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Effects of dietary phytase on body weight gain, body composition and bone strength in growing rats fed a low-zinc diet☆

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Abstract

Phytic acid, a major phosphorous storage compound found in foodstuffs, is known to form insoluble complexes with nutritionally essential minerals, including zinc (Zn). Phytases are enzymes that catalyze the removal of these minerals from phytic acid, improving their bioavailability. The objective of the present study was to determine the ability of dietary phytase to affect body weight, body composition, and bone strength in growing rats fed a high phytic acid, low Zn diet. Rats (n = 20) were fed either a control (AIN-93) or phytase supplemented (Natuphos, BASF, 1,500 phytase units (FTU)/kg) diet for a period of 8 weeks. Phytase supplementation resulted in increased ($P < .05$) bone and plasma Zn, but no change in plasma inorganic phosphorous or bone levels of Ca, Fe, or Mg. The addition of phytase to the diets resulted in a 22.4% increase ($P < .05$) in body weight at the end of the study as compared with rats fed a control diet. Dual x-ray absorptiometry (DXA) revealed that phytase supplementation resulted in increase lean body mass (LBM, $P < .001$) and increased bone mineral content (BMC, $P < .001$) as compared with feeding the control diet. Bone studies indicated that femurs and tibias from phytase supplemented rats had greater mass ($P < .05$) and were stronger ($P < .05$) than rats fed the control diet. This data suggest that the addition of phytase to low Zn diets results in improved Zn status, which may be responsible for beneficial effects on growth, body composition, and bone strength.

Keywords: Phytase; Zinc; Dual X-ray absorptiometry; Bone strength; Rats

1. Introduction

Phytic acid (PA, *myo*-inositol hexakisphosphate) is a major phosphorous (P) storage compound found in cereals, legumes and seeds [1]. The structure of PA includes six ionizable protons that allow for the formation of insoluble complexes with multivalent cations, including calcium (Ca), zinc (Zn), copper (Cu), magnesium (Mg) and iron (Fe) [2,3]. The ability of PA to form insoluble complexes with these nutritionally essential minerals results in diminished bioavailability. In particular, Zn bioavailability is diminished by PA [4–6], and numerous reports have demonstrated reduced Zn absorption and status in animals consuming feed with high levels of PA [7–9]. Furthermore, high-PA diets have been associated with reduced Zn status in humans [10,11]. The implications of diminished Zn status due to poor dietary bioavailability are severe, as Zn is an essential trace element known to incorporate into over 300 proteins, including those responsible for DNA and RNA replication, protein synthesis and gene transcription [12].

Phytases are phosphohydrolases that catalyze the removal of P and other multivalent cations from PA [13]. Many mammals, including rats, pigs and humans, have endogenous phytase activity within the small intestine,

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although this phytase seems to impart a minimal role in PA hydrolysis [3]. Although endogenous phytase activity is greater in rats than in humans, the increased PA digestibility observed in rats fed P-deficient diets does not seem to be due to endogenous intestinal phytase activity [14]. The addition of supplemental phytase to the diet is effective in improving PA digestibility, as recent studies have demonstrated the ability of dietary phytase to improve micronutrient status, body weight and bone strength in animals [15–18]. Zinc status and growth are affected by dietary phytase, as addition of microbial phytases to PA-rich soy-based diets has been shown to enhance Zn utilization and increase body weight gain in growing rats [19,20].

The effects of dietary phytase on body composition and bone health in animals fed a low-Zn diet have not been investigated. As important roles of Zn nutrition in growth [21,22] and bone health [23,24] have been described, we hypothesized that increasing Zn bioavailability by providing dietary phytase to growing rats fed a low-Zn diet would affect body composition and bone strength. Interestingly, we found that the addition of phytase to low-Zn diets improved Zn status without affecting other essential trace elements, and resulted in increased lean body mass (LBM), increased bone mineral content (BMC) and greater bone strength.

2. Methods and materials

2.1. Animals and diets

Male Sprague–Dawley rats ($n=10$ per dietary treatment group) were housed individually in polycarbonate cages in a constant temperature (25°C) animal room with a 12-h light/dark cycle and given free access to feed and distilled water. All experiments were approved by the Institutional Animal Care and Use Committee at the United States Army Research Institute of Environmental Medicine. In conducting the research described in this report, the investigators adhered to the *Guide for Care and Use of Laboratory Animals* as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. Diets were prepared by Research Diets (Brunswick, NJ) and were based upon the AIN-93 laboratory rodent formulation [25], with no Zn added to the mineral mix. Soy protein was the major protein source, and PA (Na-phytate, Sigma-Aldrich, St. Louis, MO) was added as 0.3% of the complete diet. Rats were fed either control or phytase-supplemented (Natuphos; BASF, Mt. Olive, NJ) diets ad libitum for a period of 8 weeks. Phytase was added to the diet to achieve a final activity of 1500 phytase units (FTU)/kg. We chose these levels of PA and phytase based upon data from previous studies that demonstrated improvements in Zn status in rats consuming similar diets [19,20]. The diets provided approximately 6.04 mg/kg Zn, as determined by flame atomic absorption spectroscopy (AAS), and did not differ between dietary treatment groups. The components of the diets appear in Table 1.

Body weight and feed consumption were measured (PM 30; Mettler Instruments, Hightstown, NJ) at regular intervals throughout the study. Following the 8-week feeding period, rats were euthanized under carbon dioxide. Blood was extracted from the right ventricle into heparinized tubes, and the plasma was stored at −20°C for biochemical assays. Both legs were dissected away from the hip joint, carefully cleaned of adherent tissue and wrapped with gauze soaked in 0.9% saline, and bones were stored at 4°C prior to mineral analysis and biomechanical testing.

2.2. Micronutrient status

Zinc status was assessed in plasma using flame AAS (Perkin Elmer 2380, Norwalk, CT). Plasma samples were diluted eightfold with 5% nitric acid (trace metal grade, Fisher Scientific, Pittsburgh, PA). Zinc standards, prepared from a reference solution (Fisher Scientific) in 5% nitric acid, were used as an internal control. All analysis was conducted in acid-washed glassware. Recovery tests were performed to confirm the accuracy of abovementioned method, and the recovery of Zn was 106±1.1% ($n=5$).

Plasma inorganic P (PiP) concentrations were used as an indicator of P status and were determined according to the method of Gomori [26]. All chemicals were purchased from

Table 1
Components of rat diets

Diet ingredient	Control (g/kg)	Phytase (g/kg)
Cornstarch	495.7	495.7
Maltodextrin 10	125.0	125.0
Sucrose	100.0	100.0
Soy protein	140.0	140.0
DL-Methionine	1.8	1.8
Soybean oil	40.0	40.0
Cellulose	50.0	50.0
Vitamin mix[a]	10.0	10.0
Mineral mix[b]	35.0	35.0
PA[c]	3.0	3.0
Phytase[d]	–	0.15

[a] Prepared according to the AIN-93 formulation [25].
[b] Prepared according to the AIN-93 formulation [25] with no Zn added; components include (g or mg/kg mix) calcium carbonate anhydrous (40.0% Ca), 357 g; potassium phosphate monobasic (22.8% P, 28.7% K), 250 g; potassium citrate (36.2% K), 28 g; sodium chloride (39.3% Na, 60.7% Cl), 74 g; potassium sulfate (44.9% K, 18.4% S), 46.6 g; magnesium oxide (60.3% Mg), 24 g; ferric citrate (16.5% Fe), 6.06 g; sodium *meta*-silicate · $9H_2O$ (9.9% Si), 1.45 g; manganous carbonate (47.8% Mn), 0.63 g; cupric carbonate (57.5% Cu), 0.30 g; chromium potassium sulfate · $12H_2O$ (10.4% Cr), 0.275 g; boric acid (17.5% B), 81.5 mg; sodium fluoride (45.2% F), 63.5 mg; nickel carbonate (45% Ni), 31.8 mg; lithium chloride (16.4% Li), 17.4 mg; sodium selenate anhydrous (41.8% Se), 10.25 mg; potassium iodate (59.3% I), 10.0 mg; ammonium paramolybdate · $4H_2O$ (54.3% Mo), 7.95 mg; ammonium vanadate (43.6% V), 6.6 mg.
[c] Phytic acid added as Na-phytate.
[d] BASF, Natuphos; 10,000 U/g.

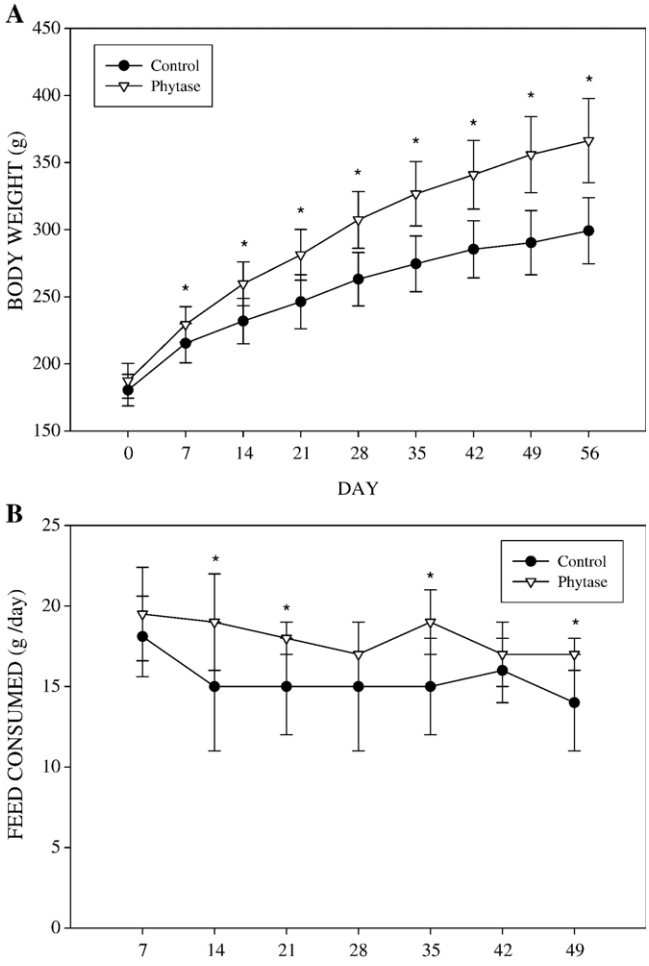

Fig. 1. Body weight gain (A) and feed intake (B) in rats fed control and phytase-supplemented diets for a period of 8 weeks. Asterisks (*) indicate significant differences ($P<.05$) between dietary treatment groups. Values are means ($n=10$ per group)±S.D.

Sigma-Aldrich. Briefly, samples were deproteinated with 12.5% trichloroacetic acid and assayed using Elon (*p*-methylaminophenol sulfate). Samples were then analyzed using a Bio-Tek PowerWave HT microplate scanning spectrophotometer (Bio-Tek, Winooski, VT).

Because dietary phytase is known to affect the absorption of many divalent cations, concentrations of Zn, Fe, Mg and Ca were determined in bone. Bones underwent digestion in concentrated nitric acid prior to mineral analysis using inductively coupled plasma — optical emission spectrometry (ICP-OES; Western Analysis, Salt Lake City, UT).

Table 2
Effect of phytase supplementation on mineral status following the 8-week feeding period

	Control	Phytase	*P* value
Plasma			
PiP (mg/dl)	8.02 (±0.68)	8.07 (±0.53)	.865
Zn (μg/ml)	0.54 (±0.13)	0.72 (±0.14)	.006
Bone			
Ca (mg/g bone)	148.3 (±7.1)	143.82 (±7.8)	.193
Mg (mg/g bone)	2.66 (±0.16)	2.75 (±0.19)	.319
Fe (μg/g bone)	81.6 (±36.6)	64.6 (±32.8)	.290
Zn (μg/g bone)	65.4 (±7.9)	75.0 (±4.9)	.004

2.3. Dual energy X-ray absorptiometry

Immediately prior to and following the 8-week feeding period, body composition and BMC were assessed using dual energy X-ray absorptiometry (DXA) analysis. Briefly, rats received an anesthetic by intraperitoneal injection of a 1-ml/kg mixture of 40 mg/ml ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA), 10 mg/ml xylazine (Xyla-Ject; Phoenix Scientific, St. Joseph, MO) and 1.5 mg/ml acepromazine (Boehringer Ingelheim, St. Joseph, MO). Once sedation was confirmed, rats were transferred to a Prodigy fan beam densitometer (GE Lunar, Madison, WI) with a small animal, high-resolution scan module. Small animal software (enCore Version 7.53.002, 2003; GE Lunar) was utilized for the determination of LBM, fat mass (FM) and BMC. All animals were scanned on the same day by the same operator.

2.4. Bone strength

Femur and tibia bones were kept on ice until determination of breaking strength using a 5-kN Flexure Fixture, configured for three-point bend tests and attached to an Instron Universal Testing Machine Model 4502 equipped with a 10-kN load cell (Instron, Canton, MA). The crosshead speed was 50 mm/min, and the data sampling rate was 10 samples/s. Maximum tolerated force and stress at maximum load were determined in femurs and tibias using Series IX, v 8.08.00 software (Instron).

2.5. Statistical analysis

Statistical analysis was performed using commercially available statistical software (SPSS 12.0; SPSS, Chicago, IL). Descriptive statistics are presented as means±S.D. For body weight data, differences were assessed using analysis

Table 3
Effect of phytase supplementation on body composition as assessed using DXA analysis

	Control	Phytase	*P* value
Body weight			
Week 0 (g)	180.4 (±11.8)	187.4 (±12.9)	.226
Week 8 (g)	299.2 (±24.7)	366.0 (±31.4)	<.001
LBM			
Week 0 (g)	164.5 (±11.7)	162.9 (±9.6)	.743
Week 8 (g)	248.9 (±21.0)	310.0 (±9.9)	<.001
FM			
Week 0 (g)	9.00 (±4.71)	11.9 (±5.08)	.203
Week 8 (g)	29.0 (±10.27)	29.9 (±12.74)	.864
BMC			
Week 0 (g)	3.25 (±0.29)	3.40 (±0.29)	.267
Week 8 (g)	7.95 (±0.65)	9.43 (±0.88)	<.001

Table 4
Effects of supplemental phytase on bone mass and length following the 8-week feeding period

	Control	Phytase
Femur		
Mass (g)	1.05 (±0.12)	1.20 (±0.13)*
Length (mm)	35.6 (±1.38)	36.5 (±3.55)
Tibia		
Mass (g)	0.87 (±0.09)	1.02 (±0.11)*
Length (mm)	40.1 (±1.61)	42.3 (±1.06)*

Asterisks (*) indicate significant ($P<.05$) differences as compared to the control group.

of variance with repeated measures. All other comparisons were made using the Student t test. A minimum P value of .05 was the necessary condition for statistical significance.

3. Results

3.1. Body weight and feed intake

Rats fed diets supplemented with phytase were heavier ($P<.05$) at the end of the 8-week feeding period as compared to rats fed the control diet (Fig. 1A). Final body weights were 22.4% greater in phytase-supplemented rats as compared to rats fed the control diet. The differences in body weight became significant ($P<.05$) by day 7 of the feeding period and remained so throughout the remainder of the study. Rats fed the control diet ate less than rats fed the phytase-supplemented diet at various time points during the study (Fig. 1B). This effect was significant ($P<.05$) on days 14, 21, 35 and 49.

3.2. Micronutrient status

Plasma and femur were used for the determination of Zn status at the end of the 8-week feeding period (Table 2). Although plasma Zn is commonly used as an indicator of Zn status, bone Zn has been utilized as a more reliable index of Zn status in animal studies [27]. Zinc status was improved by dietary phytase, as both plasma and femur Zn were significantly greater (33.3% and 14.7%, respectively; $P<.05$) in the phytase-supplemented rats as compared to rats fed control diets. Concentrations of other minerals known to be affected by dietary phytase were investigated as well. Plasma inorganic P, an indicator of P status, was not different between the two dietary treatment groups (Table 2). Furthermore, the concentrations of Ca, Mg and Fe were not different between dietary treatment groups in bone, as determined using ICP-OES analysis.

3.3. Body composition

To assess diet-induced changes in body composition, DXA analysis was performed immediately prior to the 8-week feeding period and immediately prior to euthanization. Dietary phytase had a positive effect on LBM, as LBM was 24.5% greater ($P<.001$) in the phytase-supplemented rats as compared to rats fed the control diet at the end of the 8-week feeding period (Table 3). Dietary phytase had little effect on FM, as there was no difference between the dietary treatment groups at the end of the feeding period. Bone mineral content was increased (18.6%, $P<.001$) in the phytase-supplemented rats as compared to rats fed the control diet.

3.4. Bone mass, length and strength

Phytase-supplemented rats had greater ($P<.05$) femur and tibia mass than rats fed the control diet, although femur length was not significantly different between treatment groups (Table 4). The femurs of phytase-supplemented rats were stronger than those from control-fed rats, as they tolerated more force (Fig. 2A, $P<.05$) and greater stress (Fig. 2B, $P<.05$). Additionally, the tibias of phytase-supplemented rats were longer ($P<.05$) than those of rats fed the control diet (Table 4), and the maximum tolerated force was higher (Fig. 2A, $P<.05$) in tibias from these animals as compared to controls.

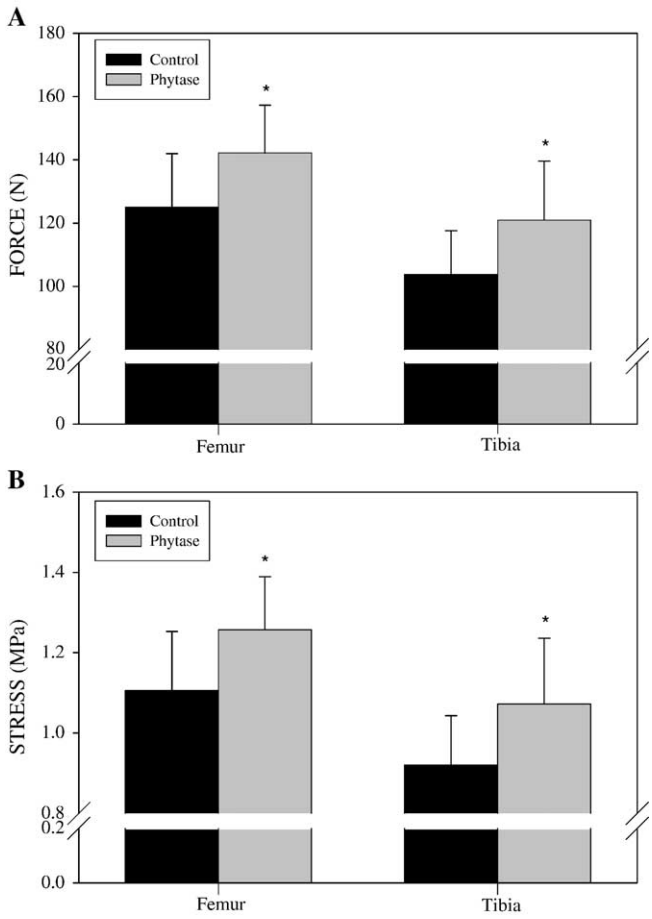

Fig. 2. Effect of dietary phytase supplementation on maximum tolerated force (A) and stress at maximal load (B) in rat femurs and tibias. Bone biomechanics were assessed using the Instron Universal Testing Machine Model 4502 equipped with a 10-kN load cell (Instron). Asterisks (*) indicate significant differences ($P<.05$) between dietary treatment groups. Values are means ($n=10$ per group)±S.D.

4. Discussion

The goal of the present study was to assess the ability of dietary phytase to improve Zn status, body weight gain, body composition and bone strength in growing rats fed a low-Zn diet. Inclusion of phytase to the diets of these rats significantly improved Zn status as demonstrated by an increase in both plasma and femur Zn concentrations, but caused no change in the status of other minerals, as PiP and bone Ca and Mg were not different between dietary treatment groups. Collectively, our data indicate that supplementing growing animals fed a low-Zn diet with phytase can produce anabolic effects, as demonstrated by gains in LBM, and these anabolic effects are accompanied by functional improvements as manifested by increases in bone strength.

Taken together, the improved Zn status and lack of differences in P, Ca and Mg status between dietary treatment groups suggest that the positive effects of phytase supplementation are the result of increased Zn nutriture. Increased body weight was seen with this improvement in Zn status, as phytase-supplemented rats gained significantly more weight than the control rats over the length of the study. Although phytase can hydrolyze phytate to its lower inositol phosphates, which could effect growth, we believe that the improvement in Zn status was mainly responsible for increased body weight in the phytase-supplemented animals. Wyss et al. [28] demonstrated that even under optimal hydrolysis conditions in vitro, *Aspergillus niger* phytase was unable to reduce PA past di- and triphosphate *myo*-inositol. In our animal model, we would expect the hydrolysis to be even less efficient, thereby producing fewer lower inositol phosphates. Although the growth-stimulating effect of supplemental phytase is similar to that reported by Rimbach and Pallauf [19], ours is the first work, to our knowledge, to investigate the effects of dietary phytase on body composition in growing rats using DXA analysis. Our findings indicate that supplemental phytase had a positive effect on body composition, as LBM was increased and there was no change in FM over the 8-week period.

Dietary Zn deficiency has long been associated with deficiencies in growth and development [29]. Although severe Zn deficiencies that are associated with growth impairments are most often reported in developing nations, poor growth due to mild Zn deficiencies have also been reported in developed nations, including the United States [30]. The results of the present study suggest that adding phytase to a low-Zn diet improves Zn status and enhances growth, particularly due to the accretion of lean tissue. Supplemental phytase tended to result in increased LBM as animals grew over the 8-week feeding period. This finding is reminiscent of human studies, in which supplemental Zn was shown to result in improved growth and greater *Z* scores for LBM in children [31], and tracer studies that demonstrate the positive association between total body Zn and LBM [32].

Feeding animals supplemental phytase seemed to limit the accretion of FM over the 8-week feeding period, as FM was similar between dietary treatment groups at the end of the study, regardless of the significantly larger body size of the phytase-supplemented animals. Although the mechanism remains unclear, reduced Zn status has been associated with increased FM in animals [33] and obesity in humans [34,35]. One possible mechanism explaining the association of reduced Zn status and increased FM is the important role of Zn in maintaining insulin sensitivity. Insulin resistance, a primary characteristic of type II diabetes, is associated with obesity and an overall inability of insulin to stimulate glucose uptake and metabolism at the tissue level. Zinc imparts a critical role in insulin metabolism, as Zn concentration in pancreatic islet cells is related to the synthesis, storage and secretion of insulin [36], and Zn confers a greater binding affinity of insulin to cell membranes and reduces insulin degradation [37]. In fact, Zn-deficient rats are resistant to exogenous insulin injections [38], and dietary Zn supplementation attenuates hyperglycemia in db/db mice, which are known to develop hyperglycemia and obesity over time [39]. The role of Zn status in the maintenance of hormones regulating feed intake may also affect the accretion of fat in Zn-deficient animals. Zinc status is known to affect the expression of neuropeptide Y (NPY), a hormone that has a profound impact on feeding behavior [40]. Changes in NPY levels in Zn-deficient rats may be responsible for the increased preference for fat in these animals [41], a factor that could contribute to long-term changes in body composition.

The addition of supplemental phytase to the diets had a major impact on bone mass, length and strength. The improved bioavailability of dietary Zn led to higher levels of Zn in both the plasma and bones of phytase-supplemented rats, whereas there was no diet-induced difference in the accumulation of Ca, Mg or Fe in bone. The increase in BMC, as determined by DXA analysis, seen with dietary phytase supplementation is similar to the increase in BMC in rats fed elevated levels of dietary Zn [42]. In the present study, femurs and tibia of the phytase-supplemented rats were significantly stronger than those of the control rats. Other researchers have identified differences in bone strength related to other trace mineral deficiencies. Medeiros et al. [43] showed that femurs from rats fed Fe- or Cu-deficient diets required less force to fracture. In our study, the improvement in bone strength was coupled with an increase in the length and rigidity in tibias from rats fed supplemental phytase. A similar increase in longitudinal bone growth with increased dietary Zn was seen by Seco et al. [42]. Although the role of trace minerals in bone turnover and metabolism will require further study, our results provide data suggesting that improving the bioavailability of Zn could have profound effects on bone integrity. We

hypothesize that the improvements in bone biomechanics observed with supplemental phytase may be due to increased expression of Zn-dependent enzymes found in bone, including alkaline phosphatase and collagenase.

One of the earliest manifestations of Zn deficiency in animals is reduced feed intake [33]. In the present study, rats fed the control diet consumed less feed than rats fed the phytase-supplemented diet at various time points. Because the use of pair feeding is known to cause changes in NPY and norepinephrine, which affect bone metabolism in animals [44], we chose to give rats ad libitum access to the experimental diets. Although ad libitum feeding makes it difficult to distinguish whether the accretion of LBM or improvements in bone strength are due to improved Zn status or increased energy intake, several studies have addressed this issue and demonstrated that Zn deficiency has a greater, and different, impact on protein metabolism and bone turnover than caloric restriction [45,46]. For example, Hosea et al. [46] clearly demonstrated that although Zn deficiency and diet restriction in a pair-fed group both resulted in osteopenia, Zn deficiency had a more severe impact. Additionally, these changes in bone integrity occurred through different mechanisms, with Zn deficiency limiting bone formation, whereas caloric restriction increased bone resorption. Changes in body weight and body composition have also been clearly attributed to improvements in Zn status through the use of pair-feeding experiments, as White [33] demonstrated improvements in the efficiency of feed conversion and FM in pair-fed Zn-adequate rats.

In conclusion, the major findings of this study indicate that supplementing rat diets with phytase results in improved Zn status, increased body weight, improved body composition and stronger bones. Furthermore, dietary phytase did not affect P status or the accumulation of other divalent cations in bone. These results demonstrate the importance of Zn bioavailability in the diet and underscore the role of Zn status in maintaining human and animal health. Although the effects of supplemental phytase on Zn absorption and status have not been tested in humans, the addition of phytase to the diets of human volunteers has been shown to significantly increase Fe absorption [47,48]. As such, the use of supplemental phytase at doses similar to those utilized to improve Zn nutriture in the present study and others (19–20, 1000–2000 FTU/kg) could be an alternative to the addition of supplemental Zn to the diet of individuals that may experience moderate Zn deficiency.

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References

[1] Harland BF, Oberleas D. Phytate in foods. Wld Rev Nutr Diet 1987;52:235–59.
[2] Torre M, Rodriguez AR, Saura-Calixto F. Effects of dietary fiber and phytic acid on mineral availability. Crit Rev Food Sci Nutr 1991;30:1–22.
[3] Zhou JR, Erdman Jr JW. Phytic acid in health and disease. Crit Rev Food Sci Nutr 1995;35:495–508.
[4] Oberleas D, Harland BF. Phytate content of foods: effect on dietary zinc bioavailability. J Am Diet Assoc 1981;79:433–6.
[5] Wise A. Phytate and zinc bioavailability. Int J Food Sci Nutr 1995;46:53–63.
[6] Lonnerdal B. Dietary factors influencing zinc absorption. J Nutr 2000;130:1378S–83S.
[7] Oberleas D, Muhrer ME, O'Dell BL. Effects of phytic acid on zinc availability and parakeratosis in swine. J Anim Sci 1962;21:57–61.
[8] Oberleas D, Muhrer ME, O'Dell BL. Dietary metal-complexing agents and zinc availability in the rat. J Nutr 1966;90:56–62.
[9] Welch RM, House WA, Ortiz-Monasterio I, Cheng Z. Potential for improving bioavailable zinc in wheat grain (*Triticum* species) through plant breeding. J Agric Food Chem 2005;53:2176–80.
[10] Turnlund JR, King JC, Keyes WR, Gong B, Michel MC. A stable isotope study of zinc absorption in young men: effects of phytate and α-cellulose. Am J Clin Nutr 1984;40:1071–7.
[11] Manary MJ, Hotz C, Krebs NF, Gibson RS, Westcott JE, Broadhead RL, et al. Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. Am J Clin Nutr 2002;75:1057–61.
[12] Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Phys Rev 1993;73:79–118.
[13] Lei XG, Porres JM. Phytase enzymology, applications, and biotechnology. Biotechnol Lett 2003;25:1787–94.
[14] Moore RJ, Veum TL. Adaptive increase in phytate digestibility by phosphorous-deprived rats and the relationship of intestinal phytase (EC 3.1.3.8) and alkaline phosphatase (EC 3.1.3.1) to phytate utilization. Br J Nutr 1983;49:145–52.
[15] Pallauf J, Pippig S, Most E, Rimbach G. Supplemental sodium phytate and microbial phytase influence iron availability in growing rats. J Trace Elem Med Biol 1999;13:134–40.
[16] Stahl CH, Han YM, Roneker KR, House WA, Lei XG. Phytase improves iron bioavailability for hemoglobin synthesis in young pigs. J Anim Sci 1999;77:2135–42.
[17] Gentile JM, Roneker KR, Crowe SE, Pond WG, Lei XG. Effectiveness of an experimental consensus phytase in improving dietary phytate-phosphorous utilization by weanling pigs. J Anim Sci 2003;81:2751–7.
[18] Walz OP, Pallauf J. The effect of the combination of microbial phytase and amino acid supplementation of diets for finishing pigs on P and N excretion and carcass quality. Arch Anim Nutr 2003;57:413–28.
[19] Rimbach G, Pallauf J. Enhancement of zinc utilization from phytate-rich soy protein isolate by microbial phytase. Z Ernahrungswiss 1993;32:308–15.
[20] Rimbach G, Brandt K, Most E, Pallauf J. Supplemental phytic acid and microbial phytase change zinc bioavailability and cadmium accumulation in growing rats. J Trace Elem Med Biol 1995;9:117–22.
[21] Nishi Y. Zinc and growth. J Am Coll Nutr 1996;15:340–4.
[22] MacDonald RS. The role of zinc in growth and cell proliferation. J Nutr 2000;130:1500S–8S.
[23] Ovesen J, Moller-Madsen B, Thomsen JS, Danscher G, Mosekilde L. The positive effects of zinc on skeletal strength in growing rats. Bone 2001;29:565–70.
[24] Rossi L, Migliaccio S, Corsi A, Marzia M, Bianco P, Teti A, et al. Reduced growth and skeletal changes in zinc-deficient growing rats are due to impaired growth plate activity and inanition. J Nutr 2001;131:1142–6.

[25] Reeves PG, Nielson FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[26] Gomori G. A modification of the colorimetric phosphorous determination for use with the photoelectric colorimeter. J Lab Clin Med 1942;27:955–60.
[27] Bobilya DJ, Johanning GL, Veum TL, O'Dell BL. Chronological loss of bone zinc during dietary zinc deprivation in neonatal pigs. Am J Clin Nutr 1994;59:649–53.
[28] Wyss M, Brugger R, Kronenberger A, Remy R, Fimbel R, Oesterhelt G, et al. Biochemical characterization of fungal phytases (*myo*-inositol hexakisphosphate phosphohydrolases): catalytic properties. Appl Environ Microbiol 1999;65:367–73.
[29] Prasad AS. Zinc in growth and development and spectrum of human zinc deficiency. J Am Coll Nutr 1988;7:377–84.
[30] Hambidge KM, Hambidge C, Jacobs M, Baum JD. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatr Res 1972;6:868–74.
[31] Friis H, Ndhlovu P, Mduluza T, Kaondera K, Sandstrom B, Michaelsen KF, et al. The impact of zinc supplementation on body growth and body composition: a randomized, controlled trial among rural Zimbabwean schoolchildren. Eur J Clin Nutr 1997;51:38–45.
[32] Watson WS, Mitchell KG, Lyon TD, Kerr N. A two-compartmental model for zinc in humans. J Trace Elem Med Biol 1999;13:141–9.
[33] White CL. The effect of zinc deficiency on the body composition of rats. Biol Trace Elem Res 1988;17:175–87.
[34] Chen MD, Lin PY, Sheu WH. Zinc status in plasma of obese individuals during glucose administration. Biol Trace Elem Res 1997;60:123–9.
[35] Marreiro Ddo N, Fisberg M, Cozzolino SMF. Zinc nutritional status in obese children and adolescents. Biol Trace Elem Res 2002;86:107–22.
[36] Zalewski P, Millard S, Forbes I, Kapaniris O, Slavotinek S, Betts W, et al. Video image analysis of labile Zn in viable pancreatic islet cells using specific fluorescent probe for Zn. J Histochem Cytochem 1994;42:877–84.
[37] Arquilla ER, Packer S, Tarmas W, Miyamoto S. The effect of zinc on insulin metabolism. Endocrinology 1978;103:1330–449.
[38] Quarterman J, Mills CF, Humphries WR. The reduced secretion of and sensitivity to insulin in zinc-deficient rats. Biochem Biophys Res Commun 1966;25:354–8.
[39] Simon SF, Taylor CG. Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med 2001;226:43–51.
[40] Shay NF, Mangian HF. Neurobiology of zinc-influenced eating behavior. J Nutr 2000;130:1493S–9S.
[41] Kennedy KJ, Rains TM, Shay NF. Zinc-deficiency changes preferred macronutrient intake in subpopulations of Sprague–Dawley outbred rats and reduces hepatic pyruvate kinase gene expression. J Nutr 1998;128:43–9.
[42] Seco C, Revilla M, Hernandez ER, Gervas J, Gonzalez-Riola J, Villa LF, et al. Effects of zinc supplementation on vertebral and femoral bone mass in rats on strenuous treadmill training exercise. J Bone Miner Res 1998;13:508–12.
[43] Medeiros DM, Ilich J, Ireton J, Matkovic V, Shiry L, Wildman R. Femurs from rats fed diets deficient in copper or iron have decreased mechanical strength and altered mineral composition. J Trace Elem Exp Med 1997;10:197–203.
[44] Huntington CE, Shay NF, Grouzmann E, Arseneau LM, Beverly JL. Zinc status affects neurotransmitter activity in the paraventricular nucleus of rats. J Nutr 2002;132:270–5.
[45] Fernandez-Madrid F, Prasad AS, Oberleas D. Effect of zinc deficiency on nucleic acids, collagen, and noncollagenous protein of the connective tissue. J Lab Clin Med 1973;82:951–61.
[46] Hosea HJ, Taylor CG, Wood T, Mollard R, Weiler HA. Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. Exp Biol Med 2004;229:303–11.
[47] Sandberg AS, Hulthen LR, Turk M. Dietary *Aspergillus niger* phytase increases iron absorption in humans. J Nutr 1996;126: 476–80.
[48] Hurrell RF, Reddy MB, Juillerat MA, Cook JD. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nutr 2003;77:1213–9.