ELSEVIER

Journal of Nutritional Biochemistry 15 (2004) 206–209

THE JOURNAL OF Nutritional Biochemistry

Large doses of zinc oxide increases the activity of hydrolases in rats

József Szabó[a], Mihály Hegedus[a], Géza Bruckner[b,*], Emma Kósa[a], Emese Andrasofszky[a], Erzsébet Berta[a]

[a]*Institute of Animal Breeding, Nutrition and Laboratory Animal Science, Faculty of Veterinary Sciences, Szent István University, Budapest, Hungary*
[b]*Division of Clinical Nutrition and Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY 40536-0003, USA*

Abstract

The effect of pharmacological doses of zinc oxide (1000; 2500; 5000 mg per kg diet) and two levels of dietary protein on pancreatic and intestinal hydrolase activity in rats were studied. It was hypothesized that ZnO would increase intestinal and pancreatic hydrolase enzyme activity. Male Wistar rats, averaging 64 g body weight, were randomly allocated to dietary treatments (chow diets- meeting all NRC requirements) containing 10% or 15% protein supplemented with additional ZnO (above 100 mg/kg $ZnSO_4$) as follows: 0.0; 0.1; 0.25; 0.5% w/w. Water and food were provided *ad libitum*. Animals were fed the diets for 10 days and body weights were recorded; after decapitation blood and organ samples were collected. Amylase, lipase, trypsin, and total protease activity of pancreatic homogenates and small intestinal contents were determined. ZnO supplementation dose dependently increased the plasma Zn concentration and significantly increased amylase, lipase, trypsin and total protease activity in pancreatic homogenates and small intestinal contents. The statistical analysis showed significant protein and ZnO interaction on the activity of amylase in the pancreas, and amylase, trypsin and total-protease in the small intestinal content. Therefore ZnO at high dietary concentration may influence the digestion of nutrients via increased hydrolase activity.

Keywords: Zinc oxide; Nutrition; Amylase; Lipase; Trypsin

1. Introduction

It has been shown that a pharmacological dose (2500 ppm) of ZnO added to the diet increased performance and reduced scouring and death losses immediately after weaning [1,2,3,4], however, the mechanism of action is obscure.

Zinc is an essential element and the recommended physiological doses for growing pigs are between 50 to 100 mg per kg feed dry matter [5]. Deficiency of zinc in swine is manifested mainly as parakeratosis [6]. Excess dietary zinc (as low as 170 mg/kg purified diet) has been shown to be toxic [7]. In the chick pancreatic aberrations were indicated by lowered activities of major digestive enzymes (approximately 20% of normal level), elevated plasma amylase activities and elevated tissue Zn concentration [7]. However, the tolerable level of zinc is dependent, among other factors, on the source of the element. In weaning pigs, for example, zinc carbonate (2000 ppm zinc) added to the diet for one month, produced zinc toxicosis [8], in contrast, the same level of ZnO was not toxic [9]. The lower toxicity of ZnO may be explained by the lower bioavailability of Zn from ZnO than from other sources such as $ZnCO_3$ or $ZnSO_4$. The relative bioavailability of ZnO for chickens [10] and pigs [11] fed corn-soybean meal diets was 61% and 67% of $ZnSO_4$, respectively.

Therefore, the primary aim of this study was to test the hypothesis that ZnO, at doses greater than NRC requirements, would increase amylase, lipase, trypsin and total-proteolytic activities in the pancreas and in small intestinal contents in a dose dependent response. The ZnO effect was tested at two levels of dietary protein.

2. Methods and materials

Forty-eight, weaned, male, Wistar, SPF rats (LATI, Gödöllõ, Hungary) were used in the experiment. Animals were housed individually in wire bottom cages, at 21°C room temperature. Following a week adaptation period, rats (6/group): were randomly allocated to the following dietary treatments (Table 1): 1. LPC[1], 2. LPC + 1000 mg/kg ZnO,

* Corresponding author. Tel.: +1-859-323-1100 x246; fax: +1-859-257-1816.

E-mail address: gbruckner@aky.edu (G. Bruckner).

[1]Low protein control diet, 10% crude protein.

0955-2863/04/$ – see front matter
doi:10.1016/j.jnutbio.2003.09.005

Table 1
Compositions of the experimental diets

Ingredients	Low Protein Diet (LPC) (g/1000g)	High Protein Diet (HPC) (g/1000g)
Extracted soybean meal	208.6	313.0
Lard	80.0	80.0
Sunflower oil	20.0	20.0
Cellulose	50.0	50.0
Vitamin and mineral premix	50.0	50.0
ZnO supplementation	0.0; 1.0; 2.5; 5.0	0.0; 1.0; 2.5; 5.0
Starch (add up to)	1000.0	1000.0

Composition of the vitamin and mineral premix: Vitamin A 200,000 IU, Vitamin D_3 20,000 IU, vitamin E 1000 mg, Vitamin K_3 40 mg, thiamin 60 mg, nicotinic acid 800 mg, Ca pantothenate 300 mg, pyridoxine 40 mg, biotin 10 mg, folic acid 10 mg, L-ascorbic acid 2000 mg, choline chloride 16000 mg, Ca 120 g, P 100 g, Mg 10 g, K 40 g, NaCl 12 g, Fe 2000 mg, Mn 1600 mg, $ZnSO_4$ 2000 mg, Cu 200 mg, I 12 mg, BHT 150 mg, starch add up to 1000 g.

3. LPC + 2500 mg/kg ZnO, 4. LPC + 5000 mg/kg ZnO, 5. HPC,[2] 6. HPC + 1000 mg/kg ZnO, 7. HPC + 2500 mg/kg ZnO, 8. HPC + 5000 mg/kg ZnO. All diets were supplemented with $ZnSO_4$ (100 mg/kg), as part of the mineral premix. Water and food were provided *ad libitum*. Animals were fed the diets for 10 days. Body weights and feed intake were recorded. After decapitation, blood, pancreatic tissue and small intestinal content samples were collected. The pancreatic tissue and the intestinal contents were homogenized in ice-cold distilled water and centrifuged at 3000 r.p.m. for 10 min. In the supernatants protein concentration [12], amylase (Phedebas Amylase Test, Pharmacea Diagnostics AB, Upsala, Sweden), lipase [13], trypsin and total-proteolytic activities were tested. The proteolytic zymogens of the samples were activated by enterokinase (Sigma Chemical Co., St. Louis, MO, USA) at 37°C for 60 min in Na-citrate buffer (pH 5.6), trypsin activity was determined photometrically using Boehringer colorimetric test-combination (Cat. No. 125024). The total-proteolytic activity was estimated using casein as a substrate and the hydrolytic products detected with Folin-Ciocalteu reagent.

[2]High protein control diet, 15% crude protein.

Table 3
Mean body weight and feed intake of rats during the ten day experiment

ZnO supplementation of the diets (mg/kg)	Low protein diet (10%) (mg/L)	High protein diet (15%) (mg/L)
0.000	1.30	1.29
1000	1.58	1.31
2500	1.95	1.69
5000	2.03	1.92

The blood samples were collected in heparinized test tubes and centrifuged at 1500 r.p.m. for 10 min. The plasma was separated and 0.5 mL from each treatment sample pooled and stored at −20°C until the determination of Zn, Fe and Cu. Plasma samples were diluted with double distilled water and Zn, Fe and Cu concentrations were determined by flame atomic absorption spectrometry (Perkin-Elmer, Model 5000 AAS, Norwalk, CT).

STATISTICAL METHODS: The data were analyzed by one-way ANOVA followed by LSD post-hoc test, two way ANOVA to monitor interaction(s) between dietary levels of ZnO and protein, the correlation coefficients were calculated by SPSS for Windows program package [14]. Differences were considered to be significant at $P \leq 0.05$.

3. Results

The mean starting and final weights as well as the feed intakes of the experimental groups are shown in Table 2. Supplementation of ZnO resulted in a dose dependent increase of plasma Zn concentrations at both dietary protein levels, however in the HPC groups plasma Zn was consistently lower than at the LPC ones, as indicated in Table 3.

As shown in Table 4 there was significant increases in pancreatic and intestinal amylase activity with increasing levels of dietary ZnO. This effect was clearly observed in both the low and high protein fed groups.

As with amylase activity, significant increases were noted for lipase activity in both pancreatic and intestinal contents with increased levels of dietary ZnO (Table 5). The

Table 2
Mean body weight and feed intake of rats during the ten day experiment

Diets	Starting body weight (g)	Final body weight (g)	Gain (g)	Feed intake (g/rat)	Feed/gain (g/g)
LPC	63.7 ± 3.8	89.3 ± 12.0	25.7 ± 9.1	106.9 ± 16.5	4.17 ± 2.05
LPC+0.1 % ZnO	63.7 ± 4.0	94.0 ± 8.3	30.0 ± 9.5	108.5 ± 20.0	3.58 ± 0.65
LPC+0.25 % ZnO	63.7 ± 3.9	91.1 ± 4.8	27.4 ± 6.1	104.9 ± 26.9	3.83 ± 0.75
LPC+0.5 % ZnO	63.7 ± 3.9	91.8 ± 4.2	28.1 ± 5.7	109.3 ± 14.7	3.89 ± 0.64
HPC	63.7 ± 3.7	116.2 ± 7.2	52.5 ± 6.0	134.6 ± 4.0	2.56 ± 0.32
HPC+0.1 % ZnO	63.7 ± 3.7	117.8 ± 6.9	54.2 ± 8.9	134.3 ± 4.3	2.48 ± 0.40
HPC+0.25 % ZnO	63.7 ± 3.7	120.2 ± 8.1	56.5 ± 6.6	134.3 ± 5.0	2.38 ± 0.27
HPC+0.5 % ZnO	63.7 ± 3.6	118.2 ± 6.9	54.5 ± 6.7	137.2 ± 4.1	2.52 ± 0.32

Table 4
Effect of ZnO supplementation on amylase activity

Diets	Pancreatic amylase activity (U/mg protein)	Small intestinal content amylase activity (U/mg protein)
LPC	2.64 ± 0.46[a]*	0.39 ± 0.03[a]
LPC+01% ZnO	3.81 ± 0.30[b]	0.47 ± 0.03[b]
LPC+0.25% ZnO	4.38 ± 0.46[c]	0.55 ± 0.05[c]
LPC+0.5% ZnO	5.27 ± 0.58[d]	0.57 ± 0.05[c]
HPC	3.99 ± 0.49b[c]	0.40 ± 0.05[a]
HPC+0.1% ZnO	5.31 ± 0.32[d]	0.57 ± 0.06[c]
HPC+0.25% ZnO	5.47 ± 0.30[d]	0.68 ± 0.06[d]
HPC+0.5% ZnO	6.19 ± 0.43[e]	0.76 ± 0.05[d]

* Values with different letters within a column indicate significant differences ($P < 0.05$).

Table 6
Effect of ZnO supplementation on trypsin activity

Diets	Pancreatic trypsin activity (mU/mg protein)	Small intestinal content trypsin activity (mU/mg protein)
LPC	39.64 ± 1.50[a]*	44.51 ± 2.37[a]
LPC+01% ZnO	44.88 ± 2.30[b]	49.08 ± 49.08[b]
LPC+0.25% ZnO	47.25 ± 1.83[b]	54.06 ± 1.75[c]
LPC+0.5% ZnO	54.57 ± 3.53[c]	58.35 ± 0.92[ef]
HPC	64.67 ± 3.00[d]	51.37 ± 1.28[c]
HPC+0.1% ZnO	68.67 ± 5.45[e]	56.51 ± 1.25[e]
HPC+0.25% ZnO	72.12 ± 2.85[e]	58.97 ± 1.25[f]
HPC+0.5% ZnO	79.38 ± 1.97[f]	61.34 ± 1.91[g]

* Values with different letters within a column indicate significant differences ($P < 0.05$).

effect of ZnO was evident at both low and high protein levels fed.

With increased levels of dietary ZnO both trypsin and total protease activities were increased (Tables 6 & 7). Furthermore there were significant effects imparted by higher protein levels on trypsin, protease and amylase activities (Tables 4, 6 & 7).

4. Discussion

Feeding a high protein (15%) diet resulted in significantly higher body weight gains than the low protein (10%) diet. The supplementation of these diets with graded pharmacological doses of ZnO, added above the NRC recommendation did not induce significant improvements in body weight gain or feed efficiency.

The ZnO supplementation, dose dependently increased the plasma Zn concentrations at both dietary protein levels however in the HPC+ZnO groups; plasma Zn was consistently lower than in rats on LPC+ZnO diets. The increases noted in plasma Zn concentrations corresponded with the data of Hahn and Backer [2]. They found 1.3 and 1.99 mg/L plasma Zn concentration at 3000 and 5000 mg/kg ZnO supplementation, respectively. However, contrary to our findings, their data also showed improved daily weight gain when plasma concentrations were at 2 to 2.5 mg/L plasma Zn concentration. These differences may be due to different protein sources or other dietary interactions with ZnO.

The high protein diet significantly increased the amylase, trypsin and total protease activity in the pancreas and the lipase, trypsin and total protease activity in the small intestinal content. The ZnO supplementation dose dependently increased the activity of all studied hydrolase enzymes (Table 4 to 7) at each dietary protein level.

The two way ANOVA showed significant protein and ZnO interaction regarding amylase activity in the pancreas ($P < 0.05$) and amylase ($P < 0.001$), lipase ($P < 0.05$), trypsin ($P < 0.05$) and total protease ($P < 0.01$) activity in the small intestinal content. Based on our experimental data, no clear explanation can be given for the mechanism that might be involved in eliciting the stimulatory effect of ZnO on the pancreatic hydrolase activities. However, zinc has been shown to exert a crucial role in the function of many enzymes [15]. For example, Alvares and Johnson [16] found decreased protein synthesis in the parotid gland of Zn deficient rats while Perez-Jimenez et al. [17] found in-

Table 5
Effect of ZnO supplementation on lipase activity

Diets	Pancreatic lipase activity (mU/mg protein)	Small intestinal content lipase activity (mU/mg protein)
LPC	84.96 ± 9.09[a]*	118.15 ± 1.74[a]
LPC+01% ZnO	109.29 ± 11.76[b]	129.33 ± 8.24[b]
LPC+0.25% ZnO	123.33 ± 13.92[c]	143.59 ± 13.82[c]
LPC+0.5% ZnO	149.13 ± 26.45[c]	153.53 ± 8.86[c]
HPC	82.11 ± 6.77[a]	129.26 ± 8.15[b]
HPC+0.1% ZnO	111.65 ± 7.02[b]	146.46 ± 8.55[cd]
HPC+0.25% ZnO	140.63 ± 14.59[c]	155.37 ± 7.16[d]
HPC+0.5% ZnO	153.6 ± 15.20[c]	171.8 ± 9.97[c]

* Values with different letters within a column indicate significant differences ($P < 0.05$).

Table 7
Effect of ZnO supplementation on total-protease activity

Diets	Pancreatic protease activity (mU/mg protein)	Small intestinal content protease activity (mU/mg protein)
LPC (Low protein control)	294.36 ± 45.68[a]*	302.23 ± 20.90[a]
LPC+01% ZnO	365.4 ± 27.57[b]	360.36 ± 32.19[b]
LPC+0.25% ZnO	432.96 ± 45.46[cd]	449.38 ± 42.31[c]
LPC+0.5% ZnO	467.54 ± 25.78[d]	547.79 ± 25.92[d]
High protein control (HPC)	412.60 ± 59.87[c]	444.87 ± 35.00[c]
HPC+0.1% ZnO	515.81 ± 26.56[e]	529.61 ± 26.81[d]
HPC+0.25% ZnO	525.65 ± 39.15[e]	555.90 ± 31.22[d]
HPC+0.5% ZnO	601.04 ± 24.20[f]	595.50 ± 29.51[e]

* Values with different letters within a column indicate significant differences ($P < 0.05$).

creased trypsin and chymotrypsin activity in rats fed a Zn deficient diet. While our studies did not address Zn deficiency, our data and those of Perez-Jimenez et al. [17] suggests that digestive enzyme levels might be increased by a Zn deficiency as well as levels which exceed the NRC recommended dietary levels. In this experiment the control animals were given a sufficient quantity of $ZnSO_4$ to meet the NRC requirements, therefore the animals were not Zn deficient. Excess or toxic amounts of Zn have been shown to decrease pancreatic flow as well as enzyme secretions [18]. This indirectly suggests that the Zn levels feed in this experiment were not toxic since the activities of most enzymes were increased and there was no significant effect on growth rates. Furthermore, the alkaline phosphatase activity of the plasma samples of these animals showed no significant deviations to the control values (unpublished data). This is in agreement with the experimental data of Poulsen [3], and Cox and Hale [19]. They showed that 2000 and 4000 ppm zinc (ZnO) had no adverse effect on the growth and health of weanling pigs.

On this basis it can be concluded that dietary ZnO supplementation may improve the digestive capacity of rats via the dose dependent increases of hydrolase enzymes in the pancreas, and the higher activity of amylase, lipase, trypsin and total protease in the small intestinal contents.

References

[1] Kavanagh N. The case of zinc oxide. Pig International 1993:11–2 Febr.
[2] Hahn JD, Baker DH. Growth and plasma zinc responses of young pigs fed pharmacological levels of zinc. J Anim Sci 1993;71:3020–4.
[3] Poulsen HD. Zinc oxide for weanling pigs. Acta Agric Scand Sect A Animal Sci 1995;45:159–67.
[4] Smith JW, Tokach MD, Goodmand RD, Nelssen JL, Richert BD. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. J Anim Sci 1997;75: 1861–6.
[5] National Research Council. Nutrient requirements of swine (9^{th} rev. ed). National Academy Press. DC: Washington, 1988.
[6] Tucker HF, Salmon WD. Parakeratosis or zinc deficiency disease in the pig. Proc Soc Exp Biol Med 1955;88:613–6.
[7] Lú JX, Combs GF. Effect of excess dietary zinc on pancreatic exocrine function in chick. J Nutr 1988;118:681–9.
[8] Brink MF, Becker DE, Terrill SW, Jansen AH. Zinc toxicity in the weanling pig. J Anim Sci 1959;18:836–42.
[9] Hsu FS, Krook L, Pond WG, Duncan JR. Interactions of dietary calcium with toxic levels of lead and zinc in pigs. J Nutr 1975;105: 112–8.
[10] Wedekind KJ, Hortin AE, Baker DH. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J Anim Sci 1992;70:178–87.
[11] Wedekind KJ, Lewis AJ, Giesemann MA, Miller PS. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. J Anim Sci 1994:2681–9.
[12] Lowry OH, Rosebrough NJ, Farr AL, Furuta F. Protein measurement with the Folin phenol reagent. J Biol Chem 1961;193:265–75.
[13] Schön H, Rassler B, Henning N. Über die Untersuchung der exretorischen Pancreasfunction. Methoden zur Aktivitatsbestimmung von Trypsin, Chymotrypsin, Carboxipeptidase, Lipase und Diastase. Klin Wschr 1961;39:217–22.
[14] SPSS for Windows 06. 01. 04. Version, SPSS inc., 1989–1995.
[15] Mertz W. Trace elements in human and animal nutrition. London: Academic Press, 1986. p. 27.
[16] Alvares O, Johnson D. Effects of zinc deficiency on rat parotid gland. J Oral Pathol 1981;10:430–5.
[17] Perez-Jimenez F, Bockman DE, Singh M. Pancreatic acinar cell function and morphology in rats fed zinc-deficient and marginal zinc-deficient diets. Gastroenterology 1986;90:946–57.
[18] Smith BL, Embling PP. Effect of oral doses of zinc oxide on the secretion of pancreatic juice and bile in sheep. Res Vet Sci 1994;94: 58–62.
[19] Cox DH, Hale OM. Liver iron depletion without copper loss in swine fed excess zinc. J Nutr 1962;77:225–8.