BIOAVAILABILITY AND FUNCTIONALITY (BREADMAKING) OF ZINC IN VARIOUS ORGANIC AND INORGANIC SOURCES[1]

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ABSTRACT

Cereal Chem. 54(3): 496-502

Bioavailability of zinc from wheat flour and various organic (acetate and stearate) and inorganic (carbonate, chloride, oxide, sulfate, and elemental) sources was determined using young rats fed submarginal (9.5 ppm) levels of zinc. Although 9.5 ppm zinc supported maximum growth, tissue (serum and femur) zinc levels differed somewhat between sources; they were lowest, however, only in wheat-fed rats. Zinc absorption from all sources, except carbonate, did not differ much. Increasing dietary zinc level to 11.5 ppm caused a most pronounced increase in total femur zinc; some increase in serum zinc level was also noted. Based on growth response, absorption and retention (tissue concentration) of zinc, it may be inferred that zinc from all nonwheat sources was equally well available. In breads made by sponge-dough procedure, none of the zinc sources tested (2.2 mg zinc/100 g flour) exerted any adverse effect on loaf volume and general bread quality including the flavor.

The necessity of zinc (Zn) in animal and human nutrition is well established (1–4). Zn forms an essential part of many metalloenzymes (4). For the human adult, about 15 mg of Zn per day has been suggested as required (2–5). Advances in knowledge of the metabolic role of Zn (1–5) and reports on its inadequacy in the diet of a major segment of the U.S. population (3,6) have led to the suggestion that cereal-grain products be fortified (6) with Zn. However, little is known of the potential of various Zn sources to meet dietary needs. Studies were thus undertaken to examine the bioavailability and, to a limited extent, the functionality (breadmaking) of various organic and inorganic sources.

MATERIALS AND METHODS

Sprague-Dawley rats (weanling, male) averaging about 40 g initially were housed individually in mesh-bottom stainless-steel cages and in a clean-controlled environment. Diets premixed with water to minimize wastage, and deionized water were offered (9 rats per diet, preliminary experiment; 8 rats per diet, main experiment) *ad libitum* for 2 (preliminary experiment) or 4 (main experiment) weeks. Body weight gain and diet intake records were kept.

Table I lists the composition of basal (A) and test (B–J) diets. Basal (Zn-deficient) diet contained 1.25 ppm of Zn. All other diets, except diet J, contained an additional 8.25 ppm Zn; diet J contained an additional 10.25 ppm Zn.

Assessment of bioavailability was based on growth response, apparent absorption (feces collected quantitatively in the final week) and retention (tissue concentration) of Zn. Zn and calcium in diet, Zn in dried-pulverized feces, blood serum (blood collected by heart puncture), bone (femur), and kidney were determined by the method of atomic absorption spectrophotometry (7) using an IL Model 251 spectrophotometer (Instrumentation Laboratory Inc.). Bone ash

[1]Presented at the 61st Annual Meeting, New Orleans, Oct. 1976.

was determined in the right femur as described earlier (8). The entire right kidney was ashed overnight. The resultant femur and kidney ash was taken up in 10 ml of 6*N* HCl by heating for 15 min; dilutions were then made as necessary. Total phosphorus in the diet was determined by the standard AOAC method (9). Various Zn sources tested for bioavailability and functionality are listed in Table II. All sources were reagent grade.

Breads (pound loaves) were made by the sponge (flour, 70%; dry yeast, 1%; yeast food, 0.5%; and water, 45.5%) and dough (flour, 30%; sugar, 2%; shortening, 6%; nonfat dry milk, 2%; and water, 19.5%) procedure. Zn was added to the sponge at the recommended (6) level of 2.2 mg/100 g flour. All breads, following weight (after cooling) and volume (rapeseed displacement) measurements, were sliced and general bread quality based on break and shred, crumb and crust color, grain and texture, was assessed. The pH of the bread was measured by the standard AACC method (10).

RESULTS AND DISCUSSION

Although requirement for Zn from plant sources may be higher because of poor availability (3,11–13) for the growing rat, 12 ppm Zn in the diet appears to

TABLE I
Composition of Test Diets

	Zn Source			
	None	Wheat	Others[a]	
	Diet A	Diet B	Diets C-I	Diet J
Egg albumin, g	20	20	20	20
Vitamins[b], g	2	2	2	2
Alphacel[c], g	2	2	2	2
Trace minerals[d], g	1	1	1	1
Oil, g	4	4	4	4
NaCl, g	1	1	1	1
KCl, g	0.3432	0.3432	0.3432	0.3432
$CaCO_3$, g	1.2314	1.2021	1.2314	1.2314
$NaH_2PO_4 \cdot H_2O$, g	1.8819	1.6803	1.8819	1.8819
Zn source, g	...	...	variable[e]	0.0028
Wheat flour, g				
Patent	...	30.5788	...	...
Clear	...	36.1956	...	...
Wheat starch, g	66.5435	...	variable[f]	66.5407
Calcium, mg/100 g				
Native	6.9	18.6	6.9	6.9
Added	493.1	481.4	493.1	493.1
Phosphorus, mg/100 g				
Native	77.5	122.8	77.5	77.5
Added	422.5	377.2	422.5	422.5
Zn, ppm	1.25	9.50	9.50	11.50

[a]See Tables III and IV.
[b]Vitamin diet fortification mixture from ICN Pharmaceuticals.
[c]Nonnutritive fiber from ICN Pharmaceuticals.
[d]Contained (starch base): Cu, 0.5 mg; Fe, 2.5 mg; Mn, 5 mg; Mg, 40 mg; and I, 0.015 mg.
[e]To furnish 8.25 ppm of Zn.
[f]Ranged from 66.5364 to 66.5427 g to make 100 g.

be adequate (11–14). In present studies, Zn was, however, provided at a submarginal (9.5 ppm) level to induce mild deficiency so that differences, if any, in its bioavailability from different sources could be detected; others (12,13) have used dose:response (growth or total femur Zn) curves and depletion-repletion techniques (14) to assess Zn bioavailability.

The specificity of a Zn-deficient diet (diet A) was first established in a preliminary experiment (Table III). Although classical deficiency symptoms (14) did not develop in a 2-week period, extreme retardation of growth, poor diet utilization, and significantly ($P < 0.01$) lower serum Zn levels were noted in rats fed diet A as against those fed a Zn-supplemented diet (diet G). Others (14,15) have reported similar findings on Zn-deficient diet.

When Zn-supplemented diets (diet B-I; Zn, 9.5 ppm) were compared (Table

TABLE II
Effect of Zinc on Bread Quality[a]

Zn Source[b]	Specific Loaf Volume cu in./oz	General Bread Quality Score[c]	Proof Time min	Bread pH
None	10.77	3.7	68	5.30
Carbonate (53.8)[d]	10.85	3.8	65	5.30
Carbonate (53.9)	10.79	3.7	64	5.30
Chloride (44.7)[d]	10.92	3.8	66	5.28
Oxide (78.6)	10.81	3.9	68	5.30
Oxide (79.5)[d]	10.98	3.9	64	5.26
Oxide (79.5)	10.69	3.7	65	5.28
Sulfate (23.6)	11.09	3.9	71	5.24
Sulfate (36.1)[d]	11.19	3.8	69	5.30
Stearate (11.2)[d]	10.85	3.7	63	5.28
Acetate (30.0)[d]	10.92	3.9	67	5.28
Elemental (100.0)[d]	10.95	3.8	66	5.28
Elemental (100.0)	10.61	3.6	70	5.38

[a]Zn added at the level of 2.2 mg/100 g flour.
[b]Values within parentheses indicate content (%) of Zn; source added to the sponge (sponge-dough procedure).
[c]Very good, 4; good, 3; fair, 2; and poor, 1.
[d]Sources used in bioavailability studies (Tables III and IV).

TABLE III
Preliminary (2-Week) Experiment[a]

	Zn Source	
	None	Stearate
	Diet A	Diet G
Weight gain, g	18 ± 4	71 ± 4
Diet:gain ratio	5.3 ± 1.1	2.0 ± 0.2
Serum Zn, μg/dl	72 ± 16	125 ± 10

[a]Values indicate average ± standard deviation.

IV), no significant ($P > 0.05$) difference in weight gain of rats or diet:gain ratio was observed. Additional Zn in the diet (diet J, Zn, 11.5 ppm) did not improve growth or diet efficiency, further suggesting that 9.5 ppm Zn was probably adequate for maximum growth. Differences in Zn sources became apparent when tissue Zn levels were examined. Serum Zn levels differed appreciably between sources but were significantly ($P < 0.01$) lower only in wheat-fed rats. Increasing dietary Zn level (diet J) caused a slight further increase in serum Zn.

Although serum Zn levels have often been used (15,16) to assess Zn status, total femur Zn is reported (13,17) to be the parameter of choice. As for serum Zn, total femur Zn was also significantly ($P < 0.01$) lower in wheat-fed rats only, although some differences between nonwheat sources (diet C-I) were observed (Table IV). Increasing the dietary Zn level (diet J) caused a most significant ($P < 0.01$) increase in total femur Zn.

Phytate and fiber in wheat are reported (18,19) to reduce Zn absorption; however, clear (high-extraction) flour used (Table I) probably contributed only small amounts. Apparent absorption (urine collection was not made) of Zn from wheat flour was not much lower than it was from nonwheat sources. Perhaps it was much lower during the first 3 weeks when absorption was not measured, or recycling of endogenous Zn was partially prevented (19), resulting in poorer Zn retention. Absorption of Zn from carbonate was significantly ($P < 0.05$) low; regardless, over 75% of dietary Zn was absorbed from all sources. Percentage absorption, but not the amount absorbed, decreased when dietary Zn level was increased (diet J). On a casein-based diet containing 8 ppm Zn, Forbes and Yohe (20) reported an absorption of 84%.

Zn sources appeared to have no significant ($P > 0.05$) effect on the weight (fat and moisture free) or ash content of femur; this was true also for the content (%) of Zn in kidney. While the level of Zn in serum and femur increased appreciably during the experiment, no such increase in the level (%) of Zn in kidney was noted.

A number of Zn sources with Zn content ranging from 11.2 to 100% were tested for their effect on the quality of bread made by the sponge-dough procedure (Table II). None of the sources, including those examined for bioavailability, had any adverse effect on loaf volume and general bread quality, including the flavor. Effect on proof time and bread pH was minimal.

While direct carcass analysis is likely to give a more accurate assessment of bioavailability, based on growth response, and absorption and retention (tissue concentration) of Zn, it may be concluded that Zn from all nonwheat sources was equally well available; slight differences observed are probably of limited physiological significance. Unlike iron (21) and magnesium (22,23), which show great differences in bioavailability and/or functionality, depending on the source used, all potential Zn sources appear to be equally well suited for cereal fortification.

TABLE IV
Tissue Concentration and Absorption of Zinc[a,b]
(4-Week Experiment)

	Zn Source								
	Wheat	**Carbonate**	**Chloride**	**Oxide**	**Sulfate**	**Stearate**	**Acetate**	**Elemental**	**Sulfate**
	Diet B	**Diet C**	**Diet D**	**Diet E**	**Diet F**	**Diet G**	**Diet H**	**Diet I**	**Diet J**
Tissue concentration of Zn									
wt Gain, g	116 ± 10	108 ± 13	115 ± 9	120 ± 14	116 ± 10	116 ± 7	115 ± 9	108 ± 13	113 ± 8
Diet:gain ratio	2.19 ± 0.12	2.31 ± 0.13	2.23 ± 0.17	2.26 ± 0.15	2.25 ± 0.13	2.27 ± 0.11	2.27 ± 0.02	2.32 ± 0.08	2.29 ± 0.13
Serum Zn, μg/dl	111 ± 31	167 ± 18	153 ± 12	165 ± 19	140 ± 24	144 ± 20	144 ± 18	161 ± 11	172 ± 22
Femur wt, mg	222 ± 14	230 ± 15	231 ± 17	239 ± 10	229 ± 13	230 ± 18	237 ± 19	214 ± 20	229 ± 16
Femur ash, %	58.4 ± 1.1	57.7 ± 1.1	58.8 ± 0.6	58.3 ± 1.3	58.9 ± 0.5	58.6 ± 0.7	58.7 ± 0.9	59.1 ± 0.8	58.1 ± 1.2

Femur Zn, μg	22.8 ± 4.1	31.8 ± 2.5	29.6 ± 3.5	34.4 ± 4.1	34.2 ± 2.8	35.0 ± 2.9	35.8 ± 3.4	33.0 ± 2.6	46.7 ± 3.4
Kidney wt, mg	615 ± 51	474 ± 58	586 ±106	604 ± 83	528 ± 28	523 ± 73	491 ± 56	499 ± 47	466 ± 98
Kidney Zn, %	0.0016 ± 0.0002	0.0020 ± 0.0003	0.0017 ± 0.0001	0.0017 ± 0.0001	0.0022 ± 0.0002	0.0022 ± 0.0002	0.0022 ± 0.0003	0.0018 ± 0.0002	0.0020 ± 0.0002
Apparent absorption of Zn (fourth week)									
Zn intake, mg	0.771 ± 0.037	0.694 ± 0.092	0.766 ± 0.036	0.775 ± 0.058	0.730 ± 0.043	0.751 ± 0.057	0.729 ± 0.058	0.698 ± 0.097	0.907 ± 0.072
Zn absorbed, mg	0.634 ± 0.045	0.538 ± 0.086	0.683 ± 0.045	0.680 ± 0.079	0.635 ± 0.050	0.671 ± 0.052	0.633 ± 0.062	0.638 ± 0.095	0.723 ± 0.087
Zn absorbed, %	82.2 ± 2.9	75.6 ± 5.4	89.1 ± 3.3	87.1 ± 4.2	87.1 ± 3.5	89.2 ± 2.0	87.3 ± 4.7	87.8 ± 4.2	78.7 ± 5.5

[a]Values indicate average ± standard deviation.
[b]0-day values: Serum Zn (μg/dl), 100 ± 6; femur weight (mg), 47 ± 2; femur ash (%), 45.3 ± 1.7; femur Zn (μg), 10.1 ± 2.6; kidney weight (mg), 106 ± 14; and kidney Zn (%), 0.0022 ± 0.0003.

Acknowledgment

The authors thank Thomas Lehmann, Baking Technologist.

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[Received August 23, 1976. Accepted September 24, 1976]