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Effect of Zinc Oxide Supplementation on Some Serum Biochemical Values in Male Broilers

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Abstract: One hundred eight (*Ross-308*) strain chicks, were randomly divided into 3 treatments (A: basal diet, B: basal diet + 50 ZnO ppm and C: basal diet +100 ZnO ppm) with 3 replicates of 12 birds per treatment, to investigate the effect of Zinc oxide (ZnO) supplement on serum lipoproteins, lipid peroxidation and some enzymes activities in male broilers from 21 to 42 days. At the end of this period, the chicks were fasted for 6 hours and then 2 birds were randomly chosen from each pen for analysis of some serum parameters. Results revealed that in group C (which fed on 100 ppm ZnO) malondialdehyede (MDA) values were lower and total antioxidant status (TAS) and high density lipoprotein cholesterol (HDL-C) values were higher than those of other groups ($P<0.05$).Also, the levels of ZnO supplement had no significant effect on serum cholesterol (CHOL), low density lipoprotein cholesterol (LDL-C), triglyceride (TRG), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activity. In conclusion, ZnO supplement (100 ppm), improved the antioxidant defense mechanisms, HDL-C and reduced MDA levels as an index of lipid peroxidation in serum.

Key words: Zinc Oxide · Lipoprotein · lipid peroxidation and Broiler

INTRODUCTION

Zinc (Zn) has been known to be an essential nutrient for animals for many years. It is required for skeleton development, growth, skin growth and integrity, appetite, reproduction, wound healing, immune competence and many biochemical processes [1]. The recommended Zn requirement for broilers is 40 mg/kg diet for almost all countries and no difference for different stages is reported [2]. Researcher demonstrated that Zn can accumulate in bone, liver and intestine and subsequently be released for use during a period of Zn deficiency [3].

Moreover, Cunningham- Rundles *et al.* [4] indicated that Zn acts as antioxidant reducing the cell membrane damage due to radicals, which in turn according to Powell [5] alters the immunological status of the animal. The mechanism by which Zn exerts its antioxidant action is not well defined. However, it has been suggested that Zn increases the synthesis of metallothionein, a cysteine-rich protein, which acts as a free radical scavenger [4].

Zn had a significant role in reduction of malondialdehyde (MDA) levels as an index of lipid peroxidation in serum and tissues. MDA shows both mutagenic and carcinogenic effects by changing membrane properties. On the other hand, studies showed that Zn administration for 6 weeks had significantly effect on decreased the plasma level of LDL-C in human. Also, an apparent inverse linear relationship between plasma Zn levels and LDL-C levels was found [6]. Also, Zn has multiple important functions because it is a cofactor for> 200 enzymes [7]. For example, Alkaline Phosphatases are a group of enzymes found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta and the kidney (in the proximal convoluted tubules), what is measured in the blood is the total amount of alkaline phosphatases released from these tissues into the blood. ALP act by splitting off phosphorus (an acidic mineral) creating an alkaline pH. The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease [8]. Decreases in the activity of this enzyme have been reported in pig serum [9], calf serum [10]. Another enzyme is LDH; increases in the activity of this enzyme with zinc additive, have been reported in serum of bulls [11].

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In poultry, LDH plays a key role in hypoxic conditions and the incidence of ascites. The ascitic birds are expected to be hypoxemic and in oxygen debt as partial anaerobic stage of metabolism might exist in these birds. Studies showed that the activity of serum LDH was higher in healthy birds compared to ascitic birds [12].

In the current research, the effect of ZnO supplement on some serum biochemical values in male broilers was investigated.

MATERIALS AND METHODS

Chicks and Diets: One hundred eight 21-day-old male broiler chicks (*Ross308 strain*) were randomly assigned to 3 groups consisting of 3 replicates of 12 birds. Utmost care was taken to provide equal physical and environmental housing conditions (namely size of units, light, temperature and aeration). Stainless-steel feeders and plastic waterers were used. Feed and water were supplied *ad libitum*. Experimental diets, formulated according to NRC [13], included following levels of ZnO: A) control diet (no ZnO), B) 50 ZnO ppm C) 100 ZnO ppm. Birds were fed with experimental diet for grower (21- 42 d) period (Table1).

Table 1: Composition of the basal experimental diet

Ingredient	(%)
Ground yellow corn	57.00
Soybean	27.00
Fish meal	1.50
Wheat bran	1.78
Wheat starch	8.48
Sea shell meal	1.55
De Calcium Phosphate	1.39
Vitamin Prmix [1]	0.25
Minera Permix[2]	0.25
DL-methionine	0.10
Sodium Chloride	0.25
Coccidio acetate	0.05
Fine Sand	0.40
Calculated analysis	
ME kcal/ kg	2950.4
Crude protein (%)	18.44
Calcium (%)	1.01
Available P (%)	0.41
ZINC, mg/kg	96

1-Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K. 2: Composition of mineral premix provided as follows kilogram of premix: Mn, 120.000 mg: Zn, 80.000 mg: Fe, 90.000 mg: Cu, 15.000mg: I, 1,600 mg: Se, 500 mg: Co, 600 mg

Samples Procedures: The zinc contents of corn, soybean meal and fish meal were taken from Gorgulu *et al.* method [14]. At the end of period (42 d) the chicks were fasted for 6 hours and then 2 birds were randomly chosen from each pen for analysis of serum parameters. Blood samples were collected from the vena axillaries. Samples were centrifuged at 3000×rpm for 10 min and sera were collected. The analysis of serum CHOL, HDL-C, TRG levels were measured on biochemical autoanalyzer (Alcyon abbot-300, USA) by using commercially available kits. LDH and ALP activates were determined Spectrophotometerically by using commercial kits [15]. Also, LDL-C value was estimated Friedewald *et al.* [16] equation. The level of malondialdehyde (MDA) were measured in the serum with the tiobarbituric-acid reaction by the method of placer *et al.* [17] and Total Antioxidant Status (TAS) Randox (SAO) (as described in the Randox Laboratories manual) which measured the capacity of the serum to neutralise the oxidative action of free radicals.

Statistical Analyses: The data collected were subjected to an analysis of variance and any significant differences were determined. When the ANOVA revealed significant differences, Duncan's multiple range tests was performed to establish where such means differed. All the data were analyzed by ANOVA using the general linear model (GLM) procedures of the SAS Institute [18].

RESULTS

In Tables 2 and 3, the blood serum CHOL, HDL-C, LDL-C, TRG, MDA, TAS, ALP and LDH values of all the groups of male broilers are given. There was a significant difference in the serum values of HDL-C, MDA and TAS ($P<0.05$) between the male broilers in the group 3 (fed with 100 ppm ZnO) in comparison to the other groups.

Table 2: Some blood serum[1] enzymes of male broilers fed with ZnO supplement (Means±SD)

	Measurement	
Diet	*ALP (U/L)*	*LDH (U/L)*
ZnO 0.00 ppm	2234.21±101.02	601.25±11.24
ZnO 50 ppm	2228.97±104.15	605.0±15.10
ZnO 100 ppm	2223.44±121.77	614.14±14.37
P value	NS	NS

[1] n = 6 samples within each treatment group. NS=non significant

Table 3: Blood serum parameters[1] concentrations in male broilers fed with ZnO (Means± SD)

	Measurement					
Diet	*COL (mg/dl)*	*LDL-C (mg/dl)*	*HDL-C (mg/dl)*	*TRG (mg/dl)*	*MDA nmol/ml*	*TAS mmol/ml*
ZnO 0.00 ppm	149.05±5.21	106.36± 4.69	19.72± 1.02^{b}	84.83± 3.25	4.30± 0.90^{a}	0.81± 0.03^{a}
ZnO 50 ppm	141.18±6.01	103.87± 5.06	22.94± 2.47^{ab}	86.44± 4.02	4.31± 0.94^{a}	0.86± 0.04^{a}
ZnO 100 ppm	140.88±3.91	100.02± 4.75	24.27± 2.14^{a}	83.05± 4.58	4.02± 0.95^{b}	0.95± 0.05^{b}
P value	NS	NS	*	NS	*	*

[1] n = 6 samples within each treatment group

NS=non significant, * = ($p < 0.05$), ^{a,b} Means within columns with no common superscript differ significantly ($P < 0.05$).

However, the serum parameters values in the group 2 (fed with 50 ppm ZnO) did not change significantly, in comparison to the control group. Altogether, different levels of ZnO supplement had no significant effect on serum CHOL, TRG, LDL-C, LDH and ALP levels. Nonetheless, with increasing ZnO additive, the levels of serum LDH raised earlier.

DISCUSSION

The obtained results indicated that the addition of ZnO supplement to feed had no significant effect on serum CHOL, LDL-C and TRG. These findings were in agreement with the work of Uyanik *et al* [19]. They reported that serum CHOL values slightly decreased in $ZnSO_4$ supplemented groups. On the other hand, reduction in LDL parameter cause increase in HDL. In the present study, MDA values decreased significantly in only 100 ppm ZnO supplemented group, while elevated serum TAS. Our results corresponded with Zago and Oteiza [20]. They reported that the reduced lipid peroxidation in Zn-supplemented birds might be due to the multifunctional roles such as antioxidative action of Zn. Shaheen and Abd EL-Fattah [21] reported that dietary Zn deficiency caused increased lipid peroxidation and this was inhibited by Zn supplementation. Onderci *et al.* [22] reported that Zn and chromium supplementation decreased serum MDA values and increased values of antioxidant vitamins such as A, C and E in cold-stressed laying hens. Moreover, Cunningham- Rundles *et a*l. [4] indicated that serum CuZn-SOD activity may play a key role in suppressing free radicals, reached a peak with Zn added levels being 80-120 mg/kg diets. In general, two mechanisms of antioxidative action of Zn can be divided into acute and chronic effects. Chronic effects involve exposure of an organism to Zn on a long- term basis. Zinc ions may replace redox (*oxidation-reduction*) active molecules, such as iron and copper, at critical sites in cell membranes and protein structure. And also, Zn ions may induce the synthesis of metallothionein that protect against free radical oxidative damage [5]. In our study, ZnO supplementation had no significant effect on serum ALP activity. But with increasing ZnO additives, the values of serum ALP reduced earlier. This result was in accordance with the reports of Uyanik *et al.* [19] Mohanna and Nys [23]. They showed that ALP activity was decreased in zinc supplemented ($ZnSO_4$) chiks. Moreover, Watkins and Southern (1993) did not find differences in ALP activity by increasing Zn use [24]. Based on our results and literature data, In conclusion, ZnO (100 ppm) usage might cause beneficial effects on serum parameters such as LDL-C, MDA and TAS.

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