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## Effect of Dietary Supplementation with Different Level of Zinc on Sperm Egg Penetration and Fertility Traits of Broiler Breeder Chicken

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**Abstract:** The aim of the present study was to evaluate the effect of zinc as feed additive (0, 50, 75, 100 mg/kg diet) on fertility traits and sperm egg penetration of Cobb 500 broiler breeders. A total 132 of (96 females and 36 males) of Cobb 500 broiler breeder chicken, 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 of 32 females and 12 males. Treatment groups were as following: T1: Birds fed the basal diet without any addition (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. Hens were artificially inseminated with semen from the same treatments during 51, 54, 57, 60, 63 and 66 weeks of bird ages. Fertility traits involved in this study were percentages of fertility, hatchability of total eggs, hatchability of fertile egg, embryonic mortality. However, sperm egg penetration during 54, 58, 62 and 66 weeks of bird age's. Results revealed that adding zinc to birds diet results in significant ( $p < 0.05$ ) increase regarding fertility, hatchability of total eggs, hatchability of fertile eggs and sperm egg penetration and significant ( $p < 0.05$ ) decrease as regards embryonic mortality. In conclusion supplementing the diet of bird with zinc resulted in significant improvement in fertility traits and sperm egg penetration. Therefore adding zinc to the diet of birds could be used as an efficient tool for improves reproductive performance of chicken.

**Key words:** Zinc, fertility traits, sperm-egg penetration, broiler breeder chicken

### INTRODUCTION

Zinc is important for the cell division and the production of healthy sperm. It is the most critical trace mineral for male sexual function. It is needed for testosterone metabolism, testicle growth, sperm production, motility, count, reducing excess estrogen in male reproductive tissue. Zinc is needed for progesterone synthesis and a deficiency can produce excessive prolactin secretion (Brown and Pentland, 2007). A study has also shown that zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus. This structure is important for successful fertilization. However, Zinc was shown to increase sex drive and male potency (Balch, 2007). Investigation of the effects of increasing zinc concentration in the diets of Brown parent stock layers on productive, fertility and hatchability traits were carried out by Durmus *et al.* (2004). In this study, after hatching, chicks were allocated to different treatment groups which diets were formulated to contain graded concentrations of zinc (60 control), 90, 120, 150, 180, 210, mg zinc/kg of diet) throughout 62 weeks, no effect ( $p > 0.05$ ) of increasing zinc concentration on egg production, 5% egg production age, livability and hatchability rates was found. On the other hand, significant differences ( $p < 0.05$ ) were obtained with an

increase in zinc concentration with relation to egg weights, feed conversion rate and hatchability efficiency (calculated from the number of live chicks divided by the number of eggs placed in the incubator) of the fertile eggs. The results of this study suggest that diets of Brown parent stock layers should include 180 mg Zn/kg for optimal performance and hatchability traits.

Research on feeding diet supplemented with complexed zinc and manganese to broiler breeders demonstrated a statistical increase in the percent of setting eggs (88.6% vs. 87.27%) compared to breeders fed organic zinc and manganese (Zinpro, 2002; Development, 2002). In this study they also demonstrated the effects on hatchability when supplementation complexed zinc and manganese in broiler breeder diets and they found that there was numerical trend for improving in hatchability in both trials (68% vs. 73%; 83.5% vs. 84.2%). Zinc also is a necessary component of sperm structure and spermatogenesis. Feeding zinc to male breeders chicken has increased sperm concentration and sperm motility (Prasad, 1993; Zinpro, 2002). Recently, Tako *et al.* (2004) observed that in ovo injection with zinc-methionine at 17 days of incubation led to improvements in the morphological development and intestinal mucosa enzyme expression of hatching

chicks. Namra *et al.* (2009) concluded that increasing zinc over NRC (1994) recommendation (50 mg/kg diet) for laying Japanese quail hens had no significant impact on egg production, egg weight, feed conversion or economical efficiency. However, Zn supplementation as Zn-methionine at level higher than that of NRC (1994) recommendation improved fertility (96.07%). Inclusion of 50 mg Zn over NRC (1994) recommendation or 200 mg methionine hydroxyl analogue free acid (88%) to the diets of Japanese quail layers resulted in no significant amelioration respecting both of egg weight and relative weight of yolk weight, but did not improve relative weight of albumen weight, yolk color and eggshell quality (eggshell weight and eggshell thickness). However, these authors concluded that improvement in fertility rates obtained in their study may be due to the improvement in egg and semen quality and higher sexual efficiency of males and methionine may be effective for improving Zn utilization in laying chicken and broiler breeder chicken (Hassan *et al.*, 2003; El-Habbak *et al.*, 2005). Furthermore, Abdel-Galil and Abdel Samad, (2004) showed that supplementing diet with Zn (100 mg /kg diet) improved fertility rate. Also, Kout El-Kloub *et al.* (2004) reported that laying hens received diets supplemented with levels of either 100 or 150 mg zinc-methionine/kg of diet resulted in significantly higher fertility percentage, while those supplemented with ZnSO<sub>4</sub> at 50, 100 and 150 mg levels and those of 50 mg of Zn-methionine resulted in non significant increase concerning fertility percentage compared with the control group. Sperm penetration of the ovum Inner Perivitelline Layer (IPVL) is positively correlated with fertility. Greater numbers of sperm penetration holes in the IPVL are indicative of successful insemination and can be positively associated with optimal filling of the sperm storage tubules in the uterovaginal region of the oviduct (Fairchild, 2001). Causes of high embryonic mortality of birds may be attributed to the environmental conditions that must be recorded in a fashion to help troubleshoot hatchery or breeder problems that result in abnormally high embryonic mortality. Factors which can have an adverse effect on avian embryogenesis include prolonged duration of egg storage (Arora and Kosin, 1966; Coleman and Siegel, 1966; Sittman *et al.*, 1971; Mather and Langhlin, 1977; Fassenko *et al.*, 1991; Fassenko, 1996; Brake *et al.*, 1997), suboptimal conditions during egg storage (Meijerhof, 1992), season of the year (Kosin and Mun, 1965), nutrition of the hen (Wilson, 1997), nutrient availability to the embryo (Byerly *et al.*, 1932), egg size (Landauer, 1967) and age of the breeders (Christensen, 1978). Present study was conducted to determine the effect of adding different level of zinc to diet on sperm-egg penetration and fertility traits of broiler breeder chicken.

## MATERIALS AND METHODS

This study was carried out to investigate the effect of dietary supplementation with different levels of zinc on

sperm egg penetration and fertility traits. A total 132 (96 females and 36 males) of Cobb 500 broiler breeder chicken, 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 of 32 females and 12 males. Treatment groups were as following: T1: Birds fed the basal diet without any addition (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. 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 from roosters in each replicate as pooled sample, using the abdominal massage technique. The roosters were trained for artificial collection of semen according to the one man technique or individual method by Gabriel (1957). Artificial insemination of hens was conducted at 2:00 pm every day of insemination to avoid eggs with hard shell in uterus during the insemination. This process was carried out by two persons after hens have been trained for two weeks. Hens were inseminated via the deep vaginal insemination method (5-6 cm, deep of reproductive duct) using 50 ml (0.05 ml) of semen for each insemination after exposing the vaginal opening with pressure applied on the vent area (Burrows and Quinn, 1937). The eggs were collected to incubating after two days of insemination for 7 days. Six inseminations (51, 54, 57, 60, 63, 66 weeks of birds' age) were conducted throughout the experimental period. Sperm penetration of the egg was measured based on the method of Bramwell *et al.* (1995) and Al-Darraj (2001) (Fig. 1). Incubating eggs were set once a week and fertility was determined by candling after 2 week of incubation. Fertility data were collected after each insemination and fertility rate was described as the number of fertile eggs to the number of eggs placed in the incubator and was calculated as follows:

$$\text{Fertility \%} = \frac{\text{Number of fertile eggs}}{\text{Total eggs}} \times 100$$

Hatchability was determined at the end of incubation. Hatchability of fertile eggs was found via a similar way in which the number of live chicks was divided by the number of fertile eggs kept in the incubator.

$$\text{Hatchability of fertile eggs} = \frac{\text{Number of live chicks}}{\text{Number of fertile eggs}} \times 100$$

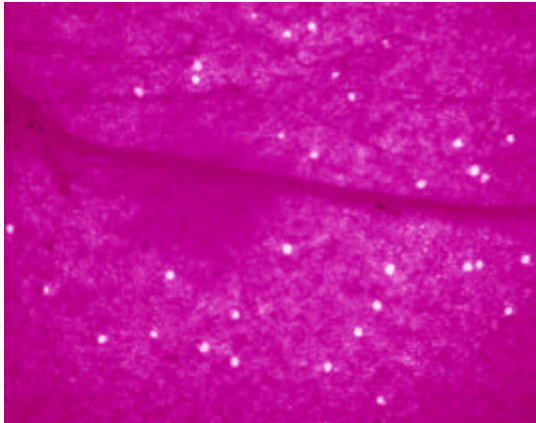


Fig. 1: Sperm penetration holes in the germinal disc region of the inner perivitelline layer

Hatchability of total eggs was calculated from the number of live chicks divided by the number of eggs placed in the incubator.

$$\text{Hatchability of total eggs (\%)} = \frac{\text{Number of live chicks}}{\text{Total eggs}} \times 100$$

Embryonic Mortality % was calculated after break open of non hatch eggs and recorded embryonic mortalities as follows:

$$\text{Embryonic mortality} = \frac{\text{Number of embryo mortality}}{\text{Number of fertile eggs}} \times 100$$

The data of this experiment were analyzed statistically using the General Linear Model procedure of SAS (2001). Significant differences between treatments means are were determined using the Duncan's multiple range test with 5% and 1% probability.

## RESULTS AND DISCUSSION

**Sperm-egg penetration:** Sperm Penetration (SP) (holes/1.5 mm<sup>2</sup>) is illustrated in Fig. 2 of four treatments groups (T1, T2, T3 and T4). It can be shown that sperm penetration results revealed the superiority ( $p < 0.05$ ) of all the different dietary treatments throughout experimental period, as compared with the control group, while no significant differences were found between T2 and T3 at 54 and 58 week of age. Significant differences were also found among the experimental dietary treatments of birds in respect of this parameter at 62 and 66wks of age. Furthermore, data of sperm penetration (Fig. 2) showed that birds fed diet supplemented with 75 mg Zn/kg of diet (T3) had the highest value (80.00) and control group was recorded the least value (31.00). This amelioration for the sperm penetration of the experimental group fed diets supplemented with the different levels of zinc (50, 75,

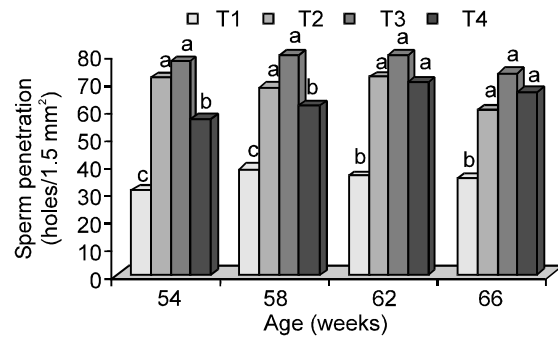


Fig. 2: Effect of dietary supplementation with zinc on sperm penetration rate (holes/1.5 mm<sup>2</sup>) of Cobb 500 broiler breeder chicken at 54, 58, 62 and 66 weeks of age. T1: Bird fed control diet (0 mg Zn/kg of diet) and T2, T3 and T4: represented birds fed diet supplemented with 50 mg Zn/kg of diet, 75 mg Zn/kg of diet and 100 mg Zn/kg of diet, respectively. <sup>a,b</sup>Bars within week of age that lack common letters refers differ significant ( $p < 0.05$ )

100 mg Zn/kg diet), may be attributed to the improvement in sperm storage tubules that found in uterovaginal junction (Donoghue, 1996), sperm mobility or motility (Donoghue *et al.*, 1998). Motile and morphologically normal spermatozoa that bypass the vagina enter the Semen Storage Tubules (SST) located at the uterovaginal junction UVJ. It has been suggested that sperms are exposed to factors including, zinc, calcium and glutamate, in the SST that suppress sperm motility, sperm metabolism, the enzyme acrosome and stabilize the plasmalemma and other sperm membranes (Bakst, 1994; Holm *et al.*, 2000). Also sperm concentration and quality play a significant role in sperm penetration and fertility. SP holes are positively correlated with numbers of sperm in the semen storage tubules (Brillard and Antoine, 1990; Brillard and Bakst, 1990; Wishart, 1995). Fairchild (2001) concluded that although factors such as semen quality, insemination technique and semen concentration could affect Sperm Penetration (SP) of the Inner Peri Vitelline Layer (IPVL), hen influences should also be considered as a factor in the determination of IPVL SP holes.

**Fertility traits:** Percentages of fertility and hatchability (Table 1) indicated that the differences between the experimental groups (T2, T3 and T4) and control (T1) were significant ( $p < 0.05$ ). Treatment groups (T2, T3 and T4) significantly surpass control group (T1) as concerns percentages of fertility, hatchability of total eggs and hatchability of fertile eggs and the best values of these three traits were recorded with birds received the diet supplemented with 100 mg Zn/kg of diet (T4) (85.23%, 75.67%, 93.28%, respectively). This improvement in fertility and hatchability in zinc treatment groups may be due to the improvement in egg and semen quality and higher sexual efficiency of males for these groups.

Table 1: Effect of dietary supplementation with different levels of zinc on fertility traits of Cobb 500 broiler breeder chicken at various ages (51, 54, 57, 60, 63, 66 weeks) (Mean±SE)

Traits	Treatments				Significant level
	T1	T2	T3	T4	
Fertility (%)	51.57±6.88 <sup>(1)c</sup>	80.26±11.97 <sup>b</sup>	81.22±0.82 <sup>b</sup>	85.23±21.82 <sup>a</sup>	*
Hatchability of total eggs (%)	44.66±2.68 <sup>c</sup>	68.98±12.24 <sup>b</sup>	69.23±1.90 <sup>b</sup>	75.67±16.22 <sup>a</sup>	*
Hatchability of fertile eggs (%)	73.44±6.95 <sup>c</sup>	87.27±5.11 <sup>b</sup>	91.40±1.53 <sup>a</sup>	93.28±0.39 <sup>a</sup>	*
Embryonic mortality (%)	25.56±6.89 <sup>a</sup>	12.73±5.34 <sup>b</sup>	8.60±1.30 <sup>c</sup>	6.72±2.74 <sup>c</sup>	*

T1: Bird fed control diet (0 mg Zn/kg of diet) and T2, T3 and T4: represented birds fed diet supplemented with 50 mg Zn/kg of diet, 75 mg Zn/kg of diet and 100 mg Zn/kg of diet, respectively.

<sup>(1)</sup>Values are means, n = 6 (51, 54, 57, 60, 63, 66 wks of age).

<sup>abc</sup>Rows that do not have the same letters differ significantly (p<0.05)

Bowling *et al.* (2003) reported that the sperm mobility or the ability of sperm cell to move through a thickened solution has been related to flock fertility, with higher sperm motility associated with higher fertility. In that study, a sperm mobility assay was used to evaluate two strains of commercial broiler breeder males. High and low mobility males were identified. These roosters were periodically evaluated through 65 weeks of age. When semen from the high and low mobility roosters were used to artificially inseminate hens, fertility was higher in hens given the high motility semen, or this amelioration in fertility of dietary supplementation with zinc may be attributed to low protein in seminal plasma, where, Thurston *et al.* (1992) deduced that high protein concentration in seminal plasma has been related to reduced fertility in turkeys. On the other hand, this improvement in fertility may be due to significant increment (p<0.05) in sperm penetration of the ovum Inner Perivitelline Layer (IPVL) which is positively correlated with fertility (Bramwell *et al.*, 1995; Robertson *et al.*, 1998; Wishart and Staines, 1999; Hazary and Wishart, 1999; Fairchild, 2001). McDaniel *et al.* (1996) demonstrated decreases in broiler breeder fertility when males were exposed to excess ambient heat. Declines were concluded to be due fewer sperm stored in the utero-vaginal junction, storage sperm tubules. Values for hatchability traits of the treatment groups are presented in Table 1. Hatchability and fertility rates differed (p<0.05) between different treatment groups (T2, T3 and T4). No significant differences were found between T2 and T3 groups with respect to percentages of fertility and hatchability of total eggs.

Moreover, hatchability of fertile eggs and embryonic mortality were determined in the present study at six times 51, 54, 57, 60, 63 and 66 weeks of age and the averages were calculated for these traits. Hatchability of fertile total eggs was affected by the inclusion of graded concentrations of Zn in the diets. Zinc treatment groups recorded the highest values (p<0.05) in hatchability of fertile eggs (87.27, 91.40, 93.28) vs. 73.44 for control group (T1) and the lowest values of percentage of embryonic mortality as compared with control group (12.73, 8.60, 6.20) vs. 25.56. No significant differences were found between T3 and T4 in both percentage of

embryonic mortality and hatchability of fertile eggs. These results are agreed with previous work of Durmus *et al.* (2004), but disagree with Kidd *et al.* (1992) who reported that supplementing the basal diet with 152 mg Zn/kg of diet in the form of ZnO or Zn- methionine had no significant differences in hatchability or had minimal effect on hatchability and fertility. This decline in fertility and hatchability of total and hatchability of fertile eggs in control group (0mg Zn/kg of diet) in our present study may have been due to the decrease in the sex hormones (estrogen and progesterone) in blood plasma of the this birds during the period from 45 wk-66 wk of age (unpublished data). On the other hand, Stahl *et al.* (1986; 1990) and Kidd *et al.* (1993) reported that zinc supplementation had no negative effect on hatchability traits.

As far as fertility and hatchability traits are concerned, it can be suggested that a Zn concentration of 100 mg Zn kg<sup>-1</sup> in the diet is required to obtain optimal fertility, hatchability and sperm-egg penetration traits in broiler breeder chickens. Trace mineral deficiencies such as zinc have been found to cause impaired growth, abnormal development of all the major organ systems and in extreme deficiencies, death of the embryo (Savage, 1968; Richards and Steel, 1987), as well as deficiencies of specific trace minerals can be readily induced in developing avian embryos by feeding insufficient amounts of the mineral to laying hens, which, in turn, produce eggs that are depleted in trace minerals (Richards and Steel, 1987).

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