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Effect of Dietary Zinc Supplementation on Some Seminal Plasma Characteristics of Broiler Breeders' Males

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Abstract: The aim of the present study was to evaluate the effect of zinc (Zn) as feed additive on certain seminal plasma traits of broiler breeders' males. A total of 36 Cobb 500 broiler breeders males, 45 weeks old were used in this study. These birds were randomly distributed equally into four dietary treatments with three replicates each. Each treatment group constituted 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 revealed that adding zinc to the diet of roosters (T2, T3 and T4) resulted in significant ($p<0.05$) decrease in seminal plasma glucose at 54, 58, 62 and 66 weeks of age and protein during 54, 58 and 66 weeks of age and concerning the total means of these two traits in comparison with T1 group. However, feeding diets containing zinc (T2, T3 and T4) resulted in significant ($p<0.05$) increase in seminal plasma Alkaline Phosphatase (ALP) activity and significant ($p<0.05$) decrease in seminal plasma Aspartate Aminotransferase (AST) and Alanine Aminotransferasr (ALT) activity and cholesterol concentration during 58 and 66 weeks of roosters age and with relation to the total means of these traits as compared to T1 group. In conclusion, supplementation of the broiler breeder males with zinc caused significant improvement in seminal plasma traits included in this study. Therefore, dietary zinc supplementation can be used as active tool for enhance reproductive performance of roosters.

Key words: Zinc, seminal plasma traits, broiler breeder males

INTRODUCTION

It is well known that zinc and others trace elements (calcium, copper, magnesium, selenium) play a very vital role in affecting various parameters of semen, among trace elements increasing evidence of a direct relationship of zinc was found with seminal parameters (Stanweell *et al*., 1983). In the study of Barber *et al*. (2005) to evaluate the Sperm Quality Index (SQI) and sperm viability as affected by various levels and sources of Se, Mn and Zn when added *in vitro* to broiler breeder semen. *In vitro* treatments consisted of the following sources and levels of minerals: control, no minerals added to sperm; Seleno L-methionine, 4 levels ranging from 8.78-7.896 mg/L; Sodium selenite, 4 levels ranging from 8.78-7.89 mg/L; $MnSO_4$, 8 levels ranging from 6.500- 65.000 mg/L; Zn 180 (Zinpro corporation), 4 levels ranging from 0.65-650 mg/L and $ZnSO_4$, 4 levels ranging from 0.65-650 mg/L. The addition of 7.896 mg of sodium selenite/L to semen was determinate to sperm motility. Also, $MnSO_4$ adversely affected SQI and sperm viability at concentrations of 6.500 mg/L and greater, sperm viability was improved when 650 mg/L of Zn 180 was added to semen. Sperm motility was increased by exposure semen to Zn 180 at 650 mg/L and $ZnSO_4$ at levels of 65 and 650 mg/L. As a result, this study suggested that zinc enhance spermatogenesis and improve semen quality. In another study of Ali *et al*. (2007) to find the relationship between zinc concentrations in blood and seminal plasma with various semen parameters of fertile and infertile men; the results of this study showed significant differences in serum and seminal zinc levels in normospermic, Oligospermic and azoospermmic men. Zinc seminal plasma showed a positive correlation with sperm count and sperm motility in normospermic and oligospermic and negative correlation with volume, pH, WBC count in all three groups (oligospermic, 30 infertile male, azoospermic, 28 infertile male and 25 fertile male, as a normosporic). There was no correlation was found with sperm morphology. Vendryes (2007) reported some useful recommendations for increasing sperm count such as supplement the diet with zinc, vitamin C and B-complex and omega-3 fatty acids. Xu *et al*. (1993) showed that the relationship of trace element concentration in seminal plasma and blood sperm density, motility and morphology and semen volume is

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as follows: The concentrations of elements were in the following descending order: Zn > Se > Pb > Cd. Except for Zinc, the concentrations were generally higher in blood than in seminal plasma. The mean concentration of zinc in seminal plasma (Zn Sp) was about 30 times higher than that in blood (Zn B). A significant inverse correlation was observed between blood cadmium levels (Cd B) and sperm density ($r = 0.24$, $p<0.05$) in oliozoospermic men (sperm density below 20 million/ml) but not in normospermic. However, men cadmium in seminal plasma (Cd sp) was also associated with low semen volume ($r = 0.29$, $p<0.05$). These findings suggest that cadmium may have an effect on the male reproductive system. In control, positive correlations were observed between concentrations of selenium and zinc in seminal plasma (Se sp and Zn sp) and sperm density in normospermic men but not in oligozoospermic men. The correlation coefficients with sperm density of Se sp and Zn sp were 0.35 ($p<0.05$) and 0.41 ($p<0.01$), respectively. The concentrations of lead in blood (Pb B) or seminal plasma (Pb sp) did not appear to have any correlation with the sperm parameters studied. In another study to investigate the effect of calcium, magnesium, zinc and copper in blood and seminal plasma on semen traits in men, Wong *et al*. (2001) showed that the concentrations of calcium, magnesium, zinc and copper in blood and seminal plasma were not different between the subfertile and fertile groups. Weak correlations were demonstrated between blood plasma zinc concentration and sperm count ($rs = 0.18$), sperm motility ($rs = 0.15$) and abnormal sperm morphology ($rs = 0.13$). Zinc and magnesium concentrations in seminal plasma correlated weakly with sperm count ($rs = 0.17$ and $rs = 0.16$, respectively) and copper concentration in blood plasma with motility ($rs = 0.25$). Strong correlations were found between calcium, magnesium and zinc in seminal plasma. As the studies regarding the effect of zinc on seminal plasma of birds are lacking, so this study was conducted to evaluate the effect of dietary supplementation with different levels of zinc on certain seminal plasma traits of broiler breeder males.

MATERIALS AND METHODS

Thirty six Cobb500 broiler breeders' males were obtained from the Erbil Poultry Project, Iraqi Kurdistan Region and the study was carried out there. The birds were randomly divided into four groups. One group of birds consumed diet not supplemented with zinc (0 mg zn/kg diet, T1; control), while another groups of birds consumed 50, 75, 100 mg of pure zinc (Himedia-India)/kg of diet (T2, T3, T4), respectively. Each treatment group constituted 9 males. Each group of birds was further divided into three replicates (3 cocks in each subgroup). Birds were raised on floors pens under similar environmental, managerial and veterinarian conditions. Birds were kept in a closed house; artificial lighting and drinking by nipples were provided through the experimental period (22 weeks). Commercial ration were provided for birds during experimental period. Birds were maintained under 16 hr light and 8 hr dark and mean temperature of 18-21°C during the whole period of study.

Semen was collected using the abdominal massage technique from all males in each treatment. Semen was collected in 5 ml tubes and then centrifuged at 1000 rpm for 10 min, seminal plasma separated and frozen at -25°C till analysis. Seminal plasma cholesterol was enzymatically measured using cholesterol esterase and cholesterol oxidase according to the enzymatic method described by Allain (1974). Seminal plasma glucose determination 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. Seminal plasma protein was 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. Colorimetric method was used to determine Alkaline Phosphatase (ALP) activity according to Kind and King (1954). Seminal plasma Glutamate Oxaloacetate Transaminase (GOT), which also called Asparte Aminotransferase (AST) and Glutamate Pyruvate Transaminase (GPT), which also called Alanine Transaminase (ALT) activities were determined by using the colorimetric method according to Reitman and Frankel (1957). Commercial kits (Randox Laboratories Ltd., United Kingdom) were used for this purpose.

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

As shown from Table 1 adding zinc to the ration of roosters (T2, T3 and T4) results in significant ($p<0.05$) decrease in seminal plasma glucose concentration during 54, 58, 62 and 66 weeks of age and respecting the total mean of this trait in comparison with control group (T1). However, T4 (100 mg zn/kg of diet) recorded the lower seminal plasma glucose concentration than other treatment groups. This decline in seminal plasma glucose in zinc treatment groups as compared to control group may be attributed to the increase of sperm activity and its metabolism. Al-Darraji (2007) reported that

Table 1: Effect of dietary zinc supplementation on seminal plasma glucose (mg/dl) (Mean±SE) of broiler breeder males

	Treatments			
Age (week)	T1	T2	T3	T4
54	81.20±00.57A	75.33±01.20B	73.66±02.66B	74.89±07.39B
58	79.23±00.50A	71.87±21.72B	64.66±16.43B	68.39±13.54B
62	51.03±09.19A	40.54±10.47C	45.46±03.59B	48.49±10.24B
66	42.25±04.97A	38.46±01.48B	38.42±03.68B	31.05±09.72C
Total means	64.42±25.67A	56.55±23.60B	55.55±34.05B	55.69±20.26B

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 zinc supplementation on seminal plasma protein (g/dl)(Mean±SE) of broiler breeder males

	Treatments			
Age (week)	T1	T2	T3	T4
54	2.40±0.30A	1.53±0.40B	1.43±0.12B	1.25±0.02C
58	3.79±0.68A	2.57±0.48B	2.84±0.33B	2.07±1.30C
66	4.80±0.73A	3.55±1.41B	3.53±1.04B	2.59±0.91C
Total means	3.66±2.25A	2.55±2.66B	2.60±0.74B	1.97±2.67C

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 ($p<0.05$)

chicken spermatozoa can convert glucose in seminal fluid to lactate by metabolism process under aerobic and non aerobic conditions. Also, this decrease of seminal plasma glucose may be resulting from the high increment in mass activity and individual motility of spermatozoa and the decrease in dead and abnormal spermatozoa as. Hammond *et al*. (1965) and Al-Darraji *et al*. (2002b) found highly significant ($p<0.01$) negative correlation between motility, sperm density and glucose concentration in seminal plasma and indicated that spermatozoa utilized the glucose in their metabolism processes. Mann (1964) reported that there was significant positive correlation between glycolysis rate and motility and sperm density. Al-Darraji (2001b) concluded that the highly significant negative correlation between numbers of spermatozoa and glucose concentration in seminal plasma suggested the utilization of glucose by spermatozoa.

Results also revealed that dietary zinc supplementation (T2, T3 and T4) resulted in significant decrease in seminal plasma protein concentration during 54, 58 and 66 weeks of age and with respect to the total means of this trait which being (3.66, 2.55, 2.60 and 1.97 for T1, T2, T3 and T4, respectively). The decrease in seminal plasma protein concentration can be used as a predictor of fertility and hatchability as it was found highly significant negative correlation between protein concentration in seminal plasma with fertility and hatchability of fertile eggs or total eggs (Thurston, 1976; Thurston *et al*., 1992). Al-Darraji *et al*. (2002a,b) found highly significant negative correlation between seminal plasma protein concentration and motility and semen concentration. The results of the present study are in agreement with the results of the authors mentioned above, as the decrease in seminal plasma protein concentration was associated with the increase in fertility and hatchability in birds fed diet containing zn (Amen and Al-Daraji, 2011b). Thurston (1976) has shown that the number of abnormal germinal cells spermiophages present in turkey semen increased as the seminal protein concentration increased. Al-Darraji (2001a) reported that numbers of the spermatozoa exhibiting progressive motility and the germ cells concentration showed highly significant negative correlation with the total seminal plasma protein content both in fresh and frozen-thawed semen samples. Furthermore, it is widely accepted that the number, viability, motility, survival and storage properties of spermatozoa are influenced by seminal plasma protein (Al-Darraji *et al*., 2001).

Results also denoted that the supplementation of broiler breeder males ration with different levels of zinc (T2, T3 and T4) resulted in significant ($p<0.05$) increase in seminal plasma ALP activity and significant ($p<0.05$) decrease in AST and ALT activities and cholesterol concentration during 58 and 66 weeks of age and concerning the total means of these seminal plasma traits as compared to control group (T1) (Table 3 and 4). When sperm cell membrane damaged, AST and ALT enzymes are released into the extra cellular medium (Al-Darraji *et al*., 2002a). Al-Darraji *et al*. (2001, 2002a) found highly significant positive correlation between dead spermatozoa, abnormal spermatozoa and acrosomal abnormalities with seminal plasms AST, ALT and LDH activities. Al-Darraji *et al*. (2002b) reported that there was significant correlation between seminal plasma AST activity and ALT activity following cellular disruption. Brown *et al*. (1971) examined several enzymes and selected AST and ALT release as the best indicator of cellular damage. Buckland (1971) mentioned that the observed increase in AST and ALT activities of seminal plasma and semen during storage may be due to structural instability of the sperm. Al-Darraji (2001a) indicated that the ALT activity in seminal

Table 3: Effect of dietary zinc supplementation on seminal plasma ALP, AST and ALT activity and cholesterol concentration (Mean±SE) of broiler breeder males at 58 and 66 weeks of age

	Treatments			
	58 week of age			
Traits	T1	T2	T3	T4
ALP activity (IU/L)	62.79±08.39B	85.62±08.52A	83.00±18.45A	81.53±10.23A
AST activity (IU/ml)	140.66±02.88A	103.66±13.86B	90.33±07.96B	97.66±15.50B
ALT activity (IU/ml)	29.33±11.60A	17.33±00.88B	18.66±02.72B	20.00±07.63B
Cholesterol concentration (mg/dl)	38.30±09.20A	24.33±02.18B	29.00±06.08B	24.66±01.45B
	Treatments			
	66 week of age			
Traits	T1	T2	T3	T4
ALP activity (IU/L)	71.83±11.22B	80.16±12.77A	83.20±16.48A	86.13±17.32A
AST activity (IU/ml)	186.33±01.85A	104.33±14.44B	101.33±11.21B	119.33±14.70B
ALT activity (IU/ml)	23.76±09.12A	17.75±04.75B	17.13±05.46B	16.16±02.20B
Cholesterol concentration (mg/dl)	32.74±19.60A	28.30±10.23B	22.47±17.80C	22.39±02.00C

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 4: Effect of dietary zinc supplementation on total means of seminal plasma ALP, AST and ALT activity and cholesterol concentration (Mean±SE) of broiler breeder males

	Treatments			
	Total means of seminal plasma traits			
Traits	T1	T2	T3	T4
ALP activity (IU/L)	67.31±06.39B	82.89±3.860A	83.10±0.141A	83.83±03.25A
AST activity (IU/ml)	163.49±32.29A	103.99±0.473B	95.83±7.770B	108.49±15.32B
ALT activity (IU/ml)	26.54±03.93A	17.54±0.296B	18.04±0.869B	18.33±02.36B
Cholesterol concentration (mg/dl)	35.52±03.93A	26.31±2.800B	25.73±4.610B	23.52±01.60B

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 ($p<0.05$)

plasma was very weak as compared to the AST activity. Al-Darraji (2000b) found a positive correlation between activities of AST and ALTT in seminal plasma and percentages of dead and abnormal spermatozoa. On the other hand, Al-Darraji *et al.* (2000a; 2002b) found a significant positive correlation between ALP activity and spermatozoa livability and concentration. Differences in seminal plasma ALP activity for control and zinc treatments closely resembled differences in spermatozoa livability, mass activity and individual motility and spermatozoa concentration. The highest spermatozoa livability, motility and concentration were noticed in semen samples of T2, T3 and T4 groups, as compared with T1 group on 58 wks and 66 wks of age (Amen and Al-Daraji, 2011a), which support high seminal plasma ALP activity, as compared with control group (T1), in which ALP activities were the lowest (Table 3 and 4). Al-Darraji *et al.* (2001) reported that both of alkaline and acid phosphatase (ALP and ACP) are involved in the metabolism of spermatozoa via the hydrolysis of carbohydrates. Also Bell and Lake (1962) mentioned that both of ALP and ACP are included in sperms metabolism via carbohydrates hydrolysis. Moreover, Al-Darraji *et al.* (2002a) found highly significant positive correlation between the amount of ALP in the seminal plasma and the number of spermatozoa per ejaculate.

As shown from Table 3, a lower seminal plasma cholesterol concentration was observed in dietary zinc supplementation treatments (T2, T3 and T4) at 58 and 66 weeks of age and respecting the total mean of this trait as compared with control group (T1). Davis (1976) suggested that the higher cholesterol concentration in seminal plasma may be inhibiting fertility process through inhibition of interfusing membranes during acrosome reaction. Ansah and Buckland (1982) found a significant negative correlation between cholesterol level in semen and seminal plasma with fertility rates for fresh and frozen- thawed semen. These findings are in agreement with the results of this study when zinc treatments groups (T2, T3 and T4) recorded the lower values of seminal plasma cholesterol concentration (Table 3 and 4) in comparison with T1 group and recorded at the same time the highest fertility and hatchability rates and lowest embryonic mortality ratio as compared with Ti group (Amen and Al-Daraji, 2011b).

Conclusion: In conclusion, dietary zinc supplementation resulted in significant improvement in seminal plasma traits included in this study of broiler breeder males.

Therefore, zinc can be used as one of efficient feed additives for improve reproductive performance of bird males.

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