Published in final edited form as:
J Nutr Biochem. 2010 February ; 21(2): 147–152. doi:10.1016/j.jnutbio.2008.12.005.

Yogurt protects against growth retardation in weanling rats fed diets high in phytic acid

Lisa M. Gaetke, PhD, RD, **Craig J. McClain, MD**[1], **C. Jean Toleman, MS, RD, LDN**[2], and **Mary A. Stuart, PhD, RD**[3]

Mary A. Stuart: mary_a_stuart@sjhlex.org

Department of Nutrition and Food Science, University of Kentucky

[1] University of Louisville, Department of Internal Medicine, Vice-President of Research and Professor, Departments of Medicine, Pharmacology and Toxicology, University of Louisville and Louisville Veterans Administration Medical Center, Louisville, Kentucky 40292

[2] Consultant, 3511 Cemetery Circle, Knoxville, Maryland 21758

[3] Appalachian Outreach Program, Saint Joseph Healthcare, One St. Joseph Drive, Lexington, Kentucky 40504, Phone: (859) 313-2065, Fax: (859) 313-3145

Abstract

The purpose of this study was to determine the affects of adding yogurt to animal diets which were high in phytic acid (PA) and adequate in zinc (38 µg Zn/g). The PA:Zn molar ratio was 60:1. Zinc status was determined by documenting growth and measuring the zinc concentration in bone (tibia) and plasma. For 25 days, 6 groups (n=6) of Sprague-Dawley weanling rats were fed one of the following AIN-76 diets. Half of the diets contained PA. Four of the diets contained yogurt with either active or heat-treated (inactive) cultures added at 25% of the diet. Diets: (without PA) 1) AIN, 2) AIN with active yogurt, 3) AIN and inactive yogurt; and (with PA) 4) AIN with PA, 5) AIN with PA plus active yogurt, and 6) AIN with PA plus inactive yogurt. Body weight, weight gain, and zinc concentration in bone and plasma were measured, and feed efficiency ratio (FER) was calculated. Rats fed diets with PA and yogurt had normal growth compared to the control group. Growth retardation was evident in the group fed the diet with PA and no yogurt. This group had significantly lower body weight compared to all other groups ($p<0.05$). Rats fed diets with PA, with or without yogurt, had significantly lower zinc concentration in bone and plasma ($p<0.05$). Adding yogurt to diets high in PA resulted in normal growth in weanling rats, however, zinc concentration in bone and plasma was still sub-optimal.

Keywords

zinc; yogurt; phytic acid; zinc status; zinc absorption

Corresponding Author: Lisa Gaetke, PhD, RD, Department of Nutrition and Food Science, University of Kentucky, 119 Funkhouser Building, Lexington, Kentucky 40506-0054, Phone: (859) 257-1031, FAX: (859) 257-3707, lgaetke@email.uky.edu.
Reprint requests: Same as corresponding author

INTRODUCTION

Zinc deficiency develops as a result of poor zinc bioavailability as well as an inadequate dietary intake of zinc (1–10). Diets composed primarily of plant products contain inhibitors of mineral absorption, such as phytic acid or phytate (PA), polyphenols, oxalate, and fiber, with PA being the most potent inhibitor of zinc absorption (2–10). PA, found in products made from soy, peanuts, whole grain cereals, rice, wheat, and corn, reduces the amount of zinc available for absorption by binding with zinc in an insoluble complex in the gastrointestinal tract (2–7). Rats fed diets that contain large amounts of PA have impaired zinc absorption, growth retardation, and compromised zinc status (3–6,8). In humans, PA plays a similar role in poor zinc bioavailability such that humans consuming a diet composed primarily of plant products require a higher daily zinc intake (9–13).

Zinc is an essential trace mineral required for normal growth, protein metabolism, the function of zinc metalloenyzymes, and immune function (1). Zinc deficiency in animals causes anorexia, weight loss, poor feed efficiency, and growth retardation (1–2,14). In humans, symptoms of zinc deficiency include stunted growth, hypogonadism, low birth weight, alopecia, skin lesions, immune deficiencies, night blindness, impaired taste and appetite, poor wound healing, diarrhea, depressed mental function, and behavioral disturbances (1,2,15).

Various agricultural, food-based, and dietary strategies, such as plant genetics, biofortification of plant-based staple foods, aquaculture, food processing procedures, and consumption of animal products, have been implemented to limit PA’s zinc binding ability and improve zinc bioavailability (16–19). Following such procedures, as when bread (20–22) or sorghum products are fermented (5,23) or leavened (22) or oats are malted and soaked (24), zinc absorption improved.

Yogurt is a fermented dairy product produced with the organisms *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The nutritive value of yogurt reflects that of milk, although the protein and carbohydrate in yogurt are usually more digestible (25–26). In most animal studies, yogurt increased growth and feed efficiency in normal rats compared to normal rats fed milk or other unfermented dairy products (27–28). A recent human study showed that when milk or yogurt was added to plant-based diets, zinc absorption was increased (29). This informative study in adults was undertaken at that stage of the life cycle when all growth has been completed.

The purpose of this study was to determine the affects of adding yogurt to animal diets which were high in phytic acid (PA) and adequate in zinc (38 mg Zn/g). The PA:Zn molar ratio was 60:1. In rats, growth and zinc concentrations of bone and blood are indicators of zinc status (1–2,14). The most obvious indicator of zinc status is normal growth in the young of any species (15).

METHODS AND MATERIALS

Animals

Weanling, male, Sprague-Dawley rats (Harlan Sprague-Dawley Inc. Indianapolis, IN) with initial weights of 57–61 g were housed individually in stainless steel wire bottom cages in a temperature controlled room (22 ± 0.5°C) with a 12-hr light/dark cycle. During a 4-day adjustment period, all rats were trained to eat between 0800–1200 h. This study was approved and performed in accordance with the guidelines for the care and use of laboratory animals with both the University of Kentucky’s Internal Animal Care and Use Committee (IACUC) and the Veterans Administration Medical Center in Lexington, KY.

Diets

All rats had free access to the AIN-76 purified diet (Table 1) (ICN Biochemicals/MP Biomedicals, Solon, OH) and variations thereof. Zinc content of the diets was 38 μg Zn/g (adequate). Sodium phytate (Sigma Chemical Co., St. Louis, MO) was added to the zinc-adequate diet for a PA/Zn molar ratio of 60:1. Yogurt was made at the University of Kentucky Dairy Laboratory using plain Dannon® yogurt as the fermentation starter. Cultured yogurt contained 20% solids (25 μg Zn/g). Diets were prepared such that calorie and zinc content were not significantly different.

General procedures

Rats were divided randomly into six groups (n=6). For 25 days, 6 groups of weanling rats (n=6) were fed one of the following AIN-76 diets. Half of the diets contained PA. Four of the diets contained yogurt with either active or heat-treated (inactive) cultures being substituted for 25% of the AIN diet. See Table 2 for diet descriptions and codes for treatment groups.

Diets were placed in shallow glass food cups with stainless steel follow-through disks to reduce food spills. To prevent spoilage, yogurt was added to specific diets immediately before the feeding period and cups were removed at the end of 4 hrs. Deionized water was freely available in plastic bottles with silicone stoppers. Feed consumption and weight gain were recorded throughout the experiment.

All rats were humanely killed on day 26. Rats were deprived of food for 12 hr, anesthetized with sodium pentobarbital and exsanguinated by cardiac puncture. Heparinized blood and tibia (bone) were collected for analyses.

Analytical methods

Tissue samples were prepared using a nitric-acid-hydrogen peroxide wet digest as previously described (5). Zinc, copper, and iron concentrations in plasma, bone (tibia), and diets were determined by flame atomic absorption spectrophotometer (Perkin Elmer Model 5000, Norwalk, CT). Integration time was 2 s with an air-acetylene flame. Wavelength was set at 213.9 nm and spectral band width was 0.7 nm.

Statistical methods

A repeated measure analysis of variance (ANOVA) with time as a repeated measure (within subject) factor and treatment group as a between-subjects factor was conducted for body weight, weight gain, feed consumption, and food efficiency ratios. The main effects of time and group and the interaction between time and group were included in the model and tested for significance using Wilks' Lambda F multivariate test. For outcomes with a significant interaction effect the one-way ANOVA with group as the only effect in the model was conducted for each time point. If the F-test was significant, the pairwise comparisons between the group means were performed. Body zinc was measured only once, at the end of the experiment, and therefore a one-way ANOVA with treatment group as the only factor in the analysis was conducted to compare the body zinc means across groups. SPSS version 15 was used for the analysis. Statistical significance was determined by a $p<0.05$.

RESULTS

Data are reported by 5-day periods for the 25 days during which the rat groups received the experimental diets. At baseline, the mean body weight for all rats was 58.42 ± 6.9 g (SD). The group means were not significantly different at the start of the study (Table 3).

The interaction between time and group was significant for all outcomes: body weight, weight gain, and food efficiency ratios, implying that the differences between groups varied with time. Following the one-way ANOVA analyses, the results were as follows:

Body weight

As shown in Table 3, there were no statistically significant differences among the groups in body weight at baseline or at the end of the first 5-day study period (baseline: $F(5,30)=0.045$, $p=0.999$, 5-day: $F(5,30)=2.145$, $p=0.087$). At the end of the study, the control group with PA (CP) had a significantly lower mean body weight than that of all other groups ($p<0.05$).

Weight gain

As to weight gain, there were significant differences among groups during the first two 5-day periods (1st period: $F(5,30)=8.326$, $p<0.001$; 2nd period: $F(5,30)=10.594$, $p<0.001$). The control group with PA (CP) gained significantly less weight than all other groups ($p<0.002$). At the end of the 5th period, the control group (C) and the AIN + active yogurt (A) had a significantly greater mean weight gain than all groups with PA. The group receiving AIN + inactive yogurt (I) had a mean weight gain higher than groups receiving PA, but the differences were not statistically significant (Table 4).

Food efficiency ratios (FER)

The FER, a calculated value, is defined as the amount of food (g) required to produce a 1 g increase in body weight. By the second period (Day 10) the control group with PA (CP) had a significantly higher mean FER than all other groups, indicating that the rats in this group had to eat proportionally more food per gram of weight gain. For the remaining time periods, there were no definitive differences in FER among groups (Table 5).

Bone and plasma mineral concentrations

The rat groups fed diets containing phytic acid PA (CP, AP, IP) had lower mean bone and plasma zinc concentrations ($p<0.05$) compared to groups fed diets without phytic acid (Table 6). There were no significant differences for copper and iron in bone and plasma (data not shown).

DISCUSSION

This study evaluated adding yogurt to rat diets high in phytic acid (PA) in order to determine if yogurt would ameliorate the negative effects that PA has on normal growth and biochemical markers for zinc status. The most overt indicator of poor zinc status is growth retardation.

Our data showed that rats fed diets with PA, plus active or inactive yogurt, grew equally as well as rats fed diets without PA. Considering only the 3 groups with PA, the most important, and totally unexpected outcome of the study was that the two groups of rats receiving yogurt and PA (AP, IP) grew decidedly better than the control group (CP) with PA alone (no yogurt). However, these same two groups with yogurt and PA (AP, IP), had no better zinc status, as measured by bone or plasma, than that of the control rats with PA (CP). Though the presence of yogurt in the diet did not improve zinc absorption, transport, or storage, this data supports the fact that the yogurt did contribute in some way to normal growth.

In this study, there are several important findings that emerged. First, rats fed diets with PA and added yogurt grew as well as control rats fed diets without PA. Yogurt often has been considered a food that provides numerous health benefits (25–26) due to changes brought about by the fermentation process (26–28). The proteins in yogurt have been linked to a growth-stimulating factor, which was associated with *Streptococcus thermophilus* rather than fermentative changes in the milk (27–28,30). It also has been suggested that the growth factor in yogurt was β-galactosidase, which would reduce the amount of undigested lactose in the intestine (27). Reducing the level of lactose in milk has been shown to improve considerably the growth rate of animals (30,31). There may be some intrinsic component of yogurt which, in part, allowed for normal growth in spite of sub-optimal zinc absorption and status.

Second, the data showed that rats fed diets with PA and added yogurt had no better zinc status, as measured by bone and zinc concentration, than rats fed diets with PA alone. It would be expected that the bone (tibia) zinc, a storage site, would be lower in animals fed diets with PA, with available zinc being used for growth. Likewise it would be expected that the bone zinc in the group not receiving yogurt would be even lower, and this was not true.

Poor zinc bioavailability as a result of PA in the diet in both animal and human studies has been studied and reviewed (2–13). The PA:Zinc (Zn) molar ratio has been proposed as a reliable indicator of zinc bioavailability from PA rich foods. A PA:Zn molar ratio of 10:1 to 15:1 induced marginal zinc deficiency, and at 20:1 growth rates were reduced (4).

Procedures that degrade PA have been studied as a means to improve zinc absorption and reduce the PA:zinc molar ratio (5,16–23). Rats fed fermented sorghum gruel (5) or meal (23) had significantly better zinc absorption than rats fed other non-fermented products. In these studies, fermentation reduced the PA:Zn ratio from 45 in the raw sorghum seeds to 24 in the fermentation product. Zinc absorption was greatly improved in rats fed whole wheat bread fermented with a sourdough starter compared to rats fed whole wheat bread fermented with yeast (20).

Our study used diets adequate in zinc (38 μg Zn/g) with PA added for a PA:Zn molar ratio of 60. The rats consuming a diet with PA and yogurt had normal growth but sub-optimal zinc concentrations in bone and plasma. In contrast to previous studies by others (2,7,10,32), in our study yogurt did not facilitate zinc absorption. The reason for this result remains unclear.

Animals have demonstrated the ability to adapt to high levels of PA in the diet (10,33). In our study, there was a positive effect of yogurt on food efficiency ratios (FERs) during the first 10 days of the study. By the end of the study, however, there were no differences in FERs among groups. These findings suggest that the rats adapted to the diets with PA over time and functioned adequately with lower zinc concentrations. Rats (34) and humans have demonstrated some ability to adapt to lower zinc stores. Essatara et al. (35) reported plasma zinc concentrations in Moroccan subjects that were lower than the range accepted as normal for subjects in the United States. Moroccan subjects ate diets high in PA (PA:Zn molar ratio range from 33 to 47), yet they had normal growth and appeared healthy. Perhaps the fermented foods in their diet accounted for normal growth in spite of lower plasma zinc concentrations.

Third, and unique to this study, the affects of active versus heat-treated (inactive) yogurt were compared. The bacteria-induced fermentation process in yogurt reduces lactose to absorbable glucose, galactose and lactic acid (36–38). Organic acids, such as lactic acid, have been shown to enhance zinc absorption by forming soluble ligands with zinc (2,7,10,32). Lactic acid bacteria in sourdough cultures have been shown to degrade PA (39), and lactic acid fermentation increased some mineral solubility in vitro (21–22). In our study, however, the microbial or bacterial action could not be the factor affecting the nutrient value of the respective diets, since both the active and inactive yogurt gave similar results.

Thus, we conclude that there is some component in yogurt (both active and heat-treated) or some mechanism by which yogurt facilitates normal growth and at the same time off-sets the PA effects, allowing for normally developed rats which showed suboptimal zinc status and not true zinc deficiency. Further research is needed to identify the growth-promoting factor(s) in yogurt.

Acknowledgments

Financial support

Veterans Administration Career Development Award, Veterans Administration Merit Review Award, NIH R01 AA10496, NIH R01 AA10762.

We wish to thank Helena Truszczynska, Ph.D. and Dr. Richard Kryscio, Ph.D. for their statistical help and Nancy Meyer, M.S., R.D., for her zinc expertise.

References

1. McClain CJ, Kasarskis EJ, Allen JJ. Functional consequences of zinc deficiency. Prog Food Nutr Sci 1985;9:185–196. [PubMed: 3911268]
2. Lonnerdal B. Dietary factors influencing zinc absorption. J Nutr 2000;130:1378–1383.
3. Oberleas D, Harland BF. Phytate content of foods: Effect on dietary zinc bioavailability. J Am Diet Assoc 1981;79:433–6. [PubMed: 7288050]
4. Oberleas D, Muhrer ME, O'Dell BL. Dietary metal complexing agents and zinc availability in the rat. J Nutr 1966;90:56–62. [PubMed: 4958459]
5. Stuart MA, Johnson PE, Hamaker B, Kirleis A. Absorption of zinc and iron by rats fed meals containing sorghum food products. J of Cereal Science 1987;6:81–90.
6. Stuart SM, Ketelsen SM, Weaver CM, Erdman JW. Bioavailability of zinc to rats as affected by protein source and previous dietary intake. J Nutr 1986;116:1423–1431. [PubMed: 3760999]
7. Krebs NF. Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr 2000;130:1374S–7S. [PubMed: 10801946]
8. Zhou JR, Fordyce EJ, Raboy V, Dickinson DB, Wong MS, Burns RA, Erdman JW. Reduction of phytic aid in soybean products improves zinc availability in rats. J Nutr 1992;122:2466–2473. [PubMed: 1453231]
9. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 2003;78:633S–9S. [PubMed: 12936958]
10. Gibson RS, Yeudall F, Drost N, Mtitimuni B, Cullinan T. Dietary interventions to prevent zinc deficiency. Am J Clin Nutr 1998;68:484S–7S. [PubMed: 9701165]
11. Fredlund K, Rossander-Hulthen L, Isaksson M, Almgren A, Sandberg AS. Absorption of zinc and calcium: dose-dependent inhibition by phytate. Journal of Applied Microbiology 2002;93:197–204. [PubMed: 12147067]
12. Sandstrom B, Almgren A, Kivisto C, Cederblad A. Effect of protein level and protein source on zinc absorption in man. J of Nutr 1989;119:48–53. [PubMed: 2492337]
13. Hunt JR, Matthys LA, Johnson LK. Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk. Am J Clin Nutr 1998;67:421–430. [PubMed: 9497185]
14. Essatara MB, Levine AS, Morley JE, McClain CJ. Zinc deficiency and anorexia in rats: normal feeding patterns and stress induced feeding. Physiol and Behav 1984;32:469–474. [PubMed: 6589654]
15. Mertz, W. Trace Elements in Human and Animal Nutrition. 5. Orlando, FL: Academic Press, Inc; 1986. Zinc; p. 1-137.
16. Sandberg AS. Bioavailability of minerals in legumes. Brit J of Nutr 2002;88:S281–5. [PubMed: 12498628]
17. Hurrell RF. Influence of vegetable protein sources on trace element and mineral bioavavilability. J Nutr 2003;133:2973S–2977S. [PubMed: 12949395]
18. Raboy V. Progress in plant breeding low phytate crops. J of Nutr 2002;132:503S–505S. [PubMed: 11880580]

19. Murphy SP, Allen LH. Nutritional importance of animal source foods. J Nutr 2003;133:3932S–3935S. [PubMed: 14672292]
20. Lopez HW, Duclos V, Coudray C, Krespine V, Feillet-Coudray C, Messager A, Demigne C, Remesy C. Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. Nutrition 2003;19:524–530. [PubMed: 12781853]
21. Svanberg U, Lorri W, Sandberg AS. Lactic fermentation of non-tannin and high-tannin cereals: effects on in vitro estimation of iron availability and phytate hydrolysis. J Food Sci 1993;8:408–412.
22. Turk M, Carlsson N, Sandberg A. Reduction in the levels of phytate during whole meal bread making: effect of yeast and wheat phytases. J of Cereal Science 1996;23:257–264.
23. Hirabayashi M, Matsui T, Yano H. Fermentation of soybean meal with Aspergillus usamii improves zinc availability in rats. Biol Trace Elem Res 1998;61:227–234. [PubMed: 9517493]
24. Larsson M, Rossander-Hulthen L, Sandstrom B, Sandberg A. Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Brit J of Nutr 1996;76:677–688. [PubMed: 8958002]
25. Vass A, Szakaly S, Schmidt P. Experimental study of the nutritional biological characters of fermented milks. Acta Med Hung 1984;41:157–161. [PubMed: 6431392]
26. Adolfsson O, Meydani SN, Russell RM. Yogurt and gut function. Am J Clin Nutr 2004;80:245–256. [PubMed: 15277142]
27. Hitchins AD, McDonough FE. Prophylactic and therapeutic aspects of fermented milk. Am J Clin Nutr 1989;49:675–684. [PubMed: 2648797]
28. Hargrove RE, Alford JA. Growth rate and feed efficiency of rats fed yogurt and other fermented milks. J Dairy Sci 1978;61:11–19.
29. Rosado JL, Diaz M, Gonzalez K, Griffin I, Abrams SA, Preciado R. The addition of milk or yogurt to a plant-based diet increases zinc bioavailability but does not affect iron bioavailability in women. J Nutr 2005;135:465–468. [PubMed: 15735079]
30. Wong NP, McDonough FE, Hitchins AD. Contribution of *Streptococcus thermophilus* to growth-stimulating effect of yogurt on rats. J Dairy Sci 1983;66:444–449. [PubMed: 6841747]
31. Gorbach SL. Lactic Acid bacteria and human health. Ann of Med 1990;22:37–41. [PubMed: 2109988]
32. Desrosiers T, Clydesdale F. Effectiveness of organic chelators in solubilizing calcium and zinc in fortified cereals under simulated gastrointestinal pH conditions. J Food Proc Preserv 1989;13:307–319.
33. Lonnerdal B, Jayawickrama L, Lien EL. Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups. Am J Clin Nutr 1999;69:490–496. [PubMed: 10075335]
34. House WA, Welch RM, Van Campen R. Effect of phytic acid on the absorption, distribution, and endogenous excretion of zinc in rats. J Nutr 1982;112:941–953. [PubMed: 7077425]
35. Essatara, MB.; Faddouli, J.; Aoui, L. Phytate, Ca, P, Fe, Zn, and Cu levels in the major food items and diets in Morocco. TEMA 5 (Proceeding); Aberdeen-Scotland. 1984.
36. Broussalian J, Westhoff D. Influence of lactose concentration of milk and yogurt on growth rate of rats. J Dairy Sci 1983;66:438–443. [PubMed: 6841746]
37. Kolars JC, Levitt MD, Aouji M, Savaiano DA. Yogurt-an autodigesting source of lactose. N Engl J Med 1984;310:1–3. [PubMed: 6417539]
38. Pochart P, Dewit O, Desjeux JF, Bourlioux P. Viable starter culture, β-galactosidase activity, and lactose in duodenum after yogurt ingestion in lactase-deficient humans. Am J Clin Nutr 1989;49:828–831. [PubMed: 2497633]
39. Shirai K, Revah-Moiseev S, Garcia-Garibay M, Marshall VM. Ability of some strains of lactic acid bacteria to degrade phytic acid. Lett Appl Microbiol 1994;19:366–369.

Table 1

Composition of AIN-76 Basal Diet

Ingredient	**%**
Sucrose	50.0
Casein Purified High Nitrogen	20.0
Corn Starch	15.0
Fiber (Alphacel non-nutritive bulk)	5.0
Corn Oil	5.0
AIN-76 Mineral Mix[2]	3.5
AIN-76 Vitamin Mix[3]	1.0
DL-Methionine	0.3
Choline Bitartrate	0.2

[1]ICN Nutritional Biochemicals/MP Biomedicals, Solon, OH

[2]Mineral Mixture (g/kg of mixture): Calcium Phosphate Dibasic 500, Potassium Citrate Monohydrate 220, Sodium Chloride 74, Potassium Sulfate 52, Magnesium Oxide 24, Ferric Citrate(16–17% Fe) 6 Manganous Carbonate(43–48% Mn) 3.5, Zinc Carbonate (70% ZnO) 1.6, Chromium Potassium Sulfate 0.55, Cupric Carbonate (53–55% Cu) 0.3, Potassium Iodate 0.01, Sodium Selenite 0.01, Sucrose, finely powdered 118.

[3]Vitamin Mixture (per kg of mixture): Pyridoxine Hydrochloride 700 mg, Thiamine Hydrochloride 600 mg, Riboflavin 600 mg, Cholecalciferol 2.5 mg, Folic Acid 200 mg, D-Biotin 20 mg, Menaquinone 5 mg, Cyanocobalamin 1 mg, DL-alpha-Tocopherol Acetate (250 IU/g) 20 g, Nicotinic Acid 3.0 g, D-Calcium Pantothenate 1.6 g, Retinyl Palmitate 1.6 g, Sucrose, finely powdered 972.9 g.

Table 2

Diet descriptions and codes for treatment groups.

Group Code	Diet Description Without Phytic Acid (PA)	Group Code	Diet Description With Phytic Acid (PA)
C	AIN Control	CP	AIN Control + PA
A	AIN + Active yogurt	AP	AIN + Active yogurt + PA
I	AIN + Inactive yogurt	IP	AIN + Inactive yogurt + PA

Table 3

Mean body weight for all groups[1,2,3]

Group Code	Mean Body Weight (g)					
	Day 0	Day5	Day 10	Day 15	Day 20	Day 25
C AIN Control	59.2 ± 6.5	76.3 ± 9.4	104.0 ± 11.2[a]	126.5 ± 10.4[a]	147.3 ± 11.6[a]	178.7 ± 15.0[a]
A AIN + Active Yogurt	59.0 ± 7.2	81.8 ± 10.9	109.3 ± 11.0[a]	132.0 ± 13.1[a]	154.0 ± 15.6[a]	181.8 ± 17.3[a]
I AIN + Inactive Yogurt	58.5 ± 6.6	81.7 ± 11.8	105.5 ± 12.9[a]	129.3 ± 13.0[a]	153.7 ± 13.6[a]	179.8 ± 16.2[a]
CP AIN Control +PA	57.8 ± 8.3	62.8 ± 14.2	78.7 ± 19.1[b]	97.0 ± 23.9[b]	116.0 ± 26.4[b]	138.7 ± 29.8[b]
AP AIN + Active Yogurt + PA	57.5 ± 8.4	78.3 ±13.9	102.8 ± 15.4[a]	126.2 ± 16.3[a]	148.0 ± 19.3[a]	169.5 ± 17.7[a]
IP AIN + Inactive Yogurt + PA	58.5 ± 7.8	79.7 ± 10.7	105.0 ± 11.9[a]	128.0 ± 15.4[a]	153.5 ± 16.9[a]	177.0 ± 20.6[a]

[1] Values are means ± SD, n = 6 for each group.

[2] Within columns, values having different superscripts are significantly different, $p<0.05$.

[3] Columns with no superscripts indicate not significant ANOVA F-test for comparing group means at the corresponding time point.

NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 4

Mean weight gain per 5-day period for all groups[1,2,3]

Group Code	Mean Weight Gain (g) per 5-day Period				
	Day 5	Day 10	Day 15	Day 20	Day 25
C AIN Control	17.2 ± 3.9[a]	27.7 ± 2.3[a]	22.5 ± 1.9	20.8 ± 4.5	31.3 ± 5.6[a]
A AIN + Active Yogurt	22.8 ± 4.4[a]	27.5 ± 2.3[a]	22.7 ± 3.8	22.0 ± 3.1	27.8 ± 2.9[ab]
I AIN + Inactive Yogurt	23.2 ± 6.3[a]	24.3 ± 3.1[a]	23.3 ± 3.1	24.3 ± 1.2	26.2 ± 4.3[abc]
CP AIN Control +PA	6.2 ± 7.3[b]	16.2 ± 5.4[b]	18.3 ± 6.0	19.0 ± 5.9	22.7 ± 5.5[bc]
AP AIN + Active Yogurt + PA	20.8 ± 5.9[a]	24.5 ± 3.0[a]	23.3 ± 2.4	21.8 ± 4.1	21.5 ± 2.3[c]
IP AIN + Inactive Yogurt + PA	21.2 ± 4.1[a]	25.3 ± 1.5[a]	23.0 ± 5.2	25.5 ± 3.4	23.5 ± 4.9[bc]

[1]Values are means ± SD, n = 6 for each group.

[2]Within columns, values having different superscripts are significantly different, $p<0.05$.

[3]Columns with no superscripts indicate not significant ANOVA F-test for comparing group means at the corresponding time point.

Table 5

Mean food efficiency ratios (FER) per 5-day period for all groups[1,2,3]

Group ID		Mean Food Efficiency Ratio (FER) per 5-day Period			
	Day 5	Day 10	Day 15	Day 20	Day 25
C AIN Control	2.2 ± 0.3	2.1 ± 0.4[a]	2.5 ± 0.3	3.2 ± 0.7[ac]	2.4 ± 0.2[a]
A AIN + Active Yogurt	1.5 ± 0.1	1.7 ± 0.1[a]	2.3 ± 0.2	2.7 ± 0.2[b]	2.4 ± 0.2[a]
I AIN + Inactive Yogurt	1.5 ± 0.3	1.9 ± 0.2[a]	2.3 ± 0.2	2.5 ± 0.1[b]	2.6 ± 0.3[ac]
CP AIN Control +PA	3.5 ± 5.0	3.0 ± 1.3[b]	2.7 ± 0.9	2.8 ± 0.6[bc]	2.6 ± 0.4[ac]
AP AIN + Active Yogurt + PA	1.5 ± 0.4	1.8 ± 0.1[a]	2.2 ± 0.2	2.7 ± 0.4[b]	2.9 ± 0.4[bc]
IP AIN + Inactive Yogurt + PA	1.5 ± 0.1	1.8 ± 0.1[a]	2.3 ± 0.4	2.4 ± 0.2[b]	2.9 ± 0.3[bc]

[1]Values are means ± SD, n = 6 for each group.

[2]Within columns, values having different superscripts are significantly different, $p<0.05$.

[3]Columns with no superscripts indicate not significant ANOVA F-test for comparing group means at the corresponding time point.

Table 6

Zinc Concentrations in Bone and Plasma[1,2]

Group ID	Bone (Tibia) Zinc (μg/g)	Plasma Zinc (μg/dL)
C AIN Control	209.96 ± 11.67[a]	171.10 ± 7.02[a]
A AIN + Active Yogurt	199.40 ± 6.85[a]	167.20 ± 11.51[a]
I AIN + Inactive Yogurt	206.23 ± 9.97[a]	160.93 ± 8.55[a]
CP AIN Control+PA	137.37 ± 22.19[b]	141.00 ± 12.34[b]
AP AIN + Active Yogurt + PA	127.98 ± 7.03[b]	134.80 ± 6.69[b]
IP AIN + Inactive Yogurt + PA	137.20 ± 10.1[b]	139.10 ± 11.44[b]

[1]Values are means ± SD, n = 6 for each group.

[2]Within columns, values having different superscripts are significantly different, $p<0.05$.