Effect of Phytic Acid Level in Soy Protein Based Infant Formulas on Mineral Availability in the Rat

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Infant formulas were prepared from either phytate-containing soy protein isolate or a soy protein isolate with 86% of the phytate removed. Six formula diets were made by adjusting calcium and phosphorus concentrations to high, medium, and low. Each diet was fed to 10 male weanling rats for 3 weeks. Rats fed the phytate-containing diets did not differ from those fed phytate-reduced diets with similar calcium and phosphorus concentrations in weight gain or in femur and carcass calcium, phosphorus, zinc, iron, copper, and magnesium contents. Fat-free dry femur weights were significantly less in rats fed the low-calcium, low-phosphorus diets, regardless of the phytate content. Concentrations of zinc, iron, and copper per gram of femur ash were all higher in rats fed the low-calcium diets than in rats fed the high-calcium diets. This study showed that availability of minerals from a typical soy protein isolate infant formula is not affected by the phytic acid concentration at the concentrations of calcium present in these formulas.

Soy-based infant formulas are prepared from soybean protein sources that contain phytic acid. High dietary levels of phytic acid decrease the bioavailability of calcium, iron, and zinc in humans and other animal species (Davies and Olpin, 1979; Reinhold et al., 1973; Widdowson and McCance, 1942; Nightingale, 1975; Davis et al., 1962; Makdani et al., 1975; Morris and Ellis, 1980; O'Dell and Savage, 1960; Oberleas et al., 1966). In addition, phytic phosphorus may not be nutritionally available (Taylor, 1965).

Although autoclaving hydrolyzes phytic acid, heat processing of soy formulas may not degrade phytic acid (O'Dell et al., 1969). If phytic acid is structurally intact in the soy formulas, then the nutritional availability of phytic phosphorus and the effect of phytic acid on the bioavailability of calcium and trace minerals from the soy formulas need to be evaluated. For formula-fed infants, the formula provides the only source of essential minerals. Therefore, the effect of phytic acid on mineral availability is of special concern for the infant consuming soy formulas.

Even though phytic acid is present in soy formulas, it cannot be assumed that it interferes significantly with mineral availability. A number of formula factors may influence mineral availability: for example, the level of each mineral in the formula, especially calcium (O'Dell et al., 1964; Nahapetian and Young, 1980), the amount of phytic acid (Likuski and Forbes, 1965), an association of phytic acid with protein (O'Dell, 1969; Katzer et al., 1959), the type of protein (Smith et al., 1962; Nielsen et al., 1966), and heat treatment (O'Dell, 1969; Kratzer et al., 1959; Smith et al., 1960). Thus, the effect of phytic acid on mineral availability from a soy formula can only be assessed by comparing the effects of feeding phytate-containing and phytate-free soy formulas.

The purpose of this study was (1) to determine the amount of phytic acid in a typical infant formula made with soy protein isolate, (2) to determine in the rat if phytic acid decreases the bioavailability of minerals from soy protein isolate formulas, and (3) to determine how three calcium levels in soy formula modify the effect of phytic acid on mineral bioavailability.

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MATERIALS AND METHODS

Determination of Phytic Acid. The phytic acid contents of soy protein isolate and soy protein isolate formulas were determined by a modification of the method of Wheeler and Ferrell (1971). The modification was an adjustment of the soy formula or isolate to pH 7.5 before the protein was precipitated with trichloroacetic acid (TCA).

Removal of Phytic Acid from Soy Protein Isolate. Phytic acid was removed from soy protein isolate by ultrafiltration (Romicon LTCX, Romicon, Inc). Soy protein isolate (5.75 kg) was first suspended in 40 L of 10% NaCl and the pH adjusted to 7.5 with 5% NaOH to dissolve the isolate. The initial sodium concentration of the dissolved isolate was approximately 40 000 ppm. To remove phytate, the dissolved isolate was pumped through an ultrafiltration unit continuously for 5 days. To be assured that protein was not crossing the membrane, the diafiltrate was monitored for protein by adding approximately 1 mL of the filtrate to 5 mL of 5% TCA solution. If no precipitate formed, the filtrate was considered free of protein. The solution was ultrafiltered until no trace of phosphorus could be detected in the concentrate. Diafiltration was continued until the concentration of the sodium ion in the filtrate fell below 1500 ppm. The sodium content of the dialyzed concentrate was 10 mg/g of protein. The dialyzed isolate was stored and kept frozen until needed for the preparation of the test formulas.

Phytic acid content was reduced by 86%. Thus, the term phytate-reduced soy protein isolate was used to describe the formulas prepared from it.

Preparation and Evaluation of Formulas. Four soy-based infant formulas, two phytate-containing and two phytate-reduced, were prepared by Ross Laboratories (Columbus, OH) according to standard manufacturing procedures for Isomil concentrated liquid. One each of the phytate-containing (PH) and phytate-reduced (RH) formulas contained similar high concentrations of calcium and phosphorus and the others (PL and RL) contained similar low concentrations of calcium and phosphorus. Except for calcium and phosphorus contents, the four formulas were designed to contain similar amounts of nutrients per deciliter: 4.0 g of protein, 7.2 g of fat, 13.6 g of carbohydrate, 10 mg of magnesium, 2.4 mg of iron, 20 mcg of iodine, 0.5 mg of zinc, 0.3 mg of copper, 0.04 mg of manganese, 64 mg of sodium, 150 mg of potassium, 120 mg of chloride, and oil- and water-soluble vitamins as found in Isomil concentrated liquid. Formulas were supplemented with L-methionine (20 mg/g of protein) to meet the requirement of the rat. The concentrations of nutrients in diet PH (Table I) were those of Isomil CL except for zinc. Because removing phytic acid from the soy isolate may have altered the quality of the protein of the soy protein, the amino acid content and protein efficiency ratios (PER) of the formulas were compared. Amino acid profiles were determined on a Durrum amino acid analyzer (Model 0500). Phytate-containing and phytate-reduced

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Table I. Composition of Formula Diets (per 100 g of Dry Diet)[a]

	phytate-containing diet			reduced-phytate diet		
	PH	PM	PL	RH	RM	RL
fat, g	26.0	26.8	27.3	21.3	21.8	21.9
protein, g	15.0	15.4	15.6	14.5	14.6	14.8
carbohydrate, g	54.7	54.3	53.9	60.1	59.9	60.1
energy, kcal	514	520	525	490	494	498
calcium, mg	551	424	291	557	414	273
phosphorus, mg	408	284	157	383	261	141
iron, mg	14.8	14.5	14.1	13.4	12.0	10.7
magnesium, mg	43.7	43.1	42.0	43.9	43.4	43.3
zinc, mg	1.59	1.76	1.91	1.54	1.63	1.73
copper, mg	1.28	1.30	1.32	1.26	1.26	1.28
phytic acid, mg	318	324	330	39	38	40
phytic phosphorus, mg	89.8	91.3	92.9	10.9	10.9	11.3
phytic phosphorus, %	22.0	32.1	59.2	2.8	4.2	8.0
phytate to zinc molar ratio	19.8	18.4	17.1	2.6	2.3	2.3

[a] Formulas: PH, phytate-containing, high calcium; PM, phytate-containing, medium calcium; PL, phytate-containing, low calcium; RH, reduced phytate, high calcium; RM, reduced phytate, medium calcium; RL, reduced phytate low calcium.

isolates were similar in amino acid contents.

PER was determined by a modified AOAC method (AOAC, 1970) using 21-day-old weanling male rats, Charles River strain, weighing 44–67. Two groups of 13 rats, matched by weight, were housed in individual stainless steel cages. The two phytate-containing formulas and the two reduced-phytate formulas were blended and supplemented with minerals (salt mix XIV) and vitamins (AOAC vitamin mix) (Taklab, Madison, WI) to assure that diets met nutrient requirements of the rat. Carbohydrate was added to adjust protein to 10% of the diet. PER (mean ± SD) of the rats receiving the phytate-containing diet and reduced-phytate diet were, respectively, 2.25 ± 0.22 and 2.37 ± 0.28 g gained/g of protein consumed. There was no statistical difference between the protein efficiency ratios values of the two groups. Thus, the protein quality of the soy isolate as measured by the modified AOAC PER method was not affected by the phytate removal.

Mineral Availability. Sixty 21-day-old weanling male rats (Charles River) weighing between 44 and 56 g were divided into six groups of 10 each by weight and housed in individual stainless steel wire cages. The four formulas were used to prepare the six formula diets used in the rat feeding study by blending PH and PL to make diet PM and blending RH and RL to make diet RM (Table I). The formula diets were therefore referred to as PH, phytate-containing, high calcium; PM, phytate-containing, medium calcium; PL, phytate low calcium; RH, reduced phytate, high calcium; RM, reduced phytate, medium calcium; and RL, reduced phytate, low calcium. Slight additions of carbohydrate and magnesium were made to four diets after formula manufacture to permit similar protein concentrations (12% of total calories) and trace mineral contents. Diets were supplemented with AOAC vitamin mixture at 1 g/100 g of diet, and except for calcium and phosphorus concentrations in diets PL and RL, diets met estimated nutrient requirements of a growing rat (National Academy of Sciences, 1978). Calcium and phoshorus ratios were similar in all six diets.

The rats were fed ad libitum daily for 3 weeks. Feed intake was weighed daily. Demineralized water was furnished.

Rats were weighed weekly. At the end of the feeding period, rats were killed with chloroform and then immediately eviscerated. After removal of the left femur, the carcass and femur were weighed, placed in polyethylene bags, and stored frozen at −18 °C.

Analytical Procedures. Femurs were thawed, scrubbed with cheesecloth to free the bone from any adhering tissue, and dried in porcelain crucibles, and fat was extracted with ethyl ether in a Soxhlet apparatus for 24 h. The defatted femur was transferred to a weighed platinum crucible, 1 mL of concentrated nitric acid added, the crucible placed in a hot-air oven until the femur was charred, and then the femur ashed in a muffle furnace (540 °C) until constant weight. The ash was dissolved in 4 mL of 2 N hydrochloric acid and diluted to 25 mL with distilled demineralized water.

Table II. Femur Weight and Ash (mg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

diet code	total wt	fat-free dry	total ash
PH	510 ± 40[a]	247 ± 17[a]	120 ± 10[a]
RH	524 ± 61[a]	255 ± 30[a]	124 ± 12[a]
PM	503 ± 42[a]	240 ± 20[a]	117 ± 8[a]
RM	496 ± 45[a]	232 ± 19[a]	108 ± 9[a]
PL	450 ± 50[a]	180 ± 13[b]	68 ± 6[b]
RL	441 ± 45[a]	176 ± 16[b]	65 ± 6[b]

[a] 10 rats/diet. [b] Mean ± standard deviation. Vertical mass not sharing common superscript are significantly different ($P < 0.05$).

The carcass was weighed, cut into pieces, placed in a procelain crucible, and dried to constant weight. Viscera and their contents were not analyzed. The dried carcass was extracted three times with anhydrous ethyl ether, air-dried, and ground in an electric blender with 100 mL of double-distilled water. This slurry was dried in a hot-air oven at 100 °C to constant weight and then divided among five platinum crucibles. The dried defatted carcass was ashed, and the ash was dissolved in the same manner as the femur.

The calcium, zinc, magnesium, copper, and iron contents of the ashed femurs and carcasses were determined by atomic absorption (Perkin-Elmer analyzer, Model 503). Phosphorus was determined in the Technicon autoanalyzer fitted with a Hitachi Perkin-Elmer 139 spectrophotometer using a modification of the method of Fiske and Subbarow (1925). Concentrations of minerals were reported as total amounts in femur and carcass, concentration per gram of fat-free tissue and per gram of ash.

Statistical Analysis. Variables were evaluated by analysis of variance, analysis of covariance, or both. Data were further analyzed by the Studentized statistic, the Newman–Keuls test. The confidence level selected for significance was $P < 0.05$.

RESULTS

Weight Gain. Rats fed the three phytate-containing diets did not differ significantly in weight or weight gain over the study period from those fed the reduced phytate diets. Values of mean ± SD in grams of weight gain for the six diet groups were respectively 77.5 ± 5.7 and 76.6 ± 8.0 for PH and RH; 83.8 ± 9.4 and 72.6 ± 6.3 for PM and RM; and 77.2 ± 5.9 and 77.1 ± 5.2 for PL and RL.

Femur Weights and Ash. The mean femur weights were not significantly different among the diet groups. The fat-free dry femurs of rats fed the low-calcium, low-phosphorus diets (RL and PL) weighed significantly less than those of rats fed diets with higher mineral concentrations (Table II). Mean total femur ash weights of rats fed the two low-calcium diets were also significantly less than those of the groups fed diets with higher calcium concentrations. There were no difference in mean femur weights between rats fed phytate-containing diets and those fed reduced-phytate diets.

Femur Calcium and Phosphorus. The mean total amounts of calcium and phophorus in the mean femur and fat-free femur were significantly less in rats fed the low-mineral diets (RL and PL) (Table III) than in rats fed diets with higher calcium and phosphorus levels. Although the concentration of calcium in femur ash did not differ significantly among groups, the concentration of phosphorus was somewhat less in the three groups that received phytate-containing diets.

Femur Zinc, Iron, Magnesium, and Copper. Mean total zinc contents of the femur were generally highest for rats fed diets with moderate amounts of calcium and phosphorus, diets PM and RM (Table IV). Only total femur zinc content of rats fed the medium-calcium phytate-containing diet (PM) was significantly higher than total femur zinc of rats fed the high- and low-calcium diets.

Table III. Femur Calcium and Phosphorus (mg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	calcium			phosphorus		
diet code	total femur	per g fat-free, dry femur	per g femur ash	total femur	per g fat-free dry femur	per g femur ash
PH	41.0 ± 3.0[a]	168 ± 10[a]	344 ± 18[a]	21.1 ± 1.6[a]	85 ± 3[a]	176 ± 4[a]
RH	42.9 ± 5.1[a]	169 ± 8[a]	347 ± 14[a]	22.5 ± 2.2[a]	88 ± 3[a]	182 ± 6[a]
PM	40.5 ± 3.5[a]	168 ± 11[a]	345 ± 11[a]	20.6 ± 1.6[a]	86 ± 4[a]	175 ± 3[a]
RM	37.8 ± 3.1[a]	165 ± 6[a]	348 ± 13[a]	19.4 ± 1.5[a]	85 ± 4[a]	178 ± 2[a]
PL	23.0 ± 2.4[b]	127 ± 11[b]	335 ± 12[a]	12.0 ± 1.3[b]	66 ± 7[b]	175 ± 14[a]
RL	20.7 ± 2.6[b]	118 ± 11[b]	326 ± 36[a]	11.5 ± 1.2[b]	66 ± 4[b]	180 ± 6[a]

[a] 10 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

Table IV. Femur Zinc and Iron (mcg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	zinc			iron		
diet code	total femur	per g fat-free dry femur	per g femur ash	total femur	per g fat-free dry femur	per g femur ash
PH	49.9 ± 9.9[a,b]	204 ± 40[a]	418 ± 80[a]	24.2 ± 3.4[a]	98 ± 10[a]	202 ± 22[a]
RH	45.1 ± 12.5[a,b]	178 ± 34[a]	363 ± 76[a]	26.1 ± 4.5[a]	98 ± 18[a]	211 ± 30[a]
PM	53.2 ± 11.0[b]	220 ± 40[a]	451 ± 81[a]	25.2 ± 4.2[a]	104 ± 17[a]	215 ± 37[a]
RM	53.2 ± 17.5[a,b]	231 ± 73[a]	490 ± 166[a]	26.4 ± 4.0[a]	116 ± 18[a]	242 ± 45[a]
PL	35.4 ± 7.9[a]	200 ± 48[a]	524 ± 124[a,b]	31.7 ± 6.6[b]	177 ± 36[b]	463 ± 102[b]
RL	40.6 ± 12.9[a]	239 ± 59[a]	633 ± 185[b]	28.6 ± 3.2[a,b]	169 ± 17[b]	462 ± 68[b]

[a] 10 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

Table V. Femur Magnesium (mg) and Copper (mcg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	magnesium			copper		
diet code	total femur	per g fat-free dry femur	per g femur ash	total femur	per g fat-free dry femur	per g femur ash
PH	0.87 ± 0.08[a]	3.5 ± 0.2[a]	7.1 ± 0.4[a]	1.5 ± 0.5[a]	6.3 ± 2.0[a]	13.0 ± 4.1[a]
RH	0.89 ± 0.09[a]	3.4 ± 0.3[a]	7.2 ± 0.3[a]	1.3 ± 0.4[a]	5.1 ± 1.3[a]	10.4 ± 2.5[a]
PM	0.77 ± 0.11[b]	3.2 ± 0.4[b]	6.6 ± 0.8[a]	1.3 ± 0.3[a]	5.3 ± 1.3[a]	11.0 ± 2.7[a]
RM	0.75 ± 0.08[b]	3.2 ± 0.3[b]	6.9 ± 0.4[a]	1.3 ± 0.4[a]	5.5 ± 1.9[a]	11.8 ± 3.9[a]
PL	0.46 ± 0.05[c]	2.5 ± 0.2[c]	6.7 ± 0.8[a]	1.3 ± 0.4[a]	7.4 ± 1.9[a]	18.8 ± 6.6[b]
RL	0.45 ± 0.03[c]	2.6 ± 0.3[c]	7.2 ± 0.7[a]	1.1 ± 0.3[a]	6.3 ± 1.8[a]	16.9 ± 5.3[b]

[a] 10 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

Concentration of zinc per gram of fat-free femur was not significantly different among groups (Table IV). The concentration of zinc per gram of femur ash was different between the group fed the RL diet and those fed the high- and medium-calcium diets (RH, PH, RM, PM).

The rats fed the low-calcium phytate diet (PL) had more mean total femur iron than rats fed any of the other diets (Table IV). The mean concentrations of iron in femur and in femur ash were significantly higher in the lowest calcium diet groups (PL, RL).

Both mean total magnesium in femur and mean concentration of magnesium per gram of fat-free dry femur were directly related to the calcium (and phosphorus) levels of the diets (Table V). The concentrations of magnesium in femur ash did not differ among the groups.

No differences among the diet groups were found in the total amount of copper or its concentration in fat-free dry femur (Table V). However, the lowest calcium diets were associated with a significantly greater concentration of copper in femur ash than were the medium- and high-calcium diets. No relationship was seen between phytate concentration in the diet and zinc, iron, magnesium, and copper contents of the femur or their concentration in the dry fat-free femur or femur ash.

Carcass Weight, Ash, and Mineral Content. Mean carcass weights and fat-free dry carcass weights of rats fed the phytate-containing diets were slightly higher, but not statistically different from, carcass and fat-free dry carcass weights of rats on the reduced-phytate diets (Table VI). The mean amount of carcass ash was significantly lower in rats that received diets containing the lowest calcium and phosphorus concentrations than in rats fed the high-calcium diets. Carcass ash contents of rats receiving any of the three phytate-containing diets, however, were not statistically different from those receiving the reduced-phytate diets. Thus, phytate had no significant effect on weight or ash of the rat carcass, but dietary calcium and phosphorus intake did have an effect.

Table VI. Carcass Weight (g) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Concentrations of Calcium and Phosphorus[b]

diet code	weight	fat-free dry wt	ash
PH	104 ± 8[a]	24.9 ± 1.9[a]	3.8 ± 0.2[a]
RH	98 ± 13[a]	25.2 ± 2.4[a]	3.8 ± 0.3[a]
PM	112 ± 13[a]	27.3 ± 2.2[a]	3.8 ± 0.3[a]
RM	99 ± 9[a]	25.7 ± 2.1[a]	3.6 ± 0.2[a]
PL	105 ± 5[a]	25.2 ± 1.6[a]	2.8 ± 0.2[b]
RL	97 ± 10[a]	25.6 ± 2.8[a]	2.6 ± 0.2[b]

[a] 5 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.050$).

Rats fed diets lowest in calcium and phosphorus had significantly less calcium and phosphorus per carcass and per gram of fat-free dry carcass than did rats fed any of the other four diets (Table VII). Rats fed the RL diet had a significantly lower concentration of calcium per gram of carcass ash than did rats fed all other diets except PL.

Mean total zinc, iron, and copper contents per carcass and concentration of these minerals in fat-free dry carcass were not statistically different among diet groups (Tables VIII and IX). Neither phytate concentration nor calcium and phosphorus concentration in the diet had an effect on

Table VII. Carcass Calcium and Phosphorus (mg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	calcium			phosphorus		
diet code	total	per g fat-free dry tissue	per g ash	total	per g fat-free dry tissue	per g ash
PH	1006 ± 46[a]	41 ± 3.2[a]	267 ± 7[a]	677 ± 34[a]	27 ± 1.6[a]	180 ± 8[a]
RH	1027 ± 76[a]	41 ± 2.8[a]	269 ± 8[a]	689 ± 48[a]	27 ± 1.7[a]	181 ± 6[a]
PM	968 ± 62[a]	36 ± 3.8[a]	255 ± 26[a]	669 ± 31[a]	25 ± 1.9[a]	176 ± 11[a]
RM	954 ± 55[a]	37 ± 3.6[a]	262 ± 10[a]	642 ± 27[a]	25 ± 1.9[a]	178 ± 3[a]
PL	682 ± 72[b]	27 ± 2.1[b]	246 ± 22[a,b]	502 ± 52[b]	20 ± 1.8[b]	180 ± 15[a]
RL	623 ± 48[b]	25 ± 2.2[b]	235 ± 5[b]	467 ± 35[b]	18 ± 0.9[b]	176 ± 8[a]

[a] 5 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

Table VIII. Carcass Zinc and Iron of Rats[a] Fed Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	zinc			iron		
diet code	total, mg	per g fat-free dry tissue, mcg	per g, ash, mcg	total, mg	per g fat-free dry tissue, mcg	per g ash, mcg
PH	2.8 ± 0.6[a]	113 ± 22[a]	739 ± 105[a,b]	2.6 ± 0.4[a]	103 ± 13[a]	681 ± 88[a]
RH	2.6 ± 0.2[a]	104 ± 17[a]	684 ± 109[a]	2.9 ± 0.6[a]	116 ± 211[a]	767 ± 151[a]
PM	3.4 ± 0.8[a]	124 ± 32[a]	896 ± 253[a,b]	2.9 ± 0.2[a]	106 ± 6[a]	764 ± 45[a]
RM	2.7 ± 0.4[a]	107 ± 22[a]	752 ± 100[a,b]	2.9 ± 0.3[a]	115 ± 14[a]	798 ± 53[a]
PL	2.7 ± 0.4[a]	106 ± 17[a]	963 ± 174[a,b]	3.1 ± 0.4[a]	121 ± 8[a]	1095 ± 110[b]
RL	2.7 ± 0.6[a]	112 ± 30[a]	1016 ± 216[b]	2.9 ± 0.3[a]	112 ± 5[a]	1090 ± 92[b]

[a] 5 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

Table IX. Carcass Copper (mcg) and Magnesium (mg) of Rats[a] Fed Phytate-Containing and Phytate-Reduced Formulas at Three Calcium and Phosphorus Levels[b]

	copper			magnesium		
diet code	total	per g fat-free dry tissue	per g ash	total	per g fat-free dry tissue	per g ash
PH	776 ± 125[a]	31 ± 4[a]	206 ± 27[a]	36 ± 1.8[a]	1.4 ± 0.1[a]	9.6 ± 0.4[a]
RH	772 ± 195[a]	31 ± 6[a]	201 ± 38[a]	36 ± 3.0[a]	1.4 ± 0.1[a]	9.5 ± 0.2[a]
PM	740 ± 143[a]	27 ± 4[a]	194 ± 35[a]	34 ± 2.6[a,b]	1.3 ± 0.1[a,b]	9.1 ± 45[a]
RM	735 ± 23[a]	29 ± 2[a]	206 ± 10[a]	31 ± 5.5[a,b]	1.2 ± 0.2[a,b]	8.8 ± 53[a]
PL	757 ± 171[a]	30 ± 7[a]	273 ± 60[b]	29 ± 4.2[b]	1.2 ± 0.1[b]	10.6 ± 1.2[a,b]
RL	802 ± 105[a]	31 ± 4[a]	304 ± 47[b]	30 ± 2.9[b]	1.2 ± 0.3[b]	11.3 ± 1.7[b]

[a] 5 rats/diet. [b] Mean ± standard deviation. Vertical means not sharing common superscript are significantly different ($P < 0.05$).

the total zinc, iron, and copper in carcass or their concentration in fat-free carcass. However, as the dietary calcium and phosphorus decreased, zinc, iron, and copper concentrations in carcass ash increased. Zinc concentration in ash was significantly less in rats fed the RH diet than in rats fed the RL diet. Mean iron and copper concentrations in carcass ash were significantly higher in rats fed low-mineral diets, both PL and RL, than in those fed the other four diets.

Rats fed the low-calcium diets (PL and RL) had significantly less mean total magnesium per carcass than did rats fed the high-mineral diets (PH and RH) (Table IX). The mean concentrations of magnesium in carcass were significantly less, but the mean magnesium concentration in carcass ash was significantly greater in rats fed the low-mineral formulas, PL and RL, than in those fed the diets with higher mineral levels. Concentrations of zinc, iron, copper, and magnesium in the carcass ash and magnesium in the carcass were influenced by the concentration of calcium and phosphorus but not the phytate in the diet.

DISCUSSION

Phytic acid concentration of soy protein isolate formula diet was 320 mg/100 g (Table I). The sample of Isomil analyzed contained approximately 20 g of protein and 430 mg of phytic acid/L of formula as fed (20 kcal/fluid oz) or 21 mg/g of protein.

Phytic acid has been shown to reduce the availability of minerals from a soy protein diet for rats (Nightingale, 1975; Makdani et al., 1975; Likuski and Forbes, 1965). Rats fed phytate-containing diets that were designed to furnish requirements of a rat for zinc and other minerals have been shown to gain less weight, have less bone ash, and retain less zinc, copper, calcium, phosphorus, magnesium, and possibly iron than rats fed similar phytate-free diets (Nightingale, 1975; Likuski and Forbes, 1965; Oberleas and Prasad, 1970; Foy et al., 1959; Davies and Reid, 1979b; Davies and Olpin, 1979a). Thus, rats fed the three phytate-containing formula diets in this study were expected to gain less weight and have less carcass and femur ash and less of each of the minerals in femur and carcass than rats fed the corresponding reduced phytate diets. Furthermore, because the binding capacity of phytic acid is enhanced by higher calcium concentrations in the diet (Oberleas et al., 1962), rats fed the phytate-containing diet with the highest calcium level (PH) were expected to gain less weight and to have less carcass and bone trace mineral content than rats fed either phytate-reduced diets or phytate-containing diets with medium and low levels of calcium. Regardless of the level of the dietary calcium, however, rats fed the three phytate-containing diets were not significantly different in mineral content from rats fed the three reduced-phytate diets. Rats gained a similar amount of weight regardless of diet fed.

Several factors, by themselves or in combination, may have prevented phytic acid from exerting an effect on mineral bioavailability from soy protein isolate fromula in this study. The relatively low level of phytic acid in the formula, the lack of enhancement of the phytic acid binding capacity by calcium, and the presence of phytase in the intestine of the rat may have influenced the mineral bioavailability.

The low phytic acid content of the phytate-containing diets was undoubtedly a major factor for the absence of phytic acid effect on bone mineralization. Although the

phytate-containing diets contained 8 times more phytic acid than the phytate-reduced diets, the phytate content of these diets was only 0.3%. In most studies in which phytic acid has been shown to decrease growth and mineral bioavailability to rats, the diets contained substantially more phytic acid than used in the present study. Likuski and Forbes (1965) found that rats had symptoms of zinc deficiency when fed diets containing 2% phytic acid and 12 ppm zinc but showed no symptoms when fed diets containing 0.4% phytic acid. Makdani et al. (1975) reported that 1% phytic acid was required in the diet to depress growth and decrease availability of calcium, zinc, and copper to rats. The ratio of phytate to zinc affects zinc availability. Lo et al. (1981) observed that bioavailability of zinc was reduced in rats fed soy protein isolate diets containing phytate to zinc molar ratios above 12:1. The phytate to zinc molar ratio of the phytate-containing diets in the present study ranged between 17 and 20 (Table I). In spite of a phytate to zinc ratio above 12, the total zinc content and zinc concentration of dry femurs of rats fed the phytate-containing and phytate-reduced diets were not different. These femur zinc concentrations were also similar to those observed by Moncilovic et al. (1975) in rats fed diets having egg albumin as protein source, no phytate, and a similar concentration of zinc as our diets, 1.2 mg/100 g. Also, the zinc concentrations per gram of bone ash were greater in all groups than those found by Forbes et al. (1984) in rats fed diets containing 0.3% or 0.5% calcium and no phytate and much higher than in rats fed a diet similar to our diet PH containing a phytate to zinc ratio of 20 and 0.5% calcium. Unlike these authors, we saw no decrease in zinc content of bone ash with the increase of phytate to zinc molar ratio, but as they, we saw a decrease with an increase in dietary calcium.

Davies and Nightingale (Nightengale, 1975) found that rats fed diets containing 1 g of phytic acid and 5 mg of iron/100 g diet accumulated significantly less iron than rats fed a similar phytate-free diet. Hunter (1981), however, found that hemoglobin regeneration in anemic rats fed diets containing up to 4% phytic acid was similar at various dietary iron levels to that of rats fed phytic acid free diets. The rats in our study were receiving about one-tenth of this amount of phytate. Thus, that concentration of phytate in the phytate-containing diets did not affect zinc or iron availability for the rat is consistent with the observation made by other researchers.

Calcium in a phytate-containing diet may decrease the bioavailability of other minerals (Likuski and Forbes, 1965). For several animal species, dietary calcium levels equal to or above requirement levels of the animal may, in the presence of dietary phytate, decrease the bioavailability of zinc, magnesium, copper, and iron (Makdani et al., 1975; Rackis, 1974; Savage et al., 1964) as well as that of phytic phosphorus (Oberleas and Prasad, 1970). At low or marginal dietary levels of of calcium, trace mineral absorption may not be affected by phytate. At these lower marginal levels, however, the calcium itself may be sufficently bound by phytate to induce a calcium deficiency in the rat (Likuski and Forbes, 1965). In the present study, dietary calcium in the six formulas was provided at three levels at, above, and below the requirement of the rat. Calcium to phosphorus ratios at all three levels of calcium intake were approximately the same.

An interrelationship between the level of calcium and the presence of phytate was not observed in this study. Rats fed the high-calcium, high-phytate diet did not differ in total femur or carcass copper, magnesium, iron, calcium, or phosphorus contents from rats fed the high-calcium, low-phytate diet. As the dietary calcium and phosphorus were decreased, the total femur and carcass magnesium, calcium, and phosphorus contents were equally less in rats fed the phytate-containing diets and those fed the phytate-reduced diets. This relationship was not apparent for iron, copper, and zinc.

Morris and Ellis (1980) found that concentration of femur zinc was significantly less in rats fed a diet containing a phytate to zinc molar ratio of 20 and 1.75% calcium than those fed a similar ratio with 0.75% calcium. In our study, the concentrations of femur zinc were as high or higher in rats fed the highest calcium level (0.5%) and a phytate to zinc ratio of 20 than that of rats in the above study fed diets with 0.75% calcium and no phytate. It appears that doubling the calcium in the soy-based formula understudy did not enhance the phytate effect on zinc bioavailability.

Phosphorus levels were similar in corresponding diet pairs, but between 22% and 59% of the phosphorus in the higher phytate diets was contributed by phytate (Table I). Phosphorus from the phytate in the diets appeared to be available to rats as shown by the similar carcass and femur phosphorus contents of rats fed the phytate-containing diets and rats fed the phytate-reduced diets. The availability of phytic phosphorus may be, in part, due to the presence of phytase in the intestine of the rat. In 1975, Bitar and Reinhold (1972) demonstrated the presence of phytase (*meso*-inositol hexaphosphate phosphohydrolase) in the small intestine of rats, chickens, and humans.

Infants given sodium phytate in their milk formula absorbed less calcium but more phosphorus than infants fed milk alone (Hoff-Jorgensen et al., 1946a). The increased phosphorus absorption may be a result of the availability of phytic phosphorus or of the increased availability of inorganic phosphorus when calcium became bound with phytic acid. Hoff-Jorgensen et al. (1946b) reported that about 70% of dietary phytic acid was hydrolyzed in the intestine of children fed diets high in phytic acid. Some of the phosphate released in this reaction may have been absorbed. Thus, the availability of phytic phosphorus, through the action of intestinal phytase on the phytate in the soy protein isolate formula, may partially explain the similarity of phosphorus content of femur and carcass in rats fed the three phytate-containing diets and rats fed the three phytate-reduced diets.

Phytate in soy formula diets did not affect weight gain, protein utilization, femur weight, or concentrations of calcium, phosphorus, zinc, iron, magnesium, or copper contents of the femur and carcass of rats. However, calcium and phosphorus levels in the formulas did influence femur weight and femur concentrations of calcium, phosphorus, zinc, iron, magnesium, and copper.

ABBREVIATIONS USED

TCA, trichloroacetic acid; PER, protein efficiency ratio; PH, phytate-containing, high-calcium diet; PL, low-calcium phytate diet; PM, phytate-containing, medium-calcium diet; RH, reduced-phytate, high-calcium diet; RL, reduced-phytate, low-calcium diet; RM, reduced-phytate, medium-calcium diet; SD, standard deviation.

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Registry No. Phytic acid, 83-86-3; magnesium, 7439-95-4; iron, 7439-89-6; zinc, 7440-66-6; copper, 7440-50-8; calcium, 7440-70-2; phosphorus, 7723-14-0.

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