

Effect of graded levels of zinc supplementation on growth performance and oxidative defense mechanism in rats

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Abstract—A study was undertaken to investigate the effect of graded levels of inorganic Zn (zinc carbonate) on growth performance and oxidative defense mechanism in weaned female Sprague Dawley rats for experimental duration of 16 weeks. Ninety weaned rats (93.83 ± 1.308 g Bwt) were randomly allotted to 30 replicates with 3 rats in each replicate which were in turn randomly allotted to 5 dietary treatments consisted of supplementing 0, 12, 24, 36 and 48 ppm Zinc carbonate to a basal diet (BD) formulated with purified ingredients without zinc. The zinc supplemented rats showed significant ($P < 0.01$) improvement in average daily gain without variation in dose of zinc supplementation. In comparison to basal diet, the feed intake in Zn supplemented rats was significantly ($P < 0.01$) higher from the 4th week and similar trend continued till the end. The lipid peroxidation was higher ($P < 0.01$) in rats fed diet with no zinc supplement. The oxidative stress markers in haemolysate (lipid peroxidation) and liver (TBARS and protein carbonyls) was higher ($P < 0.01$) in BD fed rats and their concentration reduced with Zn supplementation in a dose dependent manner up to 36ppm, while with 48ppm it increased. The reduced glutathione concentration in liver was lower ($P < 0.01$) in rats fed BD and 48ppm Zn supplemented rats, while it increased gradually from 12 to 36ppm Zn supplementation. The activity of glutathione peroxidase and reductase was highest in 36 and 48 ppm Zn supplemented rats followed by rats fed on 24 and 12 ppm zinc supplementation and BD. It indicates that Supplemental Zn at 12, 24, 36 and 48ppm was effective in improving the growth performance, feed intake and oxidative defense except 48 ppm Zn had negative effect on antioxidant activity.

Keywords—Antioxidants, feed intake, growth, rats, zinc.

I. INTRODUCTION

Zinc is an essential trace element for human as well as animals, and it exerts various physiological functions that influence the growth, health and reproduction in different ways. Zinc is an intracellular signaling molecule and plays an important role in antioxidant mechanism through number of proposed mechanisms [1]. This is the unique property of zinc that may have significant therapeutic benefits in several diseases in humans and livestock. In many diseases concurrent zinc deficiency may cause complication of clinical features by increasing the oxidative stress [2] since excess accumulation of free radicals than that can be handled by antioxidant enzymes can lead to chronic disorders in livestock as well as human. Though Zn is an essential micronutrient, the use of Zn supplement has been discouraged by some health professional because excessive intake of Zn has been reported to induce Cu, Mg, Mn, Fe and Se deficiencies due to their antagonistic interactions [3] as these minerals are required as cofactor for optimum catalytic activities and effective antioxidant defense mechanism of the body.

Hence, regardless of how these highly reactive oxygen species are generated, they must be dealt with in order to maintain good health and optimal productivity. Hence, our present investigation was carried out to know the influence of levels of zinc supplementation on growth performance and oxidative defense mechanism in rats.

II. MATERIAL AND METHODS

A study was conducted on 90 weaned female Sprague Dawley rats (SD) strain with an average body weight of 93.83 ± 1.308 g. The rats were housed in polypropylene cages in the Animal House of College of Veterinary Science, Hyderabad under hygienic conditions with controlled temperature (22-23°C) and photoperiod (12h/d). The rearing and care of rats and procedures adopted were according to the guidelines of

Institutional Animal Ethics Committee. The rats were provided with respective diet *ad libitum* and with free access to wholesome clean deionised water. Water was provided in polypropylene bottles having provision for nipples. The animals were randomly divided into 30 replicates with 3 rats per replicate and these 30 replicates in turn were randomly allotted to 5 dietary treatments and were fed with the respective diets for an experimental duration of 16 weeks. A basal diet (BD) based on AIN-76A rodent diet for growing rats was formulated without any Zn supplement using purified ingredients (Table 1) as a control diet. The other 4 diets were Zn supplemented diets in which Zn was supplemented to BD in the form of Zn carbonate to supply 12, 24, 36 and 48 ppm of Zn. During experimentation, weekly body weights and daily feed intake were recorded. On 83rd d of the experiment, blood was collected to assess the antioxidant enzymes. After 16 weeks of feeding trial, one rat per replicate was sacrificed and liver was collected for determining antioxidant enzymes activity in liver.

Table 1 Ingredient composition of Purified diet (AIN-76A)

Ingredient	Proportion, g/kg diet
Sucrose	500.0
Casein	200.0
Corn starch	150.0
Oil	50.0
Cellulose	50.0
Mineral mixture*	35.0
Vitamin mixture*	10.0
DL-methionine	3.0
Choline chloride	2.0

*Mineral and vitamin mixture was prepared as per specifications for AIN-76A

2.1 Oxidative stress markers and antioxidant enzyme activity in haemolysate

The blood collected in clean heparinized vacutainers was centrifuged at 2000 rpm for 15 minutes at 4°C to separate buffy coat and erythrocyte pellet. The erythrocytes were washed thrice with phosphate buffer saline (PH 7.4). The packed RBC obtained was mixed with an equal volume of phosphate buffer saline and then diluted as per requirement with distilled water.

The oxidative enzymes viz., RBC catalase, lipid peroxidation (LPx), glutathione peroxidase and glutathione reductase in haemolysate were estimated as per the procedures of Bergmeyer [4], Placer [5], Paglia and Valantine [6] and Mannervik [7] respectively and the enzyme activity was expressed as units per g Hb, nmol MDA/g Hb, µM/mg protein and µM/mg protein, respectively. The Hb and protein concentration in haemolysate was estimated colorimetrically as per the procedure described by Cannan [8] and Lowry [9].

2.2 Oxidative stress markers in liver

After 16 weeks of experiment, one rat per replicate was sacrificed. Immediately after slaughter, liver was removed and perfused with normal saline (0.9%) to reduce red blood cell contamination. The samples were then fixed in liquid nitrogen and stored at -20°C for antioxidant analysis. The markers Thiobarbituric acid reacting substances (TBARS), reduced glutathione (GSH) and protein carbonyls were estimated as per the procedures of Balasubramanian [10], Moron [11] and Levine [12], respectively and was expressed as nmole per mg protein, µM GSH/mg of protein and nmole per mg protein, respectively.

2.3 Statistical analysis

The results obtained were statistically analyzed by using one way ANOVA according to the method of Snedecor and Cochran [13]. The means were compared by Duncan's multiple range test [14].

III. RESULTS AND DISCUSSION

3.1 Effect of zinc on body weight and feed intake

No effect of zinc supplementation was observed on body weight of rats up to 3rd week of study, while from 4th week onwards, dietary zinc supplementation had influence on weekly body weights. The body weight during 4th week was higher ($P < 0.01$) in zinc supplemented rats than BD fed rats and similar trend continued till the end of the feeding trial i.e. up to 16 weeks. Further this was reflected in average daily gain (ADG) which improved ($P < 0.01$) with Zn supplementation. Among the Zn supplemented rats, no effect of Zn concentration (12–48 ppm) was observed on body weights and average daily gain.

Similar to body weight changes, the feed intake was comparable among rats of various groups up to 4th week and influence of Zn supplementation was observed from 4th week. During the 4th ($P < 0.01$) and 5th ($P < 0.05$) week the feed intake was higher in rats fed 12 or 24 ppm zinc compared to BD fed rats and similar pattern was observed till the end of 16th week. The feed intake in rats fed on 48 ppm Zn diet was comparable to that of BD, but later it increased by the end of 16 weeks and was similar to the intake of other Zn supplemented rats (12-36 ppm). These results were in agreement with Sahin and Kucuk [15], who observed increased feed intake ($P < 0.01$) in Zn supplemented quails (30 and 60 ppm) compared to unsupplemented group.

In the present investigation, retardation of growth in the BD group might be due to impairment of zinc dependent enzymes that influence the cell proliferation and DNA replication but not due to decreased feed intake. The improved ADG in zinc supplemented groups is consistent with the reports of Taneja [16] and Singh [17] in rats, who observed improved growth rate in rats fed basal diet containing 20ppm Zn as $ZnSO_4 \cdot 7H_2O$ throughout the experimental period (180 days), and growth rate reduced in 40 and 80ppm supplemented diets fed rats after 150 days of experiment. In present study, no such growth depression was observed in 36 and 48 ppm Zn supplemented rats. Similarly several authors reported that rats fed zinc deficient diets gained 25% [18] and 11% [19] lower weight than the zinc supplemented (30 mg/kg diet and 40 mg/l, per os respectively) rats. In present investigation, the lower feed intake with 48ppm supplementation up to 15th week, suggested that higher concentration of $ZnSO_4$ could have a negative influence on feed intake.

3.2 Effect of zinc on antioxidant enzymes and oxidative stress markers

Lipid peroxidation is a free radical mediated chain reaction, is treated as best marker of oxidative stress [20] as its concentration increases during oxidative stress. Though it is a self perpetuating reaction its propagation can be arrested by chain breaking antioxidant. The results in the present study indicated that oxidative stress was higher in zinc deficient rats and was alleviated with Zn supplementation (Table 4 and 5). The lipid peroxidation was higher ($P < 0.01$) in rats fed diet with no zinc supplement. Supplementation of 12ppm Zn reduced the lipid peroxidation, but the effect was not significant, while lipid peroxidation was lowered in rats fed on 24 and 36ppm Zn. It implies that Zn had an ameliorative effect on lipid peroxidation, while peroxidation was increased with 48ppm of Zn supplementation. Similarly, the TBARS and protein carbonyls concentration in liver indicative of oxidative stress was lower ($P < 0.05$) in Zn supplemented rats compared to unsupplemented ones. Among the zinc supplemented rats, the TBARS and protein carbonyls content was lowest ($P < 0.05$) in rats fed 36ppm Zn followed by 24ppm Zn supplementation, and the concentration in liver of 48ppm Zn supplemented rats was higher ($P < 0.05$) compared to Zn supplementation at lower concentration (12–36 ppm).

The reduced glutathione concentration in liver was lower ($P < 0.01$) in Zn deficient rats and increased gradually with 12 to 36ppm Zn supplementation (Table 5). The concentration of reduced glutathione was highest on 36ppm Zn supplementation and thereafter its concentration gradually decreased with 48ppm Zn addition. Similarly, glutathione peroxidase activity was higher ($P < 0.05$) in rats fed Zn supplemented diet than rats fed on BD. Among the Zn supplemented rats highest activity of glutathione peroxidase was observed with 48 ppm supplementation followed by 36, 24 and 12 ppm supplementation in a dose dependent manner.

The increased levels of oxidative stress markers in zinc deficient rats and improvement in antioxidant enzyme activity, with reduced lipid peroxidation in the present investigation with Zn supplementation in dose dependent manner was observed up to 36ppm zinc supplementation. However, the reverse observed in 48ppm Zn supplemented group might be due to hyper zincemia or due to altered Cu-Zn ratio. Though zinc is an essential trace element, there are some evidences that excess zinc can cause enhanced oxidative stress [21]. The activities of Cu and Zn containing antioxidant enzymes including superoxide dismutase (SOD), cytochrome-C oxidase (CCO), catalase (CAT) and glutathione peroxidase (GPx) have been reported to diminish under Zn or Cu-deficiency or due to alteration in Cu-Zn ratio [22,23, 24]. Similar to present investigation, Singh, [17] reported that supplementation of excess zinc (40 and 80ppm Zn as $ZnSO_4 \cdot 7H_2O$) in rats resulted in significant ($P < 0.01$) increase in oxidative stress markers (LPx) in liver and kidney than in the control group supplemented with 20ppm. Further, their high concentration in excess zinc supplemented groups coincided with enzymes of antioxidant defense system i.e. SOD, CAT, glutathione-S-transferase, glutathione reductase, GSH and glucose-6-phosphate dehydrogenase whose activities were evaluated significantly less ($P < 0.01$; $P < 0.05$) in these groups after six months of dietary treatment. Several researchers reported that a deficiency of zinc led to lipid peroxidation in various tissues [25,26]. It was shown that zinc deficiency caused or increased oxidative stress in rat testicles [27], kidney [28], liver [29], and brain [30]. Similarly ameliorative effect of zinc against oxidative stress in rats was reported by several authors, who induced the stress artificially using electromagnetic field [31], cadmium [19], alloxan [32], carbon tetra chloride [33] and burns [34].

IV. CONCLUSION

Supplemental Zn at 12, 24 and 36 ppm was effective in improving the oxidative defense mechanism in a dose dependent manner. Improvement in growth rate and feed intake was observed with 12 ppm Zn supplementation, while Zn supplementation at 24-48 ppm had no further improvement on growth performance.

The supplementation of 48 ppm Zn had negative effect on antioxidant activity. It indicates that supplemental zinc above AIN-76A recommended Zn concentration (36 ppm) had negative influence on oxidative defense mechanism without affecting growth performance and feed intake. Hence it shows that judicial concentration of dietary Zn must be essential in improving the health condition in humans and livestock.

Table 2: Body weight changes (g) in rats fed diets varying in zinc concentration

Week	Zinc supplementation in ppm					SEM	P value
	0	12	24	36	48		
Start	93.83	93.83	93.83	93.83	94.18	1.321	0.999
1	117.0	113.7	123.5	115.6	116.7	1.629	0.392
2	139.2	143.4	151.1	146.2	148.4	1.647	0.222
3	160.6	165.5	171.2	168.3	170.3	1.717	0.284
4	163.5 ^b	188.3 ^a	190.1 ^a	183.7 ^a	188.9 ^a	2.614	0.004
5	178.6 ^b	199.3 ^a	205.8 ^a	201.4 ^a	202.7 ^a	2.694	0.008
6	190.6 ^b	213.8 ^a	217.8 ^a	213.2 ^a	216.1 ^a	2.879	0.013
7	200.6 ^b	225.6 ^a	225.0 ^a	226.2 ^a	223.3 ^a	2.892	0.017
8	208.1 ^a	233.2 ^b	228.4 ^b	235.6 ^b	220.5 ^{ab}	2.966	0.019
9	212.3 ^b	238.9 ^a	235.2 ^a	238.1 ^a	225.3 ^a	2.985	0.018
10	220.9 ^b	246.7 ^a	242.7 ^a	245.2 ^a	241.6 ^a	3.078	0.047
11	222.9 ^b	251.3 ^a	247.6 ^a	249.4 ^a	247.9 ^a	3.134	0.019
12	222.9 ^b	247.1 ^a	253.2 ^a	248.3 ^a	247.4 ^a	3.276	0.025
13	229.6 ^b	254.9 ^a	261.8 ^a	258.3 ^a	250.5 ^a	3.237	0.011
14	216.7 ^b	257.9 ^a	264.7 ^a	264.7 ^a	261.3 ^a	4.452	0.002
15	213.4 ^b	261.2 ^a	262.3 ^a	262.8 ^a	258.2 ^a	4.469	0.001
16	213.8 ^b	256.9 ^a	263.0 ^a	260.2 ^a	264.5 ^a	4.622	0.001
Total gain	120.0 ^b	163.1 ^a	169.2 ^a	165.2 ^a	170.3 ^a	4.763	0.002
ADG	7.50 ^b	10.19 ^a	10.57 ^a	10.32 ^a	10.65 ^a	0.298	0.002

^{abc}Means with different superscripts in a row differ significantly: P<0.01; P<0.05

Table 3: Daily feed intake in rats fed diets varying in zinc concentration

Week	Zinc supplementation in ppm					SEM	P value
	0	12	24	36	48		
1	8.91	7.91	9.33	7.98	8.64	0.199	0.103
2	11.11	11.79	11.61	12.64	12.32	0.275	0.452
3	10.82	12.56	11.90	11.87	11.49	0.200	0.073
4	11.48 ^b	12.99 ^a	13.29 ^a	12.08 ^b	11.98 ^b	0.164	0.001
5	11.38 ^b	13.13 ^a	13.02 ^a	12.68 ^{ab}	12.44 ^{ab}	0.221	0.049
6	10.91 ^b	12.56 ^a	12.52 ^a	12.61 ^a	11.30 ^b	0.206	0.006
7	10.75 ^b	12.89 ^a	13.05 ^a	13.02 ^a	12.27 ^{ab}	0.277	0.030
8	10.99 ^{bc}	13.33 ^a	12.18 ^{ab}	12.86 ^a	9.95 ^c	0.327	0.001
9	11.70 ^b	14.01 ^a	13.11 ^{ab}	12.48 ^{ab}	11.49 ^b	0.287	0.021
10	11.96 ^b	13.46 ^b	16.53 ^a	12.53 ^b	12.89 ^b	0.373	0.001
11	11.17 ^b	12.69 ^{ab}	13.77 ^a	12.76 ^{ab}	12.03 ^{ab}	0.288	0.048
12	10.85 ^b	12.42 ^{ab}	12.20 ^{ab}	12.76 ^a	11.08 ^b	0.254	0.048
13	11.41 ^b	13.03 ^a	13.63 ^a	12.17 ^{ab}	12.18 ^{ab}	0.242	0.027
14	10.72 ^b	12.69 ^a	13.22 ^a	12.41 ^a	12.08 ^{ab}	0.265	0.027
15	9.48 ^b	11.82 ^a	11.44 ^{ab}	10.44 ^a	10.59 ^{ab}	0.284	0.046
16	9.06 ^b	11.75 ^a	11.44 ^a	11.75 ^a	11.01 ^a	0.313	0.022

^{abc}Means with different superscripts in a row differ significantly: P<0.01; P<0.05

Table 6: Oxidative enzyme activities in haemolysate of rats fed diets varying in zinc concentration

Attribute	Zinc supplementation in ppm					SEM	P value
	0	12	24	36	48		
Lipid peroxidation (nM MDA/g Hb)	28.35 ^a	24.83 ^{ab}	13.92 ^c	18.47 ^{bc}	24.40 ^{ab}	1.334	0.001
Glutathione peroxidase (µM/mg protein)	0.860 ^c	1.246 ^{bc}	1.406 ^b	2.173 ^a	2.363 ^a	0.124	0.001
Glutathione reductase (µM/mg protein)	0.650^b	0.958^{ab}	0.849^b	1.273^a	0.964^{ab}	0.313	0.006

^{abc}Means with different superscripts in a row differ significantly: P<0.01

Table 7: TBARS, protein carbonyls and reduced glutathione concentration in liver of rats fed diets varying in zinc concentration

Attribute	Zinc supplementation in ppm					SEM	P value
	0	12	24	36	48		
TBARS (nM MDA/mg protein)	0.020 ^a	0.018 ^{ab}	0.013 ^{bc}	0.010 ^c	0.016 ^{abc}	0.0010	0.030
Protein carbonyls (nM/mg protein)	1.37 ^a	1.05 ^{bc}	1.05 ^{bc}	0.887 ^c	1.16 ^{ab}	0.045	0.049
Reduced glutathione (µM/mg protein)	31.28^c	143.53^b	101.07^{bc}	278.93^a	34.15^c	19.951	0.001

^{abc}Means with different superscripts in a row differ significantly: P<0.01; P<0.05

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