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Effect of Dietary Zinc on Certain Blood Traits of Broiler Breeder Chickens

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Abstract: The aim of the present study was to evaluate the effect of zinc as feed additive on some the blood plasma traits of males and females of broiler breeder chickens. A total 132 (96 females and 36 males) of Cobb 500 broiler breeder chickens, 45 weeks old were used in this study. These birds were randomly distributed into four dietary treatment groups with three replicates each. Each treatment group constituted of 24 females and 9 males. Treatment groups were as following: T1: Birds fed the basal diet without any addition of zinc (0 Zn) (Control), T2: Birds fed diet supplemented with 50 mg Zn (pure zinc)/kg of diet, T3: Birds fed diet supplemented with 75 mg Zn (pure zinc)/kg of diet and T4: Birds fed diet supplemented with 100 mg Zn (pure zinc)/kg of diet. Results indicated that dietary zinc supplementation (T2, T3 and T4) resulted in significant ($p < 0.05$) increase in blood plasma cholesterol, protein, calcium and phosphorus concentration and Alkaline Phosphatase (ALP) activity in broiler breeders males and females during 58 and 66 weeks of age and as regards the total means of these traits as compared with control group (T1). However, adding different levels of zinc to the diet (T2, T3 and T4) resulted in significant ($p < 0.05$) increase in blood plasma glucose in broiler breeders males and females during 54, 58, 62 and 66 weeks of age and concerning the total mean of this trait. In conclusion, supplementing the diet of broiler breeder chickens with zinc caused significant increase in blood plasma traits included in this study. Therefore, zinc can be used as efficient feed additive for enhance physiological status of birds.

Key words: Zinc, blood traits, broiler breeder chickens

INTRODUCTION

Plasma zinc seems to fall only when dietary intake is so low that homeostasis cannot be established without the use of some zinc from the exchangeable pool, of which plasma zinc is a component. Thus, plasma zinc is a valid, useful indicator of the size of exchangeable pool zinc; reduction in plasma zinc reflects a loss of zinc from the bones and liver and an increased risk of the development of metabolic and clinical signs of zinc deficiency (King, 1990). The levels of plasma zinc and lipoprotein organic phosphorus in mature laying hens were reported to be higher than those of immature females and mature male birds due to plasma vitellogenin (Mitchell and Rose, 1991). It was also suggested that the measurement of plasma zinc provides a simple and accurate technique for the estimation of the reproduction status in domestic fowl (Mitchell and Rose, 1991). An experiment conducted by Donmez *et al.* (2002) examined the addition of Zn into drinking water at levels 0, 125, 500, 1000 mg/kg on physiological performance of broiler chicks and found that there was no significant differences between control and Zn - supplemented groups as regards total erythrocyte count, hemoglobin amount, hematocrit, total leukocyte count and differential leukocyte count. Kaya *et al.* (2001) reported that plasma and yolk zinc

concentration were affected only by zinc supplementation. However, an interaction between Zn and vitamin-A had an effect on plasma triglyceride and phosphorus concentrations of laying hens. Furthermore, since the positive relationship between plasma zinc and egg production was observed ($r = 0.279$, $p < 0.01$), it is suggested that plasma zinc may be an indicator for estimation of egg production or hen performance (Kaya *et al.*, 2001). A study undertaken by Fu-Yu *et al.* (2007) to investigate the effect of different levels of zinc on blood parameters of Holstein Bulls involved 5 dietary treatments (0, 50, 80, 110 and 200 mg/kg of diet). The results of this study showed that the addition of zinc to the diet resulted in significant ($p < 0.01$) decrease in activity of serum GOT and GPT and serum testosterone concentration, whereas the effect of dietary supplementation of zinc on ALP and Lactate Dehydrogenase (LDH) activity and serum zinc was not significant ($p > 0.05$). However, the best results with relation to blood traits included in this study were recorded when zinc was added at the level of 110 mg/kg of diet.

A study was carried out by Ozcelik *et al.* (1995) to investigate the accumulation of toxic doses of zinc on several and their effect on performance of broiler chicks. For this purpose 60 one day-old chicks were used and

they were divided into two groups including control group and experimental group. Chicks in control group were fed with normal ration, while the experimental group was fed with diets containing 2500 ppm zinc. At the end of the fourth week, the chicks were slaughtered when the mineral levels in serum, liver, kidney, muscle and bone of the control group and the experimental group were analyzed, and it was found that the difference was statistically significant ($p < 0.001$). The Zinc concentrations in the serum, liver, kidney, muscle and bone of experimental group were 327.9%, 1004.4%, 390.2%, 68.0% and 613.6% respectively and higher than the control group. This findings support the hypothesis that liver, muscle and blood levels of zinc in chicks increased parallel with the zinc concentrations in the diet (Henry *et al.*, 1987; Watkins and Southern, 1983; Stahl *et al.*, 1989; Shan, 1993). In a another study of Chantiratikul *et al.* (2008) to investigate the effect of sodium selenate and zinc-L-selenomethionine on plasma Selenium (Se) concentration and glutathione peroxidase (G-SH-Px) activity in the Red Blood Cells (RBC) of laying hens, the dietary treatments were T1 : basal diet, T2, T3 and T4: basal diet added with 0.3, 1.0 and 3.0 mg Se from sodium selenite/kg, respectively, T5, T6 and T7: basal diets added with 0.3, 1.0 and 3.0 mg Se from zinc-L-selenomethionine/kg, respectively. The findings of this study revealed that the plasma Se concentration of hens received supplemented zinc-L-selenomethionine diets was higher ($p < 0.05$) than that of hens which received supplemented sodium selenite diets. The results of this experiment also indicated that zinc-L-selenomethionine increased plasma Se concentration than sodium selenite, however G-SH-Px activity in RBC of laying hens was not affected by Se sources. However, the purpose of the present study was to determine the effect of dietary zinc supplementation on certain blood plasma traits of broiler breeder chickens.

MATERIALS AND METHODS

One hundred and thirty six Cobb500 broiler breeders' males and females, 45 weeks old were obtained from the Erbil Poultry Project, Iraqi Kurdistan Region and the study was carried out there. The birds were divided into four groups. One group of birds consumed no zinc supplemented diet (0 mg/kg diet, T1), while another groups of birds consumed 50, 75, 100 mg pure zinc (Himedia-India)/kg diet supplemented diet (T2, T3, T4 respectively). Each treatment group constituted of 24 females and 9 males. Each group of birds was further divided into three replicates (8 hens and 3 cocks in each replicate). Birds were distributed on 12 floors pens of 3 replicates. Birds were maintained under 16 hr light and 8 hr dark and mean temperature of 18-21°C during the whole period of study. Birds received the commercial diets and water was provided by nipples through the experimental period (22 weeks). Blood samples were

collected from wing vein using sterile lancet and centrifuge tubes containing EDTA for blood collection and then centrifuged at 1000 rpm for 10 min, blood plasma separated and frozen at -25°C till analysis, where the number of birds that used for blood collection for each treatment was 15 females and 9 males.

Plasma total cholesterol was enzymatically measured using cholesterol esterase and cholesterol oxidase according to the enzymatic method described by Allain (1974). The determination of blood plasma glucose was based on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in production of hydrogen peroxide. Plasma glucose was determined according to Trinder (1969) using commercial kits of plasmatic laboratory products LTD. Plasma total proteins were determined by using colorimetric method described by Gornall *et al.* (1949). The peptide bonds of proteins react with Cu^{+2} in alkaline solution to form a coloured complex whose absorbance, proportional to the concentration of total protein in the specimen or sample, is measured at 550 nm. The Biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution. Moorehead and Briggs (1974) derived CPC (O-cresol phtalin complexone) method allows to determinate total calcium concentration in plasma, serum or urine. In alkaline solution CPC reacts with calcium to form a dark-red coloured complex whose absorbance measured at (570 nm) is proportional to the amount of calcium in the specimen (sample) by using commercial kits (Biolabo, Maizy, France). To determine blood plasma phosphorus the method without deproteinisation described by Daly and Ertingshausen (1972), modified by Gamst and Try (1980) was used. In an acid medium, phosphate ions form a phosphomolybdic complex with the ammonium molybdate. The absorbance measured at 340 nm is proportional to the concentration of phosphate ions in the sample or specimen. Colorimetric method was used to determine alkaline phosphatase activity according to Kind and King (1954). The data were analyzed statistically using the General Linear Models procedure of SAS (2001). Significant differences between treatment means are separated using the Duncan's multiple range test with 5% and 1% probability (Duncan, 1955).

RESULTS AND DISCUSSION

Blood parameters of females: As shown from Table 1 and 3 the addition of zinc to the diet of broiler breeder chickens (T2, T3 and T4) resulted in significant ($p < 0.05$) increase in blood plasma cholesterol, protein, calcium, phosphorous concentrations and ALP activity at 58 and 66 weeks of age in comparison with control group. However, dietary supplementation with different levels of zinc resulted in significant ($p < 0.05$) increase regarding total means of blood plasma cholesterol, protein, calcium, phosphorous concentrations and ALP activity

Table 1: Effect of dietary supplementation of zinc on certain blood plasma traits (Mean±SE) of broiler breeder hens at 58 and 66 weeks of age

Traits	Treatments			
	58 week of age			
	T1	T2	T3	T4
Cholesterol concentration (mg/100 ml)	115.66±23.66B	125.33±16.34A	131.00±7.50A	134.00±13.61A
Protein concentration (g/dl)	3.31±00.11B	4.30±00.28A	4.33±0.20A	4.13±00.06A
ALP activity (IU/L)	145.48±02.05C	179.63±04.73B	205.61±4.09A	222.15±37.14A
Calcium concentration (mg/100 ml)	11.76±00.55B	13.06±00.37A	13.43±0.38A	13.53±00.75A
Phosphorus concentration (mg/100 ml)	5.83±01.18B	6.16±00.13A	6.30±0.100A	6.43±00.40A

Traits	Treatments			
	66 week of age			
	T1	T2	T3	T4
Cholesterol concentration (mg/100 ml)	191.83±07.41B	218.43±65.99A	221.77±65.73A	236.70±15.98A
Protein concentration (g/dl)	5.95±01.00B	8.10±00.17A	8.22±01.86A	8.93±01.93A
ALP activity (IU/L)	144.43±36.25B	181.41±08.52A	184.22±09.55A	188.56±11.43A
Calcium concentration (mg/100 ml)	11.43±00.39B	13.00±00.14A	13.21±00.62A	13.09±00.45A
Phosphorus concentration (mg/100 ml)	4.24±00.26B	5.93±02.09A	5.99±00.71A	6.01±00.48A

T1 = Control (0 mg zn /kg of diet), T2 = 50 mg zn/kg of diet, T3 = 75 mg zn/kg of diet, T4 = 100 mg zn/kg of diet.
Means with different letters within rows differ significantly (p<0.05)

Table 2: Effect of dietary supplementation of zinc on total means of certain blood plasma traits (Mean±SE) of broiler breeder hens

Traits	Treatments			
	Total means of blood plasma traits			
	T1	T2	T3	T4
Cholesterol concentration (mg/100 ml)	153.745±53.86B	171.88±65.83A	176.38±64.18A	185.00±72.62A
Protein concentration (g/dl)	4.63±1.86B	6.20±2.68A	6.27±2.75A	6.53±3.39A
ALP activity (IU/L)	144.95±0.74C	180.52±1.25B	194.91±15.12A	205.35±23.75A
Calcium concentration (mg/100 ml)	11.59±0.233B	13.03±0.042A	13.32±0.155A	13.31±0.311A
Phosphorus concentration (mg/100 ml)	5.035±1.124B	6.045±0.162A	6.145±0.219A	6.22±0.297A

T1 = Control (0 mg zn/kg of diet), T2 = 50 mg zn/kg of diet, T3 = 65 mg zn/kg of diet, T4 = 100 mg zn/kg of diet.
Means with different letters within rows differ significantly (p<0.05)

Table 3: Effect of dietary supplementation of zinc on blood plasma glucose (Mean ± SE) of broiler breeder males and females at 54, 58, 62 and 66 weeks of age

Sex	Age (Weeks)	Treatments			
		T1	T2	T3	T4
Females	54	171.66±16.22B	193.54±4.48A	199.17±14.64A	201.16±10.09A
	58	183.72±11.16B	198.23±15.43A	207.27±40.41A	211.40±46.77A
	62	211.85±14.81B	239.69±10.18A	245.54±15.69A	245.44±12.87A
	66	205.33±12.45B	228.00±17.37A	219.60±19.29A	227.33±12.18A
	Total means	193.14±10.71B	214.86±12.33A	217.89±11.25A	221.33±15.08A
Males	54	163.55±18.56B	189.29±18.89A	193.36±18.05A	195.25±20.09A
	58	172.25±32.51B	197.70±19.99A	209.40±32.04A	211.74±1.85A
	62	185.77±16.49C	209.03±17.30B	227.89±17.36B	243.14±17.04A
	66	180.00±19.85C	201.66±12.60B	218.17±20.60B	239.00±20.01A
	Total means	175.39±10.41B	199.42±10.66A	212.20±13.55A	222.28±12.98A

T1 = Control (0 mg zn/kg of diet), T2 = 50 mg zn/kg of diet, T3 = 65 mg zn/kg of diet, T4 = 100 mg zn/kg of diet.
Means with different letters within rows differ significantly (p<0.05)

as compared to control group (Table 2). Results of this experiment also revealed that supplementation the diet of broiler breeder hens with different levels of zinc (T2, T3 and T4) resulted in significant (p<0.05) increase in blood plasma glucose at 54, 58, 62 and 66 weeks of age and concerning the total mean of this trait as compared with control group (T1) (Table 3).

These significant increases (p<0.05) in blood plasma cholesterol, protein, calcium, phosphorous and glucose concentrations and ALP activity may be attributed to the role of zinc in sex and steroid hormones synthesis and its action on the metabolism of sex steroids together with prostaglandins (Favier, 1992; Brown and Pentland, 2007). Zaghari *et al.* (2009) reported that progesterone

injection of broiler breeder pullets (20 week of age) affected plasma glucose, triglycerides and cholesterol concentrations of hens. Thus, the increase in the corticosteroids hormones secretion, epinephrine and norepinephrine lead to increase of ALP activity, but mechanism was not quietly clear (Al-Darraj, 2008). The numerous studies indicated that there were significant positive correlation were found between blood plasma ALP activity and blood calcium and phosphorus and egg production rate, egg weight and shell thickness of laying hens (Meissner, 1981; Al-Darraj, 2007). However, Freeman (1987) indicated that there was positive correlation between the increase of blood plasma corticosterone level and blood plasma cholesterol. Anshan (1993) reported that serum Alkaline Phosphatase (ALP) activity was higher in zinc-supplemented chicks than that in the control, while Anshan *et al.* (1990) found significant increase of blood plasma ALP activity in association with an increase of dietary zinc level. Champak *et al.* (2008) indicated that supplementing the diet of broiler chickens caused significant increase in serum glucose and cholesterol

concentrations and numerical increase as regards serum protein concentration in comparison with control group.

Blood parameters of males: Results from Table 4 and 5 clearly denoted that feeding diets containing different levels of zinc (T2, T3 and T4) resulted in significant increase ($p < 0.05$) in blood plasma cholesterol, protein, calcium and phosphorus concentration and ALP activity in broiler breeder males at 58 and 66 weeks of age and with respect to the total means of these traits as compared to control group (T1). However, the supplementation of broiler breeder males ration with different levels of zinc (T2, T3 and T4) resulted in significant ($p < 0.05$) increase in blood plasma glucose at 54, 58, 62 and 66 weeks of age and with relation to the total mean of this trait in comparison with control group (T1)(Table 3).

Levengood *et al.* (2000) reported that supplementing ration of Mallards ducks with different level of zinc had higher blood plasma ALP activity and calcium, phosphorus, glucose and total protein concentration

Table 4: Effect of dietary supplementation of zinc on certain blood plasma traits (Mean±SE) of broiler breeder males at 58 and 66 weeks of age

Traits	Treatments			
	T1	T2	T3	T4
58 week of age				
Cholesterol concn. (mg/100 ml)	77.66±5.45B	91.33±6.00A	96.33±9.26A	97.11±05.13A
Protein concn. (gm/dl)	4.20±0.26B	5.87±0.03A	5.99±0.26A	5.97±0.34A
ALP activity (IU/L)	127.37±3.88B	132.34±20.60B	155.98±60.57A	153.44±14.76A
Calcium concn. (mg/100 m)	7.13±1.18B	8.95±0.31A	9.03±1.65A	9.11±0.81A
Phosphorus conc. (mg/100 m)	3.85±1.07B	4.36±0.08A	4.46±0.36A	4.46±0.12A
66 week of age				
Cholesterol concn. (mg/100 ml)	83.24±44.06B	95.54±20.75A	96.16±72.66A	99.72±9.33A
Protein concn. (gm/dl)	6.54±1.94B	7.28±1.32A	7.37±1.29A	7.41±0.73A
ALP activity (IU/L)	133.77±0.52C	145.81±21.43B	167.49±34.07A	178.50±6.68A
Calcium concn. (mg/100 m)	6.98±0.46C	7.83±3.03B	8.76±1.12A	8.83±0.66A
Phosphorus conc. (mg/100 m)	3.09±0.72B	4.27±0.90A	4.41±0.30A	4.97±0.54A

T1 = Control (0 mg zn/kg of diet), T2 = 50 mg zn/kg of diet, T3 = 65 mg zn/kg of diet, T4 = 100 mg zn/kg of diet.
Means with different letters within rows differ significantly ($p < 0.05$)

Table 5: Effect of dietary supplementation of zinc on total means of certain blood plasma traits (Mean±SE) of broiler breeder males

Traits	Treatments			
	T1	T2	T3	T4
Total means of blood plasma of males				
Cholesterol concentration (mg/100 ml)	80.45±3.94B	93.43±2.97A	96.24±0.120A	98.41±1.84A
Protein concentration (gm/dl)	5.37±1.65B	6.57±0.99A	6.68±0.97A	6.69±1.01A
ALP activity (IU/L)	130.57±4.52B	139.07±9.52B	161.73±8.13A	165.97±17.72A
Calcium concentration (mg/100 m)	7.05±0.10B	8.39±0.79A	8.89±0.19A	8.97±0.197A
Phosphorus conc. (mg/100 m)	3.47±0.53B	4.31±0.063A	4.43±0.035A	4.71±0.360A

T1 = Control (0 mg zn/kg of diet), T2 = 50 mg zn/kg of diet, T3 = 65 mg zn/kg of diet, T4 = 100 mg zn/kg of diet.
Means with different letters within rows differ significantly ($p < 0.05$)

than in control group. Peretz *et al.* (2001) observed that zinc supplementation to the diet increased bone ALP in healthy men. Zinc is essential for nucleic acid synthesis and activity of many enzymes (Prasad, 1985); zinc deficiency may cause abnormalities in nucleic acid synthesis and protein many factoring. The increase of blood plasma glucose concentration found in this study may be attributed to the increase of ACTH and steroids hormones as a result of supplemental dietary Zinc (Williams, 1984).

Rashidi *et al.* (2010) showed that heat stress increased serum cholesterol, triglyceride and glucose concentrations ($p < 0.05$). Puvadolpirod and Thaxon (2000) reported that the increase in corticosteron and Adrinocorticotrophic Hormone (ACTH) level in blood, cellular and chemical composition resulted in increases in plasma concentrations of glucose, protein, triglyceride and cholesterol. These changes of glucose concentration in blood plasma may be due to direct reversing of corticosterone level changes in blood plasma (Al-Darraj and Al-Hassani, 2000). Therefore, responding these components (glucose and protein) in blood plasma as a result of zinc supplementation may be contradicted for its responding of increase concentration of corticosterone hormone (Abdul-Hassan, 2005).

The increase in blood plasma cholesterol concentration may be attributed to the factors that affect plasma cholesterol concentration-feed back control of body cholesterol. Among the important factors that affect plasma cholesterol concentration are the following: 1- An increase in the amount of cholesterol ingested each day increases the plasma cholesterol concentration slightly. However, when cholesterol is ingested, the rising concentration of cholesterol inhibits the most essential enzyme for endogenous synthesis of cholesterol, 3-hydroxy-3-methyl glutary CoA reductase. 2- a highly saturated fat diet increases blood cholesterol concentration 15-25%. This results from increased fat deposition in the liver, which then provides increased quantities of acetyl - CoA in the liver cells for the production of cholesterol and 3- tack of insulin or thyroid hormone increases the blood cholesterol level, whereas excess thyroid hormone decrease the cholesterol concentration. These effects are probably caused mainly by changes in the degree of activation of specific enzymes responsible for the metabolism of lipid substances (Guyton and Hall, 2006), or these changes of cholesterol levels in blood plasma may be due to the zincs role in enzyme action, zinc forms an integral part of several enzyme (metallo enzymes) in the body. In the body cholesterol is the source for the synthesis of steroid hormones and bile acids (Sastry, 2008). However, the increase in zinc treatments (T2, T3 and T4) regarding plasma protein concentration as compared to control group (T1) may be attributed to the hormonal

regulation of protein metabolism, for example growth hormone increased the synthesis of cellular protein, glucocosteroids increased break down of most tissue proteins. The increasing of corticosterone hormone and glucocorticoids which are secreted by the adrenal cortex increased the quantity of protein in most tissues while decreased the amino acids concentration in the plasma, as well as decreased both liver protein and plasma proteins, or may be due to the decrease of thyroxin secretion, which thyroxin increases the rate of metabolism of all cells and, as a result indirectly affects protein metabolism. Moreover, the significant increase in blood plasma ALP activity in males fed diet containing zinc (T2, T3 and T4) during the periods of this experiment as compared to control group may be attributed to the action of vitamin D₃, the active form of vitamin D₃, (1, 25 dihydroxy cholecalciferol), which has several effects on the intestine, kidneys and bones that increase absorption of calcium and phosphate into the extra cellular fluid and contribute to feed back regulation of those substances. Other effects of 1, 25 dihydroxy cholecalciferol that may play a role in promoting calcium absorption are the formation of 1-a calcium stimulated ATPase in the brush border of the epithelial cells and 2-ALP in the epithelial cells. The precise details of all those effect are unclear (Guyton and Hall, 2006). Also noticed from the present study that dietary zinc supplementation (T2, T3 and T4) caused significant increase in calcium and phosphorus concentration in blood plasma compared to the control group (T1), this may be due to the roles of both vitamin D₃ and Parathyroid Hormone (PTH) in both bone resorption and deposition. In the absence of vitamin D₃, the effect of PTH in causing bone resorption is greatly reduced or even prevented. Vitamin D₃ also increases calcium and phosphate absorption by the epithelial cells of the renal tubules thereby tending to decrease excretion of these substances in the urine (Guyton and Hall, 2006). It is suggested that zinc has an inhibitory effect on calcitonin secretion from thyroid tissue (Kaji, 2001). Nishiyama *et al.* (1991) observed that zinc infusion resulted in a decrease in serum calcitonin concentration, but did not change the concentrations of ionized calcium and PTH.

Conclusion: It may be concluded from this study that dietary zinc supplementation had significant effects on the blood plasma parameters of broiler breeder males and females. Therefore, zinc can be used as an efficient tool for enhance physiological status of broiler breeder chickens.

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