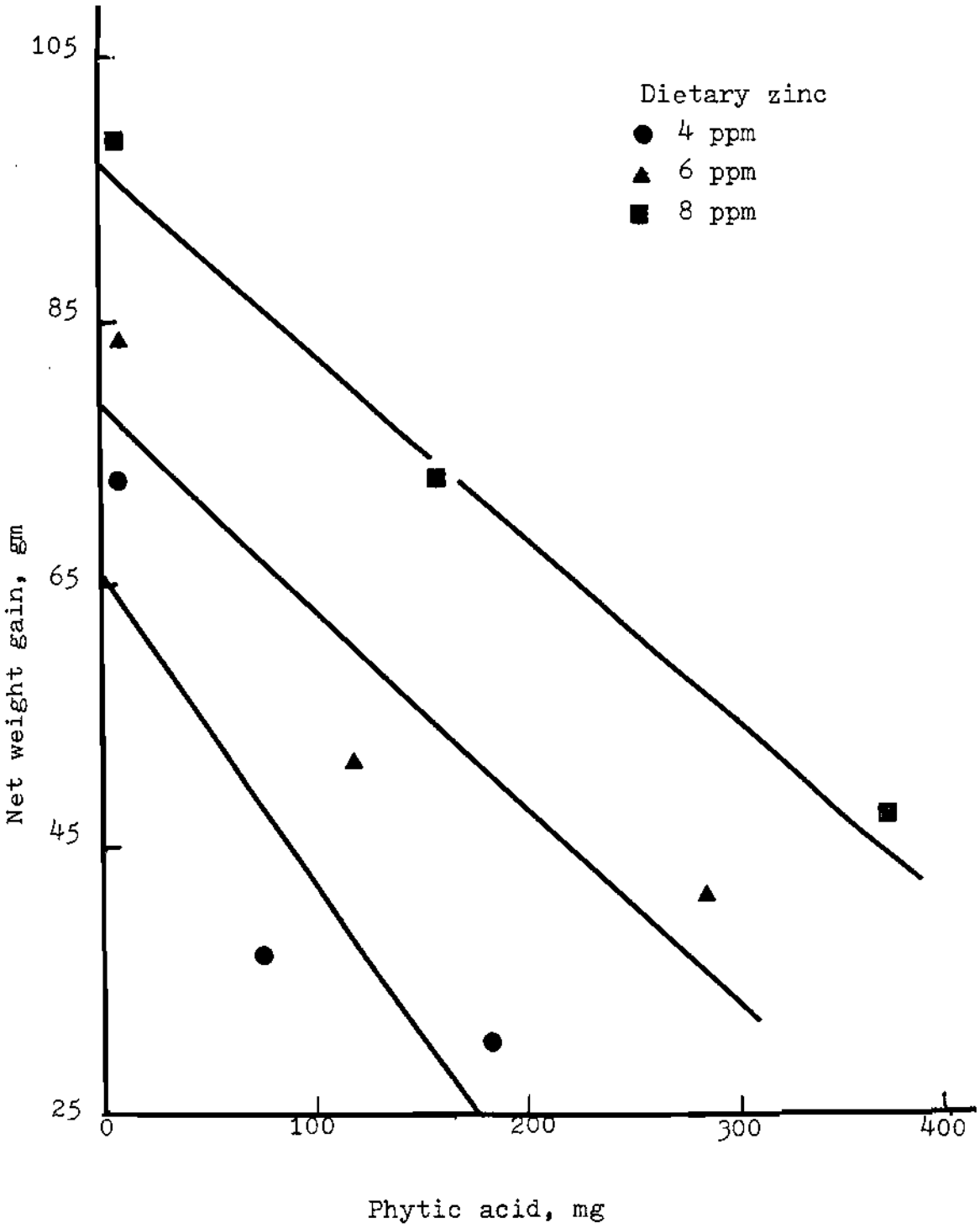


Figure 8. Graph of the relationship of net weight gain of rats and milligrams of phytic acid added to 100 grams of diet in this study.

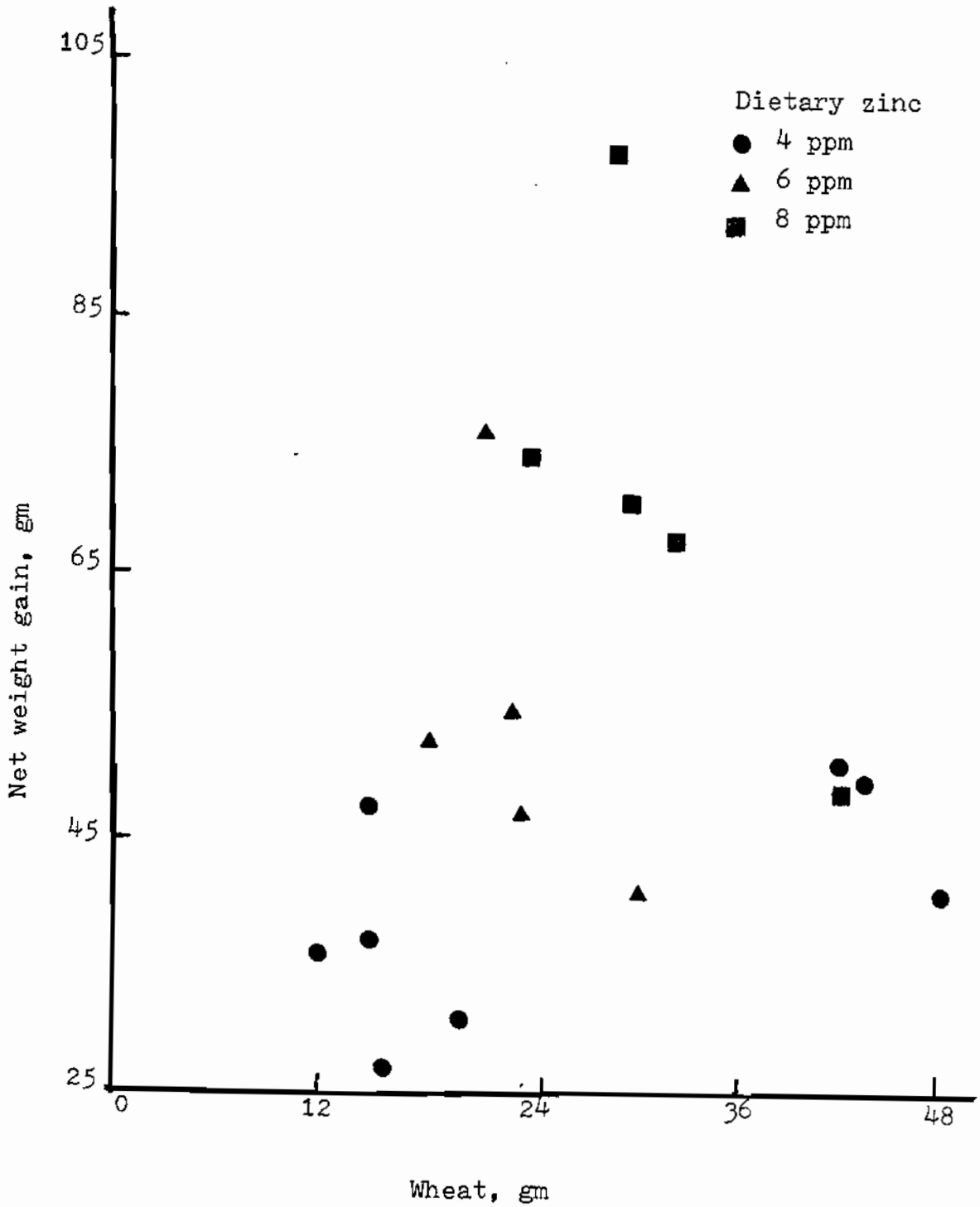


Figure 9. Graph of the relationship of net weight gain of rats and grams of wheat added to 100 grams of diet in expanded study.

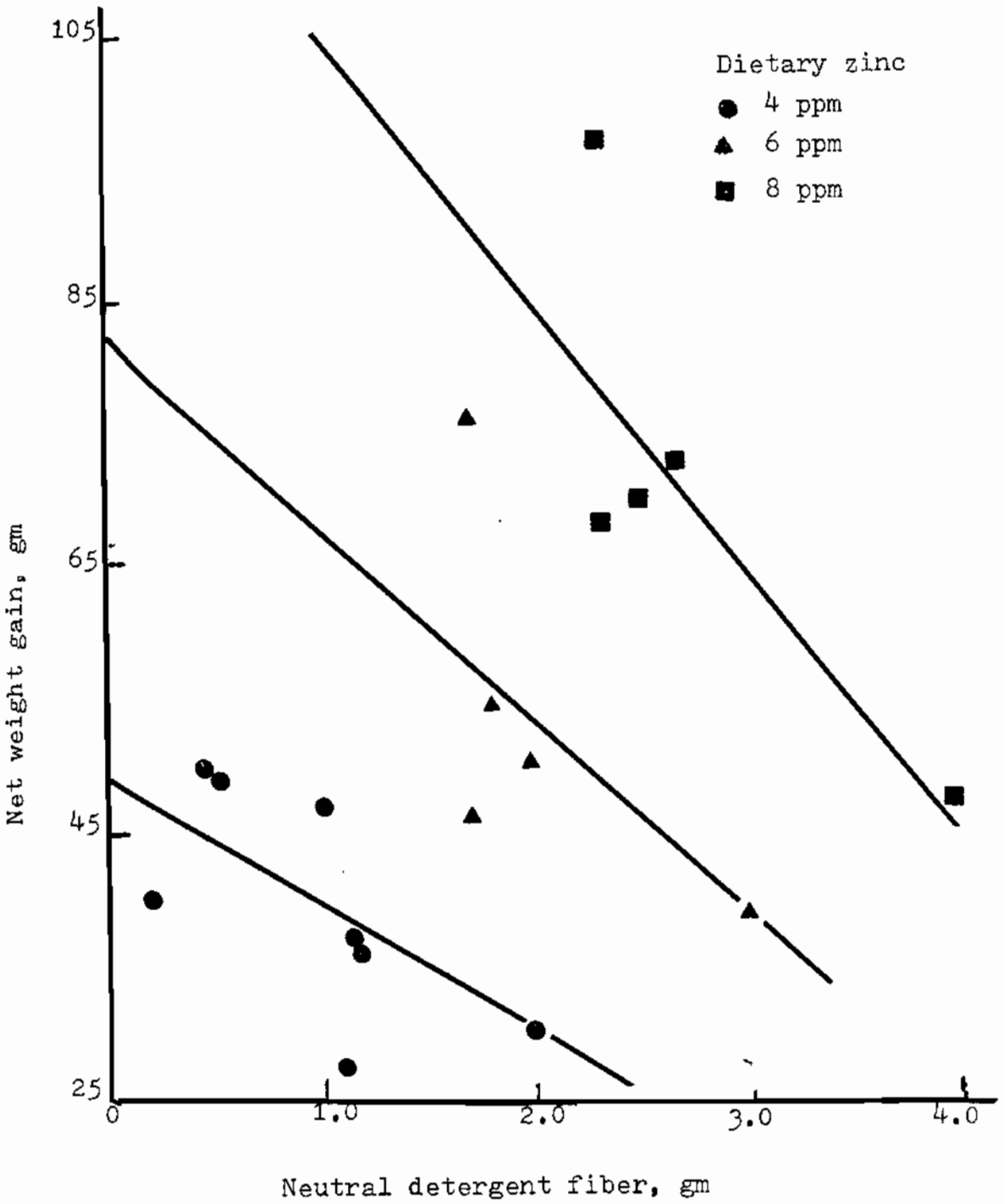


Figure 10. Graph of the relationship of net weight gain of rats and grams of added neutral detergent per 100 grams diet in expanded study.

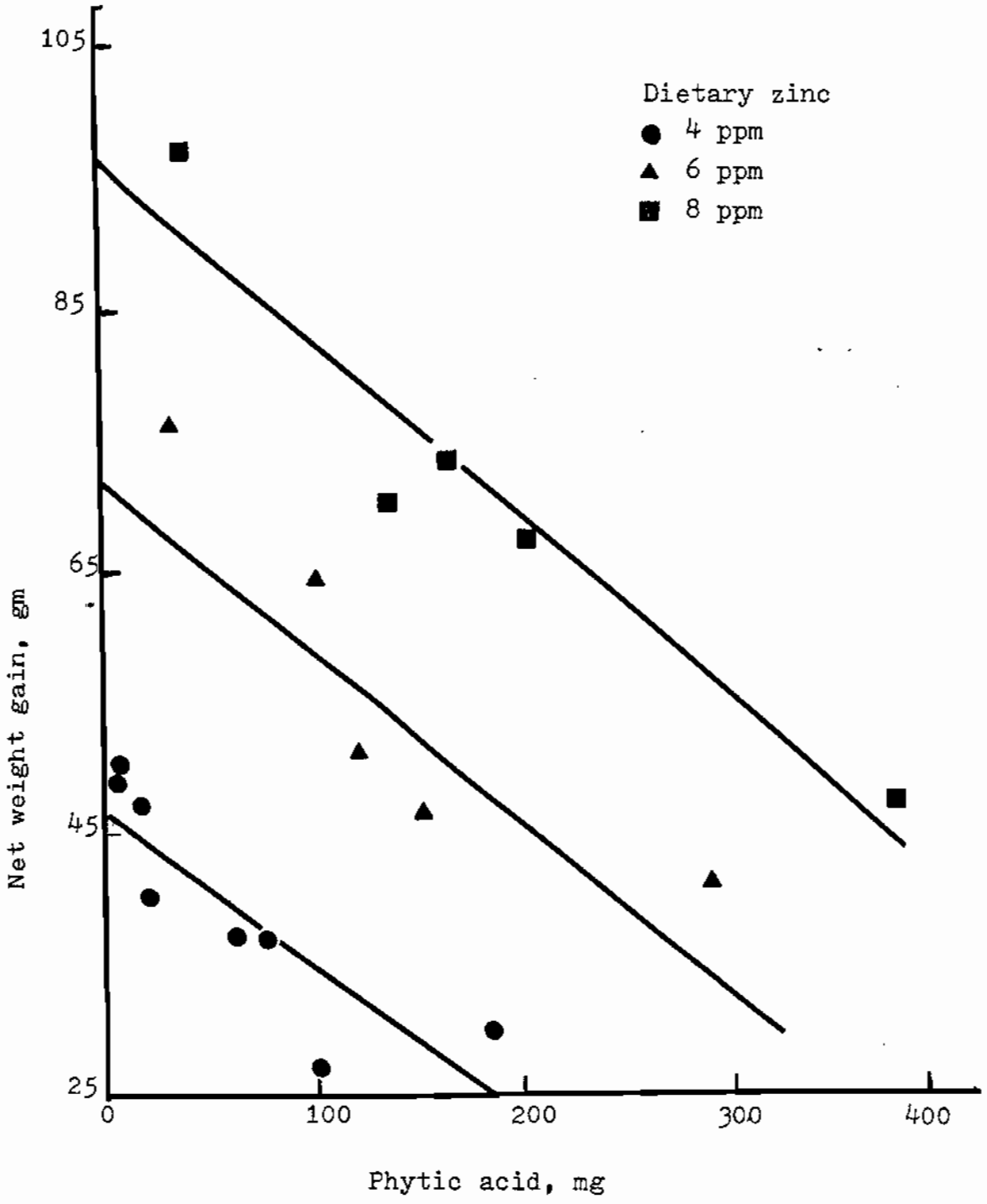


Figure 11. Graph of the relationship of net weight gain of rats with milligrams of phytic acid per 100 grams of diet in expanded study.

DISCUSSION

Wheats

Phytic Acid

Wheat phytic acid levels showed a pattern in relationship to where the wheat was grown (Table 10). Values for the cultivars grown in Nebraska were extremely high ranging from 789 mg/100 gm wheat to 1346 mg/100 gm wheat with a mean of 1072±200. Other midwestern areas were 916 mg/100 gm wheat to 1013 mg/100 gm wheat with a mean of 976±32 for Minnesota, and for Kansas 722 mg/100 gm wheat to 999 mg/100 gm wheat with a mean of 841±94. Western wheats had lower phytic acid values. Utah's were 614 mg/100 gm wheat to 997 mg/100 gm wheat with a mean of 861±133, and Montana had the lowest values with 570 mg/100 gm wheat to 848 mg/100 gm wheat with a mean of 666±110.

There was a wide range of values for the same cultivar of wheat grown in different areas. Centurk was grown in three different states, and all three phytic acid levels were different: Kansas, 777 mg/100 gm wheat, Nebraska, 963 mg/100 gm and Montana 570 mg/100 gm.

Phytic acid contributes 60-80% of the total phosphorus present in cereal grains (McCance and Widdowson, 1935). Asada et al. (1969) showed increased phosphorus in

the fertilizer caused an increase in phytic acid in rice, and this may also be the case for wheats. Nahapetian and Bassiri (1976) suggest different phytic acid levels might be related to the environmental conditions under which the wheat was grown. Phytic acid values found in the flour used for the Iranian flat bread tanok ranged from 559 mg/100 gm wheat to 883 mg/100 gm wheat depending on the season of harvest (Reinhold, 1972). These values were for a 90% extraction flour, the flours used in this study were 100% extraction. The phytic acid levels reported from the Iran were similar to those used in this study, thus suggesting a higher level of phytic acid in Iranian wheat.

Zinc

Zinc concentrations in wheat showed greater variability then did the phytic acid (Table 10). Two to two and one half fold differences were found in the wheats grown in Utah, Nebraska, and Minnesota. Wheats in Montana were uniformly low in zinc. The zinc concentrations of the cultivar, Centurk, grown in the three different states were different: Kansas, 39.9 ppm; Nebraska, 27.5 ppm; and Montana, 14.6 ppm. These differences between states and of the same cultivar may be caused by inherent zinc levels in the soil and/or added fertilizers. It appears that agricultural practices in regards to phosphate and zinc fertilizers can manipulate the phytic acid:zinc molar ratio in wheats.

Zinc Bioavailability

The relative zinc bioavailability as calculated by the three methods show a similar trend: as the phytic acid: zinc molar ratio and neutral detergent fiber increased in the diets the relative bioavailability decreased.

Phytic acid vs. Fiber

With the small sample size, using the two wheats and the control, it was not possible to distinguish between the influence of phytic acid or neutral detergent fiber on zinc bioavailability. With the expansion of the data base to include the six other wheats, the difference in influence of phytic acid and neutral detergent fiber became visible.

Results imply that phytic acid is a major factor in determining zinc bioavailability from wheat. This information that phytic acid has a greater influence on zinc availability than neutral detergent fiber is important in attempts to improve zinc availability. No techniques have been developed or are known that can decrease the fiber content of whole wheat other than by reducing the extraction level of the flour. In fact, studies have shown that cooking grains actually increases their neutral detergent fiber content (Franz et al., 1980). However, there are techniques that can decrease phytic acid levels.

Yeast fermentation is one method to achieve this. Naturally occurring phytases in yeast can reduce phytic acid levels in wheat significantly if the fermentation is

carried out long enough and with a high enough yeast content. Reinhold et al., (1974) and Franz et al., (1980) fermented wheat flours for four hours with 5% yeast[1]. These conditions are both longer and higher, respectively, than common bread making procedures used in the United States. The quantity of phytic acid destroyed by the procedures used in the United States is unknown.

Fermentation without yeast has also been shown to reduce the phytic acid content of wheat (Franz et al., 1980). This is achieved by the natural phytases that occur in wheat. Other techniques may also be developed. By further work, information can be gained on the best ways to decrease phytic acid in both commercial and home production of wheat products and improve zinc bioavailability.

---

[1]Baker's percentage.

CONCLUSIONS

The phytic acid and zinc concentrations of wheats grown in five different states varied by as much as 2 to 2½ fold. It appears that agricultural practices in regards to phosphorus and zinc can influence the phytic acid:zinc molar ratios of wheat and hence, influence zinc bioavailability.

Phytic acid was found to influence zinc bioavailability from wheat more than neutral detergent fiber. This suggests that techniques to decrease phytic acid in wheat can increase zinc bioavailability.

APPENDIX A

Procedure for Phytic Acid Determination

APPENDIX A

Procedure for Phytic Acid Determination

Reagents

1. 5% (w/v) trichloroacetic acid
2. $FeCl_3$ solution in 1.0 N HCl.
   Dissolve 2.42 gm $FeCl_3 \cdot 6H_2O$ in 1 liter of 1.0 N HCl.
3. 0.20 N HCl solution
   Dilute 8.6 ml concentrated HCl to 500 ml with deionized water.
4. 0.20 N NaOH solution
   Dissolve 3.2 gm NaOH in 200 ml deionized water. Do not keep more then one day.
5. Approximately 5 N HCl
   Dilute 100 ml concentrated HCl to 200 ml with deionized water.
6. Acetate buffer
   Dissolve 138 gm sodium acetate trihydrate and 120 ml of glacial acetic acid in deionized water. Dilute to 1000 ml with deionized water.
7. Hydroquinone solution
   Add 2.5 gm hydroquinone (photographic or reagent grade) and 1 ml of 1:1 HCl in 100 ml deionized water. A saturated solution results. Prepare fresh when any color is noticed.

8. 2,2'bipyridine solution

   Dissolve 0.100 gm of 2,2'pyridine in 4 ml of 95% ethanol and dilute to 100 ml with deionized water.

9. Iron standard

   Standards were diluted with 1.0 N HCl. Standards contained 2.0, 4.0, 10.0, 15.0, and 20.0 ug Fe/ml.

Procedure

1. Weigh out exactly 1.000 gm sample into a 50 ml pyrex centrifuge tube.
2. Add 20 ml 5% TCA to the centrifuge tube. Stopper the tube and place on shaker to be mixed 10 minutes. Centrifuge the samples 5 minutes at 2000 rpm. Decant supernatants into a 50 ml graduate cylinder. The residue is extracted a second time with a further 20 ml of 5% TCA solution under the same conditions. The supernatants are pooled and made to 50.0 ml with 5% TCA solution. The cylinder is covered and the contents mixed.
3. Transfer a 5.0 ml aliquot of the extract to a 12 ml glass centrifuge tube. Add 4.0 ml of the $FeCl_3$ solution and heat in a boiling water bath for 15 minutes. A white precipitate of ferric phytate forms. Remove the tube from the hot water bath and cool to room temperature. Centrifuge the tubes for 1 minute at 2000 rpm. Decant supernatant and discard. Wash the precipitate 3 times with 5 ml 0.2 N HCl solution. Centrifuge only

15-30 seconds. Discard supernatants. After the last washing, turn the tubes upside down and drain for 1 minute.

4. The washed precipitate is suspended in 2.0 ml of deionized water. Place tubes in boiling water bath for 2 minutes. Add 2.0 ml of 0.20 N NaOH solution to each tube. Leave the tubes in the boiling water bath for an additional 15 minutes. A red brown precipitate of ferric hydroxide forms. Remove tubes from the water bath and cool to room temperature.
5. Filter through Whatman No. 40 filter paper. Discard the filtrate. Wash the precipitate 3 times with deionized water. Each wash should be about 2-3 ml. Do not let the precipitate dry on the filter paper to prevent iron oxide formation.
6. Place 25 ml volumetric flask under the funnel. Dissolve the iron by using 5.0 ml of 5 N HCl solution. Wash the filter paper 3 times with deionized water. Bring the filtrate to volume with deionized water. Cover and mix well.
7. The reagents and sample or standards were added to a test tube in the following order.

   0.50 ml hydroquinone solution

   1.00 ml sample, standard or blank

   2.00 ml acetate buffer

   0.50 ml bipyridine solution

   Mix well and let stand 10-15 minutes.

Transfer to a 2 cm cell and read at 520 nm on a spectrophotometer.

Calculations

ug Fe/ml determined by regression

$$\frac{\text{ug Fe/ml} \times 25 \times 10}{1000} = \text{ug Fe equivalent to phytic acid in 100 gm sample.}$$

1 mmole Fe = 55.85 mg

1 mmole phytic acid = 660.08 mg

3 mmole Fe binds 1 mmole phytic acid

$$\frac{\text{ug Fe/ml} \times 25 \times 660.08}{3 \times 55.85} = \text{mg phytic acid/100 gm sample.}$$

Thus ug Fe/ml x 98.49 = mg phytic acid/100 gm sample.

APPENDIX B

Procedure for Acid Washing

APPENDIX B

Procedure for Acid Washing

The following procedure was used on all glassware used for zinc determinations.

1. Glassware was washed in Alconox detergent and rinsed 4 times in tap water.

2. Glassware was then rinsed with distilled water and air dried on racks covered with plastic sheets.

3. Dried glassware was then soaked in 6 N nitric acid bath for a minimum 12 hours.

4. The glassware was transfered to a deionized water bath to rinse off the acid.

5. The glassware then was rinsed 4 times with deionized water and left to dry as in step 2.

APPENDIX C

Modified Procedure for Neutral Detergent Fiber Determination

APPENDIX C

Modified Procedure for Neutral Detergent Fiber Determination

Reagents

1. Neutral-Detergent solution

Distilled water	1	l
Sodium lauryl sulfate	30	gm
2-ethoxyethanol (ethylene glycol monoethyl ether)	10	ml
EDTA, dihydrate crystal	18.61	gm
Sodium Borate decahydrate	6.81	gm
Disodium hydrogen phosphate anhydrous	4.56	gm

Dissolve sodium lauryl sulfate in some of the water. Add the 2-ethoxyenthanol and set the mixture aside. Add EDTA and sodium borate to a 600 ml beaker with enough of the water to dissolve it upon heating. Heat on a hot plate until dissolved. Do the same with the disodium phosphate in another beaker of water. Add the sodium borate, EDTA mixture and sodium phosphate mixture to the sodium lauryl mixture along with the rest of the water. Mix the solution carefully. Check the pH, if mixed correctly it should be 6.9 to 7.1.

2. Amylase solution

Dissolve 2 gm amylase in 100 ml distilled water. Mix

with a glass rod and filter through Whatman No. 1 filter paper. Use same day.

3. Acetone

   Use a grade free of color and residue.

Procedure

1. Use Gooch-type, high form pyrex fritted glass 30 ml capacity coarse porosity crucible for filter. Make sure they are clean and will filter well. Weigh dry crucible and keep in desiccator until ready to use.
2. Weigh out exactly 0.5000 gm of sample and place into a 600 ml Berzelius beaker which has no spout.
3. Add 50 ml of neutral-detergent solution. Place on a heater and cover with a 500 ml Erlenmyer flask with cold water in it to act as a condenser.
4. Let the sample come to a boil. At all times make sure the condenser has cool water in it.
5. Thirty minutes after onset of boiling, remove the beaker from the heat and add 50 ml of the neutral detergent solution and 2 ml of the enzyme solution. Return the beaker to the heater. Cover with a cool flask and let come back to a boil without adjusting the thermostat setting.
6. While the sample is boiling set up the filter on the suction apparatus. Turn on suction and form seal using distilled water.
7. Sixty minutes after onset of boiling filter the hot solution through the crucible. Be careful to transfer

all the solution without losing any of the fiber. Rinse with hot distilled water. With good filtering the solution is pulled through quickly.

8. Rinse crucible with near boiling water twice to remove solution. Then break suction and add 2 ml enzyme solution and hot water (70°C) to rinse. Let stand 10-15 minutes.
9. Turn on suction again to pull solution through. Rinse twice with near boiling water.
10. Rinse twice with acetone. Let suction continue until sample is dry.
11. Dry crucible overnight in a 100°C oven. Then remove from the oven. Cool to room temperature in desiccator and weigh crucible. Calculate percentage fiber.

Calculation

$$\frac{(\text{crucible weight} + \text{fiber}) - \text{crucible weight}}{\text{sample weight} \times \% \text{ dry weight}} \times 100 = \% \text{ neutral detergent fiber}$$

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THE BIOAVAILABILITY OF ZINC IN WHOLE WHEAT

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ABSTRACT

The influence of dietary fiber and phytic acid on the bioavailability of zinc to the rat was determined by comparing the neutral detergent fiber and phytic acid content of wheat to three week weight gain of rats. The amount of added wheat, phytic acid and neutral detergent fiber content of the diets was calculated for 100 gm of diet. Correlations of the wheat added, phytic acid or neutral detergent fiber with weight gain showed phytic acid was significantly negatively correlated with weight gain. There was a trend for neutral detergent fiber to have some influence on weight gain when added zinc in the diet was less than 4 ppm. Results showed that wheat phytic acid has a greater influence on zinc bioavailability in rats than dietary fiber. It was also found that phytic acid and zinc levels in whole wheat appear to be influenced by agricultural practices.

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