International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating: 4.61
VET 2025; 10(6): 327-329

www.veterinarypaper.com
Received: 14-04-2025
Accepted: 17-05-2025

K Padmaja
Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

TV Chaitanya Kumar
Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

K Jayasri
Centre for Continuing Veterinary Education Cell, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Corresponding Author:
TV Chaitanya Kumar
Department of Veterinary Biochemistry, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Impact of Zinc toxic levels on Hepatic Heme Biosynthesis in Chick Embryos

K Padmaja, TV Chaitanya kumar and K Jayasri

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i6e.2349

Abstract
Zinc as an essential trace element it is involved in various molecular and biological processes, and simultaneously toxic at high levels. Although, consequences of zinc deficiency have been recognised for many years, it is only recently that attention has been directed to the potential consequences of excessive zinc intake. All nucleated cells synthesize sufficient heme to meet the obligatory needs of mitochondrial cytochromes for cellular respiration, while certain tissues produce additional heme for their specialized roles. Biochemical studies of heme biosynthesis in experimental animals are useful to elucidate the mechanism underlying the different porphyria's. Chick embryos were treated with 25 and 75µ moles of Zinc / kg wt on 14th day of embryonic development. Embryos were sacrificed on 20th day for the collection of hepatic tissues. The levels of Aminolevulinic acid (ALA), heme and the activity of Aminolaevulinic acid synthase (ALA-S), Aminolaevulinic acid dehydratase (ALA- d) and heme synthetase were assayed in hepatic tissues of chick embryos. ALA-S activity was decreased in a dose dependent manner with zinc treatment. Whereas, ALA levels and ALA- d activity was unaltered and heme synthetase activity and hepatic heme levels were increased in a dose dependent manner with zinc treatment.

Keywords: Zinc, ALA-S, heme synthetase, heme and chick embryos

Introduction
Zinc an essential metal is also known to be an environmental pollutant [1]. The homeostatic mechanism evidently fails at higher levels of zinc intake leading to zinc intoxication [2]. Zinc toxicity causes dehydration, electrolyte imbalance, stomach pain, nausea, lethargy and muscular incoordination [3]. Zinc functions as a stabilizer of bio membranes and biostructures [4]. Zinc is required for porphyrin biosynthesis and its potential effect on cellular heme metabolism has been demonstrated [5]. Haemoglobin liberated into plasma from disrupted erythrocytes is dangerous, because it can accelerate peroxidation of lipids in the presence of H_2O_2 [6, 7]. Hence alterations or defects in enzyme activities of heme biosynthetic pathway result in profound change in these functions [8]. Most of the investigations conducted on effects of zinc have been concerned with metal mixtures [9]. Zinc supplementation has improved heme biosynthesis in rats exposed to lead has been examined extensively [10]. Whereas, very little attention was given to studies on embryonic system. Hence the present work was carried out to study the effect of toxic doses of zinc on heme biosynthesis in chick embryos.

Materials and Methods
Freshly laid Bobcock strain zero-day old eggs were procured from the Balaji hatcheries, Chittoor dt. Andhra Pradesh. They were placed in an egg incubator, the temperature of which was maintained at 37°C with 65% relative humidity. The eggs were rotated manually once in a day during incubation. Injections of zinc as $ZnSO_4$ (25 and 75µ moles/kg egg wt.) dissolved in distilled water were given in a total volume of 10µl to 14-day old chick embryos. Control embryos were injected with the same amount of distilled water. A minimum of 25 embryos were used in each group. Embryos were sacrificed on 20th day to collect hepatic tissues, because heme is predominantly synthesized in liver cells.

Hepatic ALA levels were measured according to the method of Mauzerall and Granick [11]. ALA-d activity was assayed by the method of Granick *et al*. [12], ALA-S activity was carried out according to the method of Lien and Beattie [13]. Heme synthetase was determined according to the method of Porra and Jones [14]. Heme was extracted according to Gerber and Maes [15] and estimated by the method of Christian de duve [16]. The protein content was determined by using bovine serum albumin as a standard [17]. Statistical evaluation was done as per Snedecor [8].

Results and Discussion

A major site for regulation of hepatic heme biosynthesis was proposed at the level of ALA formation, hence the primary focus appears was on ALA-S enzyme. The mechanism involved in the regulation of the activity of ALA-S is of main interest, because perturbations in its activity are linked to several inherited and acquired disorders collectively known as porphyrias in broiler chicken [19]. The ALA-S activity inhibited in a dose dependent manner with 25 and 75 µ moles of zinc concentration. (Table. 1). Similar results were reported with sodium sulphide treatment where ALA-S activity was decreased [20].

ALA-d plays an important role in the supply of blood pigment precursor in chick embryo liver and it is a key step in heme biosynthesis [21]. Hence a major site for control of hepatic heme biosynthesis is at the level of ALA formation. Zinc treatment has not altered ALA levels (Table 1) and ALA-d activity (Table 2) in chick embryos, which may be due to the requirement of zinc metal for enzyme activation [22]. Though Zinc acts as a cofactor for AlA-d enzyme, Zinc toxic levels could not increase the enzyme activity further beyond its physiological levels, this suggests that the enzyme ALA-d has been saturated with Zinc at its physiological levels and could not increase enzyme activity further with increase in zinc levels to their toxic levels.

Hepatic heme synthetase activity and heme levels increased with zinc treatment (Table 3). Though ALA levels has not increased, increased activity of heme synthetase may be responsible for elevated levels of heme production [23]. Further, elevated levels of hepatic heme with zinc treatment could be attributed to the decrease in ALA-S activity by means of feedback inhibition, because heme regulates its own production by down regulating the heme biosynthesis at the level of rate limiting enzyme ALA-S. To elucidate further, the excess heme produced due to increased activity of heme synthetase with zinc treatment has inhibited the first enzyme, ALA-S because both the enzymes ALA-S and heme synthetase are located in the mitochondrial membrane [24]. Further, many studies suggest that the intracellular heme presumed to be in the form of free heme pool is considered as ultimate mediator of heme biosynthesis control through its effect on ALA production and transport [25]. However, excess free heme is highly toxic due to its ability to promote oxidative stress and lipid peroxidation thus leading to membrane injury and ultimately apoptosis [26]. Therefore, excess heme produced and released could be reason for zinc toxicity in neurons mediated mainly by oxidative stress and zinc induced cell death may be associated with increased levels of ROS in neuron [27]. These findings were endorsed by one of our previous works where Zinc treatment to chick embryos has increased TBARS and SOD activity [28]. In contrast, few other studies have reported that heme synthetase activity measured in terms of metalloporphyrin's formed are serving as SOD mimetics and stated they could combat oxidative stress [29].

Table 1: Effect of Zinc on ALA-S activity And ALA Levels in hepatic tissues of chick embryos

Treatment with Zinc (µmoles/kg egg wt)	ALA-S Activity (nmoles of ALA formed mg protein/30 min at 37º)	ALA Levels (µmoles of ALA formed/gr wt. of the tissue)
Control	$23.30^{a} \pm 0.44$	0.67 ± 0.02
25	$17.93^{b} \pm 0.46$	0.63 ± 0.01
75	$12.00^{c} \pm 0.33$	0.64 ± 0.02

n=12, Mean ± S.E, P<0.05

Table 2: Effect of Zinc on hepatic ALA-d activity in chick embryos.

Zn treatment (µmoles/Kg egg wt.)	ALA-D Activity (nmoles of PBG formed/mg protein/hr at 37 ºC)
Control	46.00 ± 1.15
25	47.00 ± 1.20
75	48.00 ± 1.27

n=12, Mean ± S.E, P<0.05

Table 3: Effect of Zinc on hepatic heme synthase activity and heme levels in chick embryos.

Zn treatment (µmoles/Kg egg wt.)	Heme synthetase (nmoles of metalloproteins yrin formed /hr at 37 ºC)	Heme concentration (µgm heme/ gm wt. of tissue)
Control	$11.55^{c} \pm 0.11$	$571.67^{c} \pm 10.60$
25	$13.17^{b} \pm 0.19$	$678.00^{b} \pm 17.26$
75	$14.78^{a} \pm 0.21$	$872.00^{a} \pm 21.23$

n=12, Mean ± S.E, P<0.05

Conclusion

Zinc is an essential mineral and is required for normal function of many enzymes. Zinc at its toxic levels has affected the embryonic heme biosynthesis by increasing the levels of free heme, by increasing the activity of heme synthetase. Though it acts as cofactor for ALA-d enzyme, zinc toxic levels couldn't enhance this enzyme activity in embryos. Zinc by increasing the heme levels and it being the end product of heme biosynthesis, has indirectly been the cause of decreased ALA-S activity, a regulatory enzyme of this pathway. Further this study suggests that the toxic levels of zinc has mediated its deleterious effects of increased ROS production through high free heme levels, which is a ROS generator through its peroxidase activity.

Acknowledgements: Authors are thankful to SVVU and SVU for providing funding and facilities for conduct of the research.

Conflict of Interest: The authors declare no conflict of interest.

Funding: No Special Funding.

References

1. Stefanidou M, Maravelias CD, Dona A, Spiliopoulou C. Zinc: A multipurpose trace element. Arch Toxicol. 2006;80(1):1-9.
2. Agnew UM, Slesinger TL. Zinc toxicity. In: StatPearls. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK560526/
3. Plum LM, Rink L, Haase H. The essential toxin: Impact of zinc on human health. Int J Environ Res Public Health. 2010;7(4):1342-1365.
4. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol Rev. 2015;95(3):749-784.
5. Bloomer JR, Reuter RJ, Morton KO, Wehner JM. Enzymatic formation of zinc-protoporphyrin by rat liver and its potential effect on hepatic heme metabolism. Gastroenterology. 1983;85(3):663-668.
6. Galaris D, Mira D, Sevanian A, Cadenas E, Hochstein P. Co-oxidation of salicylate and cholesterol during the oxidation of metmyoglobin by H_2O_2. Arch Biochem Biophys. 1988;262(1):221-231.
7. Kanner J, Harel S. Initiation of membranal lipid peroxidation by activated metmyoglobin and methaemoglobin. Arch Biochem Biophys. 1985;237:314-321.
8. Stiles LI, Ferrao K, Mehta KJ. Role of zinc in health and disease. Clin Exp Med. 2024;24(1):1-9.
9. Anwer J, Ali S, Mehrotra NK. Antagonistic effect of zinc in lead treated developing chick embryo. Drug Chem Toxicol. 1988;11(1):85-95.
10. Santose B, Subagio HW, Suromo L, Sunomo HR. Zinc supplementation improves heme biosynthesis in rats exposed to lead. Universa Medicina. 2015;34(1):3-9.
11. Mauzerall D, Granick S. The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. J Biol Chem. 1956;219:435-439.
12. Granick JL, Sassa S, Granick RD, Levere RD, Kappas A. Studies in lead poisoning II. Correlation between the ratio of activated to inactivated δ-aminolevulinic acid dehydratase of whole blood and the blood lead level. Biochem Med. 1973;8:149-159.
13. Lien LF, Beattie DS. Detection and regulation of δ-aminolevulinic acid synthetase activity in rat skeletal muscle. Arch Biochem Biophys. 1982;215:171-178.
14. Porra RJ, Jones OT. Studies on ferrochelatase. Assay and properties of ferrochelatase from pig liver mitochondrial extract. Biochem J. 1963;87(1):181-185.
15. Gerber GB, Maes J. Heme synthesis in the lead intoxicated mouse embryos. Toxicology. 1978;9:173-179.
16. De Duve C. Spectrophotometric method for simultaneous determination of myoglobin and haemoglobin in extracts of human muscle. Acta Chem Scand. 1948;2:264-289.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. J Biol Chem. 1951;193:265-275.
18. Snedecor GW, Cochran WG. Statistical methods. 6th ed. Oxford: Oxford and IBH Publishing; 1994.
19. Hafner S, Bounous D, Kelley LC, Campagnoli R. Hepatic protoporphyrin accumulation in broilers chicken. Avian Dis. 1993;37(3):900-904.
20. Savolainen H, Jappinen P, Tenhunen R. Reversal of sulphide induced effects on porphyrin metabolism by exogenous heme. Res Commun Chem Pathol Pharmacol. 1985;50(2):245-250.
21. Healey JF, Herbert LB, Sinclair PR, Sinclair JF. Conversion of 5-aminolevulinate into heme by homogenates of human liver: Comparison of rat and chick embryo liver. Biochem J. 1985;227(3):893-901.
22. Granica AD. Trace metal and haemoglobin metabolism. Ann Clin Lab Sci. 1981;11(3):220-208.
23. Padmaja K, Somasekharaiah BV, Prasad ARK. Selenium altered regulation of heme biosynthesis in chick embryos. Drug Chem Toxicol. 1993;16:395-408.
24. Herbert LB, Sinclair PR, Sinclair JF. Hepatic heme metabolism and its control. Yale J Biol Med. 1979;52:12-37.
25. Muller EU, Vincent SH. Concepts of heme distribution within hepatocytes. Biochem Pharmacol. 1985;34(6):719-725.
26. Chiabrando D, Vinchi F, Fiorito V, Mercurio S, Tolosano E. Heme in pathophysiology: A matter of scavenging, metabolism and trafficking across cell membranes. Front Pharmacol. 2014;5(8):61.
27. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. Nat Rev Neurosci. 2005;6:449-462.
28. Padmaja K, Ramamurthi R. Effect of zinc on lipid peroxidation and antioxidant enzymes in hepatic and brain tissues of chick embryos. J Enzyme Inhib. 1997;12:281-290.
29. Grujicic J, Allen AR. MnSOD mimetics in therapy: Exploring their role in combating oxidative stress-related diseases. Antioxidants. 2024;13(12):1444. https://doi.org/10.3390/antiox13121444

How to Cite This Article
Padmaja K, kumar TVC, Jayasri K. Impact of Zinc toxic levels on Hepatic Heme Biosynthesis in Chick Embryos. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(6):327-329.