# Bioavailabilities of Zinc from Nonfat Dry Milk, Lowfat Plain Yogurt, and Soy Flour in Diets Fed to Neonatal Pigs<sup>†</sup>

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The bioavailabilities of zinc (Zn) from nonfat dry milk (NDM), lowfat plain yogurt (YOG), and soy flour (SF), relative to zinc carbonate (ZC), were measured in neonatal pigs. Dietary treatments were formulated so that 4, 8, 12, or 16  $\mu$ g of Zn/g of diet was provided by either ZC, NDM, YOG, or SF. Apparent Zn absorption was greater from NDM (83%) and YOG (87%) than from ZC (75%), which in turn was greater than from SF (49%). Relative Zn bioavailability was determined by a comparison of response curves for milligram of Zn retained per day during a 6-day balance study, plasma Zn concentrations on days 21 and 28, and femur Zn concentration. When the results for these parameters were pooled within Zn sources, the bioavailabilities of Zn from NDM, YOG, and SF were 97%, 95% and 7%, relative to ZC. Retentions of oral doses of <sup>65</sup>Zn added to diets containing ZC, NDM, or YOG were similar (91.3%, 89.0%, and 88.9%, respectively) and greater than when the diet contained SF (69.1%). Thus, Zn from SF was absorbed and utilized less effectively than Zn from YOG, NDM, or ZC. In conclusion, the bioavailabilities of Zn in NDM and YOG were very high and similar to that in ZC compared to the very low Zn bioavailability in SF.

A variety of factors are known to influence zinc (Zn) bioavailability (Solomons, 1982; O'Dell, 1985). In general, the Zn in food of plant origin is less bioavailable than that of animal origin (O'Dell et al., 1972). Plant seeds contain phytic acid which forms an insoluble complex with Zn, thereby preventing its absorption (O'Dell and Savage, 1960). Thus, even though a predominantly vegetarian diet may contain a level of Zn that is considered adequate, there may be an insufficient level of available Zn. Dairy products contain from 27 to 65  $\mu$ g of Zn/g on a dry matter basis (Consumer and Food Economics Institute, 1976), and there is some evidence that milk Zn is fairly available (Johnson and Evans, 1978). However, few studies of Zn bioavailability have been conducted with species other than the rat.

This research was undertaken to investigate the bioavailability of Zn in nonfat dry milk (NDM), lowfat plain yogurt (YOG), and soy flour (SF) compared to a standard, zinc carbonate (ZC). Several criteria were utilized to assess Zn absorption and bioavailability to enhance the accuracy of our conclusions. The pig was used as our experimental

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animal model because of the biological and digestive similarities to humans (Dodds, 1982; Miller and Ullrey, 1987).

### PROCEDURE

**Experimental Design.** A total of 80 crossbred male castrate neonatal pigs (Duroc  $\times$  Landrace  $\times$  Yorkshire; University of Missouri Swine Farm, Columbia) ranging in age from 4 to 10 days were used. A randomized complete block design incorporating a 4  $\times$  4 factorial treatment arrangement (4 Zn sources and 4 Zn concentrations) were used for the study. There were three trials, with one pig assigned to each treatment in trial 1 and two pigs assigned to each treatment (blocked according to initial weight) in trials 2 and 3. Thus, data were obtained from five pigs (replicates) on each of the 16 treatments.

Animal Care. The pigs were individually housed in stainless steel cages in an environmentally controlled room. The temperature was maintained at 32 °C during the first week and was gradually lowered to 25 °C by the fifth week. Relative humidity ranged from 40% to 60%. Artificial lighting was provided from 7:30 a.m. through 12:00 midnight. Meals were fed in stainless steel cups. During a 2-day adaptation period, the pigs were fed a semipurified liquid diet similar to the NDM diet containing 16  $\mu$ g of Zn/g of diet used in this study. The experiment was begun by feeding the pigs a low Zn (<1  $\mu$ g/g) basal diet (Table I) for 7 days to deplete body Zn reserves. Then, the pigs were fed their respective experimental diets in six daily meals every 3 h from 8:00 a.m. to 11:00 p.m. At each meal, the pigs were supplied in liquid form (1:2, dietary dry matter/deionized water).

Diet consumption was recorded at each meal. Pigs were weighed and 5-8 mL of blood was collected from the anterior vena cava prior to their 8:00 a.m. meal on days -7, 0, 7, 14, 21, and 28. All pigs were sacrificed on day 28 by lethal injection of sodium pentobarbital (Euthanasia 6, Veterinary Laboratories, Inc., Lenexa, KS). Subsequently, right femurs were removed and frozen for later Zn analysis.

Diets. The formulated diets were adequate in all dietary nutrients, except Zn, for 1-5-kg pigs (NRC, 1979). The compositions of the diets are presented in Table I. Spray-dried chicken egg whites (Kraft Foods Inc., Glenview, IL) were the protein source for the depletion diet ( $<1 \mu g$  of Zn/g) and standard diets because they are low in Zn and phytate. The egg whites were rehydrated and autoclaved to inactivate the avidin and

#### Table I. Composition of Diet-c

zn source: zn level, μg/g:		zinc ca	rbonat	e (ZC) <sup>d</sup>		nonfat dry milk (NDM)				lowfat yogurt (YOG)				soy flour (SF)			
	<1	4	8	12	16	4	8	12	16	4	8	12	16	4	8	12	16
egg white' nonfat dry milk'	24.8	24.8	24.8	24.8	24.8	19.9 9.49	15.0 19.0	10.1 28.5	5.15 38.0	21.1	17.4	13.7	10.0	20.8	16.8	12.8	8.73
yogurt <sup>#</sup> soy flour <sup>h</sup>										6.71	13.4	20.1	26.8	6.84	13.7	20.5	27.3
dextrose	23.5	23.5	23.5	23.5	23.5	21.8	20.0	19.2	16.3	22.8	22.1	21.0	20.1	22.2	20.7	19.1	17.4
corn syrup solids <sup>i</sup>	25.5	25.5	25.5	25.5	25.5	23.8	22.0	21.2	18.3	24.8	24.1	23.0	22.1	24.2	22.7	21.1	19.4
corn oil	15.3	15.3	15.3	15.3	15.3	14.9	14.4	13.9	13.5	14.4	13.2	12.5	11.6	15.9	16.5	17.2	17.9
CaHPO <sub>4</sub> ·H <sub>2</sub> O	4.22	4.22	4.22	4.22	4.22	3.76	3.29	2.83	2.37	3.91	3.60	3.29	2.99	4.17	4.11	4.00	3.82
cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.78	1.56	1.35	1.13
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.29	0.29	0.29	0.29	0.29	0.31	0.33	0.35	0.37	0.30	0.31	0.32	0.33	0.18	0.06		
MgSO4 7H2O	0.21	0.21	0.21	0.21	0.21	0.15	0.09	0.03		0.17	0.13	0.09	0.04	0.06			
CaCO <sub>3</sub>																0.04	
NaCl D,L-methionine									0.06								0.02 0.05
L-threonine L-tryptophan													0.03 0.02	0.07			
by analysis crude protein, % zinc, μg/g	20.8 0.84	20.8 5.30	20.8 10.0	20.8 13.1	20.8 18.1	19.6 4.78	18.6 8.61	19.0 12.7	25.3 16.7	19.3 4.79	18.6 8.70	20.0 12.6	20.0 16.9	20.8 4.68	21.4 8.79	21.9 12.0	22.3 15.6

<sup>a</sup> All diets were formulated to contain 1.40% lysine, 1.11% calcium, 0.94% phosphorus, and 3.22 Mcal of digestible energy per kilogram on a dry matter basis. <sup>b</sup> All diets also contained 2.0% vitamin/mineral mix of the following composition, on a dry matter basis: ferric citrate (17% Fe), 4910 mg/100 g; <sup>c</sup>CuSO<sub>4</sub>·5H<sub>2</sub>O, 131 mg/100 g; MnSO<sub>4</sub>·H<sub>2</sub>O, 68.3 mg/100 g; Na<sub>2</sub>SeO<sub>3</sub>, 3.65 mg/100 g; KlO<sub>3</sub>, 1.31 mg/100 g; L-ascorbate, 1250 mg/100 g; DL-α-tocopheryl acetate, 367 mg/100 g; nicotinic acid, 184 mg/100 g; calcium D-pantothenate, 109 mg/100 g; vitamin A acetate (500 IU/mg), 36.7 mg, 100 g; riboflavin, 25 mg/100 g; menadione sodium bisulfite, 23.8 mg/100 g; pyridoxine hydrochloride, 12.5 mg/100g; thiamin hydrochloride, 10.9 mg/100g; folic acid, 5.0 mg/100 g; D-biotin, 0.835 mg/100 g; vitamin B<sub>12</sub>, 0.184 mg/100 g; cholecalciferol (40 000 IU/mg), 0.0458 mg/100g; butylated hydroxy anisole (antioxidant) 113 mg/100g; terramycin concentrate (22.5% oxytetracycline hydrochloride), 3660 mg/100 g; Neomix 325 (71.5% neomycin sulfate), 1650 mg/100 g; dextrose was the carrier. <sup>c</sup> All diets also contained 0.18% of a premix which provided 600 mg of choline chloride/kg of diet. <sup>d</sup> Egg white diets contained 2.0% of a premix which provided 0, 4, 8, 12, or 16 µg of Zn/g of diet as ZnCO<sub>2</sub>. Dextrose was the carrier. <sup>e</sup> Spray-dried egg whites, Kraft Foods Inc., Glenview, IL. <sup>i</sup> Nonfat dry milk solids, Kraft Foods Inc., Glenview, IL. <sup>g</sup> Lowfat plain yogurt, Mid-American Farms Dairy, Lebanon, MO. <sup>h</sup> Textured vegetable protein, unflavored-nonfortified, Archer Daniels Midland Co., Decatur, IL. <sup>i</sup> Star-Dry 24R, A. E. Staley Manufacturing Co., Decatur, IL. <sup>j</sup> Solka Floc, Teklad Test Diets, Madison, WI.

trypsin inhibitors which could interfere with swine growth (Watkins, 1986). Standard diets were prepared by adding appropriate amounts of the available inorganic Zn supplement, ZC (Sullivan, 1961; Ammerman and Miller, 1972), to the basal diet, producing diets containing 4, 8, 12, and 16  $\mu$ g of added Zn/g of diet dry matter. These concentrations of Zn fall within a range that is below the requirement (Hankins et al., 1985) and were intended to result in linear improvements in response criteria associated with increasing concentration of dietary Zn. NDM (Kraft Foods) YOG (Mid-American Farms, Lebanon, MO), and SF (food grade textured vegetable protein, Archer Daniels Midland Co., Decatur, IL) replaced egg whites in the basal diet to make diets with the same concentration of Zn as in the standard diets. Zinc analysis revealed that NDM, YOG, and SF contained 24, 33, and 55  $\mu$ g of Zn/g of dry matter, respectively. All diets were formulated to contain similar concentrations of digestible energy, fiber, lysine, calcium, phosphorus, magnesium, potassium, iron, and copper. The protein sources were added in amounts that contributed the appropriate amount of Zn and met the requirement for essential amino acids (NRC, 1979). Although all diets were formulated to be adequate in protein, the crude protein concentration varied between 18% and 25% among the different diets.

Measurement of Apparent Zn Absorption. Total feces and urine were collected from individual pigs on days 20 and analyzed for Zn. A 6-day Zn balance was calculated by subtracting the Zn excreted on days 14-20 from that consumed on days 13-19.

Measurement of <sup>65</sup>Zn Retention. A pulse dose of isotopically labeled Zn (<sup>65</sup>ZnSO<sub>4</sub>, University of Missouri-Research Reactor) was fed along with a meal on day 21. <sup>65</sup>Zn (1, 2, 3, or  $4 \mu$ Ci) was mixed into the diets containing 4, 8, 12, or 16  $\mu$ g Zn/g, respectively, immediately before feeding. The isotope was diluted to 1  $\mu$ Ci/mL before being mixed with approximately 25 g of the appropriate liquid diet. After consuming this portion, the pig was fed its diet as usual. One hour after isotope administration, the pigs were placed in a whole body  $\gamma$ -scintillation detector [described by Tumbleson et al. (1968)], and the amount (disintegrations per minute) of <sup>66</sup>Zn in the animal was measured. The pigs were continued on their normal dietary regimen and assayed for radioactivity at 3, 4, 5, and 6 days postadministration. The percentage of Zn retained each day was determined by comparing daily counts with initial radioactivity, after both values had been corrected for background and counter efficiency. Log of percent isotope retained was plotted against time, and the y intercept of the regression lines was calculated to determine the percent of  $^{65}$ Zn retained from the labeled meal (Heth and Hoekstra, 1965).

Relative Zn Bioavailability. Bioavailability was calculated for each Zn source by plotting each of several indices of Zn status (growth, plasma Zn on days 21 and 28, milligrams of Zn retained during the balance period, and femur Zn concentration) against dietary Zn intake for comparison with the reference material, ZC. Gordon and Chao (1984) concluded that using actual food intake values was more appropriate than the theoretical amount of nutrient added by the test sources when calculating the relative biological value. Therefore, Zn intake was determined by multiplying the amount of food consumed during the appropriate period by the analyzed Zn content of the diet. Growth rate and bone Zn were plotted against Zn consumption days 0-27, Zn retention during the balance study was plotted against Zn consumption days 0-20, and plasma Zn concentrations were plotted against Zn consumption during the week preceding the blood sampling. These times were selected because they represent the periods during which Zn intake would have the greatest influence upon that respective parameter. The slope of the response to increasing concentration of Zn intake from each Zn source was calculated for each parameter. Relative bioavailabilities were estimated by comparing the slopes of these curves for the three test sources to that of ZC (Miller et al., 1981; Wedekind and Baker, 1990).

**Zn Analysis.** Feed samples were dried, ashed in a muffle furnace at 500 °C, treated with hot nitric acid, dry-ashed again, dissolved in hydrochloric acid, and appropriately diluted with deionized distilled water for analysis by flame atomic absorption spectrophotometry (FAAS) (Model 2380, Perkin-Elmer, Norwalk, CT). Recovery of Zn from the National Bureau of Standards' bovine liver samples was 98.31% (SE = 0.55). Feces

Table II. Average Daily Food Consumption per Pige-o

	Zn source										
Zn, µg/g	zinc carbonate (ZC)	nonfat dry milk (NDM)	lowfat yogurt (YOG)	soy flour (SF)							
4	82*	81*	100*	64ª							
8	129 <sup>a,b</sup>	1 <b>49</b> ª	183ª	87 <sup>b</sup>							
12	169ª	200 <sup>a</sup>	201ª	106 <sup>b</sup>							
16	175	226ª	206ª	101 <sup>b</sup>							
mean <sup>d</sup>	139*	164 <sup>a,d</sup>	172 <sup>b</sup>	89°							

<sup>a</sup> Values are means of the grams of dry matter consumption per day during the 28-day period which followed a 7-day depletion period. There were five pigs per treatment, except for the 8  $\mu$ g of Zn/g of diet containing SF which had n = 4. <sup>b</sup> Treatment means within a row (Zn level) that do not share a common supescript are different (P < 0.05) as determined by the least significant difference test. Standard error of the means was 21 g/day. <sup>c</sup> Linear, quadratic, and cubic effects of increasing Zn concentration from each source (within columns) were tested. Linear increases in diet consumption were observed (P < 0.05) with increasing concentration of Zn in diets containing ZC, NDM, and YOG. <sup>d</sup> Zinc source means that do not share a common superscript are different (P < 0.05) as determined by the least significant difference test. Standard error of the means was 11 g/day.

were dried, ground, and analyzed as described above. Whole femurs were cut transversely, defatted with a chloroform/ methanol (2:1) solution, and similarly analyzed. Urine was centrifuged at 1000g for 20 min and analyzed directly. Proteins were precipitated from plasma with 10% trichloroacetic acid, and the supernatant was analyzed directly by FAAS.

Statistical Analysis. The bioavailability data were analyzed as a randomized complete block in which the treatments were arranged as a  $4 \times 4$  factorial (4 Zn sources  $\times 4$  Zn concentrations). Linear, quadratic, and cubic effects of increasing Zn from each source were tested (Linear, Quadratic and Cubic Orthogonal Contrast Statistics, Agricultural Experiment Station, University of Missouri) and reported when significant (P < 0.05 or 0.01). Linear contrasts were performed on the bioavailability parameters to determine differences among Zn sources over increasing concentrations of Zn intake. Where appropriate, treatment effects were measured with the least significant difference test (Snedecor and Cochran, 1980).

#### RESULTS

Diet Consumption (Table II). Average daily diet consumption was similar (P > 0.10) among Zn sources containing 4  $\mu$ g of Zn/g of diet. When dietary Zn was 8  $\mu$ g/g, consumption of the SF diet was less (P < 0.05) than that of the NDM and YOG diets. Consumption of the SF diets was also less (P < 0.05) than that of the other three Zn sources containing 12 and 16  $\mu$ g of Zn/g of diet. Consequently, linear increases (P < 0.05) in food intake were observed as the concentration of Zn in the diet increased in pigs fed ZC, NDM, and YOG but not in pigs fed SF.

Growth Rate (Figure 1). Linear increases in average daily gains occurred with increasing Zn intake in pigs consuming diets with ZC, NDM, and YOG (P < 0.01) but not SF (P > 0.10). The linear increases in growth rate that were observed with increasing Zn intake were alike among all Zn sources (P > 0.10). The slopes of the growth responses for NDM, YOG, and SF were 124%, 114%, and 98%, respectively, compared to that of ZC.

Feed Efficiency (Table III). Diets containing SF generally supported gain less efficiently (P < 0.05) than the other three Zn sources within each Zn level. Increases in Zn concentration from all Zn sources were associated with significant linear increases (P < 0.05) in feed efficiency. The linear increases for ZC, NDM, and YOG were similar (P > 0.10) and greater (P < 0.05) than those for SF. SF also had a cubic effect (P < 0.05).

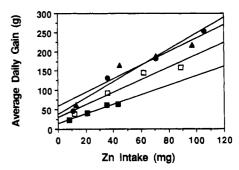


Figure 1. Average daily gain vs total amount of Zn consumed between days 0 and 27 by pigs fed diets containing 4, 8, 12, and 16  $\mu$ g of Zn/g of diet from ZC ( $\Box$ ), NDM ( $\odot$ ), YOG ( $\blacktriangle$ ), and SF ( $\blacksquare$ ). The slopes of the dose-response curves were 1.24 for NDM, 1.14 for YOG, and 0.98 for SF, relative to 1.0 for ZC.

Table III. Average Feed Efficiencies\*\*

Zn, μg/g	Zn source										
	zinc carbonate (ZC)	nonfat dry milk (NDM)	lowfat yogurt (YOG)	soy flour (SF)							
4	194 <sup>a,b</sup>	310*	366*	81 <sup>b</sup>							
8	460 <b>*</b>	589ª,b	747 <sup>6</sup>	450 <b>*</b>							
12	711•	791*	844•	287 <sup>b</sup>							
16	771•	1019 <sup>b</sup>	969 <sup>a,b</sup>	424°							
meand	53 <b>4</b> •	677 <sup>b</sup>	732 <sup>b</sup>	303°							

<sup>a</sup> Feed efficiencies were calculated as the grams of weight gained per kilogram dry matter consumed during a 28-day period which followed a 7-day depletion period. Values are means. There were five pigs per treatment, except with the 8  $\mu$ g of Zn/g of diet containing SF where n = 4. <sup>b</sup> Treatment means within a row (Zn level) that do not share a common superscript are different (P < 0.05) as determined by the least significant difference test. Standard error of the means was 76 g/kg. <sup>c</sup> Linear, quadratic, and cubic effects of increasing Zn concentration from each source (within columns) were tested. Linear improvements in feed efficiency occurred (P < 0.05) with increasing concentration of Zn in the diet from all four sources. A cubic effect was also observed for SF. The linear improvements were similar (P > 0.10) for ZC, NDM, and YOG, which in turn were greater (P < 0.05) than those obtained for SF. <sup>d</sup> Zinc source means that do not share a common superscript are different (P < 0.05) as determined by the least significant difference test. Standard error of the means was 38 g/kg.

Apparent Zn Balance (Table IV). All pigs consumed increasing amounts (P < 0.05) of Zn as their dietary Zn concentration increased, regardless of Zn source fed. However, pigs fed SF had greater increases (P < 0.01) in fecal Zn excretion with increasing dietary Zn than pigs fed ZC, NDM, and YOG. Therefore, as dietary Zn intake (milligrams per day) increased, pigs fed the ZC, NDM, and YOG diets had linear increases (P < 0.01) in the milligrams of Zn absorbed and retained per day compared with the flat response for pigs fed SF. When the values for all pigs within each Zn source were pooled ( $\bar{x}$  in Table IV), the milligrams of Zn consumed per day was less (P< 0.01) and the fecal Zn (micrograms per day) was greater (P < 0.01) for SF than for ZC, NDM, and YOG. Consequently, the milligrams of Zn absorbed per day was greater (P < 0.01) for ZC, NDM, and YOG than for SF. Pigs fed ZC also had more (P < 0.05) fecal Zn (micrograms per day) than those fed YOG, which resulted in less (P < 0.01)Zn absorption from ZC than YOG. Urinary Zn (micrograms per day) was higher (P < 0.01) in pigs fed ZC than in pigs fed NDM, YOG, and SF. Thus, the milligrams of Zn retained per day and the milligrams of Zn absorbed and retained expressed as percentages of Zn consumed were greater (P < 0.05) for NDM and YOG than for ZC, which in turn was greater (P < 0.01) than for SF.

Figure 2 presents the response curves of Zn retention

Table IV. Effect of Distary Zinc Source and Concentration on Zinc Balance from Day 14 to Day 204

Zn source: Zn level, μg/g:	zinc carbonate (ZC)						nonfat dry milk (NDM)				lowfat yogurt (YOG)					soy flour protein (SF)					pooled
	4	8	12	16	ž٥	4	8	12	16	£	4	8	12	16	£	4	8	12	16	£	SEM
Zn ingested, mg/day	0.46	1.43	2.33	3.60	1.95*	0.35	1.40	3.20	4.36	2.34ª	0.58	1.95	3.29	4.07	2.47*	0.25	0.80	1.42	1.71	1.06 <sup>b</sup>	0.42
fecal Zn, <sup>d</sup> µg/day	175	421	309	495	350*	106	272	296	304	245 <sup>a,b</sup>	215	254	205	150	206 <sup>b</sup>	85	404	680	963	557°	102
Zn absorbed, mg/day	0.28	1.01	2.02	3.10	1.60*	0.25	1.13	2 <b>.9</b> 0	4.11	2.10 <sup>a,b</sup>	0.36	1.70	3.09	3.92	2.27 <sup>b</sup>	0.16	0.39	0.74	0.74	0.53°	0.40
urine Zn/ µg/day	34	40	83	94	62•	17	38	40	30	316	14	30	27	57	32 <sup>b</sup>	22	32	39	58	39 <sup>6</sup>	12.0
Zn retained, mg/day	0.25	0.97	1.94	3.01	1.54*	0.23	1.09	2.86	4.06	2.07 <sup>b</sup>	0.35	1.67	3.06	3.87	2.24 <sup>b</sup>	0.14	0.36	0.70	0.69	0. <b>49°</b>	0.40
apparent absorption, %	56.3	74.1	85.9	84.9	75.3 <b>*</b>	70.8	77.1	91.0	93.0	83.0 <sup>b</sup>	67.2	88.7	94.9	96.9	86.9 <sup>b</sup>	62.0	45.0	<b>49</b> .5	43.7	49.4°	5. <b>94</b>
retained/ absorbed,# %	86.4	95.9	<b>9</b> 5.7	96.9	93.7•	91.0	94.7	<b>96.</b> 3	99.2	95.8 <sup>a,b</sup>	96.4	98.3	99.0	98.6	96.1 <sup>ь</sup>	83.9	83.2	91.8	88.5	87.2°	2.54
retained/ ingested,* %	49.1	71.0	82.1	82.3	71.1•	64.5	73.4	89.5	92.3	79.9 <sup>ь</sup>	64.8	87.2	93.9	95.6	85.4 <sup>ь</sup>	52.3	39.0	45.8	38.8	43.5°	6. <b>96</b>

<sup>a</sup> The balance study was conducted as described in the text. Values are means. There were five pigs per treatment, except the SF 4  $\mu$ g of Zn/g of diet which had n = 4. <sup>b</sup> The average values of the Zn sources are the means of 20 pigs, except SF which had n = 19. Zn source means with different superscripts within rows are different (P < 0.05) as determined by the least significant difference test. <sup>c</sup> Similar linear increases (P < 0.01) in Zn ingested for ZC, NDM, and YOG with increasing concentration of dietary Zn, which had greater slopes (P < 0.05) than the flatter linear response obtained for SF (P < 0.05). <sup>d</sup> SF had a linear increase (P < 0.01) in fecal Zn with increasing concentration of dietary Zn, which differed (P < 0.05) from the flat response obtained for SF. (P < 0.01) from the flat response (P < 0.01) for ZC, NDM, and YOG. <sup>e</sup> Similar linear increases (P < 0.01) for ZC, NDM, and YOG, and SF (P < 0.05) the slopes obtained for SF. (P < 0.05) the flat response obtained for NDM differed (P < 0.05) but not NDM (P > 0.05, with increasing concentration of dietary Zn, which differed (P < 0.05) but not NDM (P > 0.05), with increasing concentration of dietary Zn, which differed (P < 0.05) but not NDM (P > 0.05, with increasing concentration of dietary Zn, which differed (P < 0.05) but not NDM (P > 0.05, with increasing concentration of dietary Zn, which differed (P < 0.05) to the slopes for YOG and SF. <sup>e</sup> Similar linear increases (P < 0.05) for ZC and NDM, but not YOG or SF, with increasing concentration of dietary Zn. All Zn source had similar (P < 0.05) slopes.

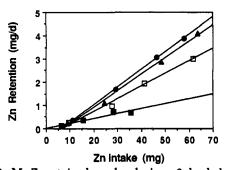


Figure 2. Mg Zn retained per day during a 6-day balance study (days 14-20) vs total amount of Zn consumed between days 0 and 20 by pigs fed diets containing 4, 8, 12, and 16  $\mu$ g of Zn/g of diet from ZC ( $\Box$ ), NDM ( $\odot$ ), YOG ( $\blacktriangle$ ), and SF ( $\blacksquare$ ). The slopes of the dose-response curves were 1.19 for NDM, 1.17 for YOG, and 0.47 for SF, relative to 1.0 for ZC.

during the balance study plotted against Zn intake, to compare relative bioavailabilities. The milligrams of Zn retained per day increased linearly with increasing dietary Zn consumed from day 0 to day 20 among pigs fed ZC, NDM, and YOG (P < 0.01) but not SF (P > 0.10). The slopes of the dose-response curves for NDM, YOG, and SF were 119%, 117%, and 47%, relative to ZC.

**Retention of** <sup>65</sup>Zn (Figure 3). The percentages of <sup>65</sup>Zn retained by pigs on the 4, 8, 12, and 16  $\mu$ g of Zn/g diets within each Zn source were alike (P > 0.05), so the values were pooled for each day. By day 3 the decline in radioactivity of the pigs was linear, apparently due to the endogenous excretion of <sup>65</sup>Zn absorbed from the pulse dose. The percentage of <sup>65</sup>Zn retained from the SF diet was less (P < 0.01) than that retained from the ZC, NDM, and YOG diets (66.0 vs 89.2, 92.8, and 86.4, respectively). The slopes of the retention curves (Figure 3) were alike (P >0.10); thus, the biological half-life of <sup>65</sup>Zn was not affected by diet.

**Plasma Zn.** Plasma Zn concentrations on day 21 (Figure 4) and day 28 (Figure 5) increased linearly (P < 0.01) with increasing amounts of dietary Zn consumed during the week immediately preceding sampling from ZC, NDM, and YOG but not SF (P > 0.10). The linear increases were similar (P > 0.10) for ZC, NDM, and YOG, which in turn were higher (P < 0.05) than the flatter slope

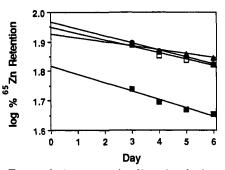


Figure 3. Extrapolating regression lines for the log of percent <sup>66</sup>Zn retained on days 3–6 back to day 0 (after administration of an oral pulse dose) indicated the percent of <sup>65</sup>Zn retained from the original meal to be 92.8% for NDM ( $\oplus$ ), 89.2% for ZC ( $\Box$ ), 86.4% for YOG ( $\blacktriangle$ ), and 66.0% for SF ( $\blacksquare$ ). Results for pigs on the 4, 8, 12, and 16  $\mu$ g of Zn/g of diet within each Zn source were pooled for each day.

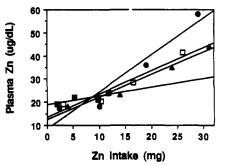


Figure 4. Plasma Zn concentration on day 21 vs total amount of Zn consumed days 14-20 by pigs fed diets containing 4, 8, 12, and 16  $\mu$ g of Zn/g of diet from ZC ( $\Box$ ), NDM ( $\odot$ ), YOG ( $\blacktriangle$ ), and SF ( $\blacksquare$ ). Slopes of the dose-response curves were 1.24 for NDM, 0.97 for YOG, and 0.28 for SF, relative to 1.0 for ZC.

obtained for SF on day 21 and the negative slope obtained for SF on day 28. Significant quadratic effects (P < 0.05) were also observed for NDM and YOG on day 21 and for NDM on day 28. The plasma Zn concentration slopes for NDM, YOG, and SF, respectively, were 124%, 97%, and 28% of that for ZC on day 21 and 72%, 80%, and -23% of that for ZC on day 28.

**Femur Zn.** The concentrations of Zn in the femurs of pigs on day 28 of the experiment were alike (P > 0.10)

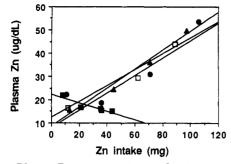


Figure 5. Plasma Zn concentration on day 28 vs total amount of Zn consumed between days 21 and 27 by pigs fed diets containing 4, 8, 12, and 16  $\mu$ g of Zn/g of diet from ZC ( $\Box$ ), NDM ( $\triangle$ ), YOG ( $\oplus$ ), and SF ( $\blacksquare$ ). Slopes of the dose-response curves were 0.72 for NDM, 0.80 for YOG, and -0.23 for SF, relative to 1.0 for ZC.

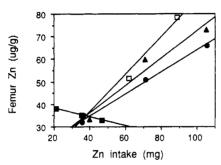


Figure 6. Zn concentration of dry, fat-free femurs on day 28 vs total amount of Zn consumed between days 0 and 27 by pigs fed diets containing 8, 12, and 16  $\mu$ g of Zn/g of diet from ZC ( $\Box$ ), NDM ( $\bullet$ ), YOG ( $\blacktriangle$ ), and SF ( $\blacksquare$ ). Relative slopes of the dose-response curves were 0.74 for NDM, 0.85 for YOG, and -0.21 for SF, relative to 1.0 for ZC.

among pigs consuming the diets with 4 and 8  $\mu$ g of Zn/g within each Zn source. Femur Zn increased (P < 0.01) linearly with increasing amount of dietary Zn consumed from day 0 to day 27 at the 8, 12, and 16  $\mu$ g of Zn/g of diet levels for pigs on the ZC, NDM, and YOG diets but not SF (P > 0.10) (Figure 6). A quadratic effect also occurred (P < 0.05) for ZC. Because the 4  $\mu$ g of Zn/g of diet values were not within the linear range of the response curves, they were not used in calculating slopes. The slopes of the lines for NDM, YOG, and SF were 74%, 85%, and -21%, respectively, relative to ZC. The slope for SF was less than that of YOG (P < 0.01), ZC (P < 0.05), and NDM (P < 0.10). The slope for NDM was also less (P < 0.05) than that for YOG.

## DISCUSSION

Relative Zn bioavailability was calculated for each Zn source on the basis of the linear response obtained between increasing Zn intake and the concentration of Zn in bone, Zn retained during a balance study, and the concentrations of plasma Zn on days 21 and 28. When the values for these criteria were pooled, the relative Zn bioavailabilities (compared with ZC) from NDM, YOG, and SF were 97%, 95%, and 7%, respectively. Thus, the Zn in ZC, NDM, and YOG is equally available, whereas most of the Zn in SF is unavailable. Plasma and bone Zn concentrations on day 28 suggest that SF had a negative impact upon Zn bioavailability by the end of our experiment.

Zn that enters the intestine, whether its origin is endogenous or dietary, enters a common Zn pool (Pekas, 1966; Nutrition Reviews, 1981). Diets with components that contain phytic acid, such as SF, impede the absorption of Zn from the intestine (O'Dell and Savage, 1960). Therefore, endogenously secreted Zn may become complexed to phytic acid in the intestine and be excreted with the feces. Additionally, Berger and Schneeman (1986) concluded that because casein was more readily digested by rats than soy protein isolate or egg white, then less carboxypeptidase (and Zn) was secreted into the gastrointestinal tract. Therefore, not only is more endogenous Zn secreted in response to the soy flour, but this Zn may become insolubly bound to phytate and excreted with the feces (O'Dell, 1979). Under this scenario, dietary SF has a net negative effect on Zn utilization and Zn status. According to Davies and Olpin (1979) and Lo et al. (1981), this might occur when the phytate: Zn molar ratio exceeds 10:1. This ratio in SF has been reported to be 39:1 (Davies and Reid, 1979). While the phytate:Zn molar ratio remained constant at all four dietary levels of SF in our study, the diet with 16  $\mu$ g of Zn/g of diet had the highest total phytate content and would be expected to sequester the most endogenous Zn. This concept is supported by the plateauing or depression in Zn balance, bone Zn concentration, and plasma Zn concentration on days 21 and 28 observed as the concentration of SF in the diet increased.

An effect upon endogenous Zn excretion should manifest itself by affecting the biological half-life of absorbed Zn. However, the slopes of the  $^{65}$ Zn retention curves are alike among the four Zn sources. This implies that they have similar biological half-lives, which might be construed as evidence that SF did not influence endogenous Zn secretion. However, 7–10 days are required before dietary  $^{65}$ Zn equilibrates with the endogenous Zn pool that is most influenced by phytate (Oberleas, 1986). This would have been undetectable in this study, because  $^{65}$ Zn retention was measured for only 6 days postadministration.

The Zn absorption and  $^{65}$ Zn retention values obtained in this experiment were relatively high compared to other published results (Johnson and Evans, 1978; Sandstrom et al., 1982; Lonnerdal et al., 1984). In our experiment, the pigs were young with a high potential for growth. All the pigs were in some degree of Zn deficiency during the time that the pulse dose of  $^{65}$ Zn was administered. Zn absorptive mechanisms are enhanced during periods of Zn deficiency (Smith and Cousins, 1980; Hallmans et al., 1987), contributing to the higher isotope retention we observed.

In conclusion, Zn was absorbed and retained very well from ZC, NDM, and YOG, but relatively poorly from SF because of the greater fecal losses. Zn bioavailability, which includes utilization (O'Dell, 1984), was similar from ZC, NDM, and YOG but negligible from SF, as determined by sensitive indices of Zn status.

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### LITERATURE CITED

- Ammerman, C. B.; Miller, S. M. Biological availability of minor mineral ions: a review. J. Anim. Sci. 1972, 35, 681–694.
- Berger, J.; Schneeman, B. O. Stimulation of bile-pancreatic zinc, protein and carboxypeptidase secretion in response to various proteins in the rat. J. Nutr. 1986, 116, 265-272.
- Consumer and Food Economics Institute. In Composition of Foods: Dairy and Egg Products, Raw-Processed-Prepared; Agriculture Handbook 8-1; Agriculture Research Service, USDA: Washington, DC, 1976.
- Davies, N. T.; Olpin, S. E. Studies on the phytate:zinc molar contents in diets as a determinant of Zn availability to young rats. Br. J. Nutr. 1979, 41, 590-603.

- Davies, N. T.; Reid, H. An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soyabased textured-vegetable-protein meat-substitutes or meatextenders. Br. J. Nutr. 1979, 41, 579–589.
- Dodds, W. J. The pig model for biomedical research. Fed. Proc. 1982, 41, 247-256.
- Gordon, D. T.; Chao, L. S. Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. J. Nutr. 1984, 114, 526-535.
- Hallmans, G.; Nilsson, U.; Sjostrom, R.; Wetter, L.; Wing, K. The importance of the body's need for zinc in determining Zn availability in food: a principle demonstrated in the rat. Br. J. Nutr. 1987, 58, 59-64.
- Hankins, C. C.; Veum, T. L.; Reeves, P. G. Zinc requirement of the baby pig when fed wet-autoclaved spray-dried egg albumen as the protein source. J. Nutr. 1985, 115, 1600-1612.
- Heth, D. A.; Hoekstra, W. G. Zinc-65 absorption and turnover in rats. I. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. J. Nutr. 1965, 85, 367-374.
- Johnson, P. E.; Evans, G. W. Relative zinc availability in human breast milk, infant formulas, and cow's milk. Am. J. Clin. Nutr. 1978, 31, 416-421.
- Lo, G. S.; Settle, S. L.; Steinke, F. H.; Hopkins, D. T. Effect of phytate:zinc molar ratio and isolated soybean protein on zinc bioavailability. J. Nutr. 1981, 111, 2223-2235.
- Lonnerdal, B.; Cederblad, A.; Davidsson, L.; Sandstrom, B. The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. Am. J. Clin. Nutr. 1984, 40, 1064–1070.
- Miller, E. R.; Ullrey, D. E. The pig as a model for human nutrition. Annu. Rev. Nutr. 1987, 7, 361–382.
- Miller, E. R.; Ku, P. K.; Hitchcock, J. P.; Magee, W. T. Availability of zinc from metallic zinc dust for young swine. J. Anim. Sci. 1981, 52, 312-315.
- NRC. In Nutrient Requirements of Swine, 8th ed.; National Academy of Sciences-National Research Council: Washington, DC, 1979.
- Nutrition Reviews. On the entero-pancreatic circulation of endogenous zinc. Nutr. Rev. 1981, 39, 162-163.
- Oberleas, D. The effect of phytate on endogenous zinc and zinc homeostasis. In *Trace Elements in Man and Animals— TEMA* 5; Mills, C. F., Bremner, I., Chesters, J. K., Eds.; Commonwealth Agricutural Bureaux: Farnham Royal, U.K., 1986.

- O'Dell, B. L. Effect of soy protein on trace mineral availability. In Soy Protein and Human Nutrition; Wilcke, B. H., Hopkins, D. T., Waggle, D. H., Eds.; Academic Press: New York, 1979.
- O'Dell, B. L. Bioavailability of trace elements. Nutr. Rev. 1984, 42, 301–307.
- O'Dell, B. L. Bioavailability of and interactions among trace elements. In *Trace Elements in Nutrition of Children*; Chandra, R. K., Ed.; Vevey/Raven Press: New York, 1985.
- O'Dell, B. L.; Savage, J. E. Effects of phytic acid on zinc bioavailability. Proc. Soc. Exp. Biol. Med. 1960, 103, 304-305.
- O'Dell, B. L.; Burpo, C. E.; Savage, J. E. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 1972, 102, 653–660.
- Pekas, J. C. Zinc 65 metabolism: gastrointestinal secretion by the pig. Am. J. Physiol. 1966, 211, 407-413.
- Sandstrom, B.; Keen, C. L.; Lonnerdal, B. An experimental model for studies of zinc bioavailability from milk and infant formulas using extrinsic labeling. Am. J. Clin. Nutr. 1983, 38, 420–428.
- Smith, K. T.; Cousins, R. J. Quantitative aspects of zinc absorption by isolated, vascularly perfused rat intestine. J. Nutr. 1980, 110, 316–323.
- Snedecor, G. W.; Cochran, W. G. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, 1980.
- Solomons, N. W. Factors affecting the bioavailability of zinc. J. Am. Dietet. 1982, 80, 115-121.
- Sullivan, T. W. The availability of zinc in various compounds to broad breasted bronze poults. *Poult. Sci.* 1961, 40, 340-344.
- Tumbleson, M. E.; Dommert, A. R.; Wescott, R. B.; Coffman, W. J. Effects of administration route upon iron-59 retention in miniature swine as measured in a 2-pi liquid scintillation whole body counter. J. Anim. Sci. 1968, 27, 83–87.
- Watkins, K. L. Egg Albumen as a Protein Source for Neonatal Pigs, Master's Thesis, University of Missouri, Columbia, MO, 1986.
- Wedekind, K. J.; Baker, D. H. Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci. 1990, 68, 684–689.

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