# EFFECTS OF DIETARY SELENOMETHIONINE SUPPLEMENTATION ON SEMEN QUALITY, FERTILITY AND ANTIOXIDANT STATUS OF COCKERELS

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# SUMMARY

he aim of this study was to determine the effect of different levels of Selenomethionine (Sel-Plex) (0.0, 0.15, 0.30 and 0.45 mg/kg diet) on semen quality, fertility and hatchability of local strain (Inshas) cockerels. Thirty six mature cockerels at 28 weeks of age were divided into four group {(G1 (0.0), G2 (0.15), G3 (0.30) and G4 (0.45)} mg/kg diet (9 cockerels each). All birds were reared under similar management and housing-condition throughout the experimental period. Results revealed that, the final live body weight (LBW) and change of body weight of cocks in group 4 (G4) was significantly higher (P<0.05) than cockerels in group G1 and G2, but did not differ significantly with group G3 or between group G1 and group G2. Daily feed intake did not affected significantly by treatments. Testosterone concentration of cockerels in group 3 (G3) was significantly (P<0.05) higher than in other groups, while the fertility rate of cockerels in G4 was significantly (P<0.05) higher than in other groups. However no significant differences between group G3 and G4 in hatchability rate. Percentages of progressive motility and livability of cockerel spermatozoa were significantly (P<0.05) higher in groups G3 and G4 as compared as in groups G1 and G2, but the opposite trend in percentages of abnormality and acrosome damage of spermatozoa. Total antioxidant capacity (TAOC) in serum of cockerel's in group 3 (G3) was significantly higher (P < 0.05) than in control G1, but no significant differences among selenium treatments, while SOD activity in serum of cockerel's was not influenced significantly by supplemented different levels of selenium. Activity Enzymes AST and ALT were significantly higher in G1 than in G3, while no significant differ among G2, G3 and G4. In conclusion, use of organic selenium supplementation (0.3 or 0.45 mg/Kg) in diet of cockerels could be recommended to improve semen quality, fertility, hatchability and oxidative status of roosters.

Keyword: Semen, fertility, hatchability, Selenium methionine, cockerels.

# INTRODUCTION

Chicken spermatozoa are characterized by comparatively high levels of 20: 4 n-6 and 22: 4 n-6 fatty acids within their phospholipids (Blesbois *et al.*, 1997). As a result of this high proportion of polyunsaturated fatty acids (PUFA) chicken semen is susceptible to lipid peroxidation, which could lead to sperm damage (Syrai *et al.*, 1998)

Imbalance between reactive oxygen species and total antioxidant capacity can cause male infertility (Kazama and Hino 2012; Drevet, 2012). Selenium as a component of glutathione peroxidase protects spermatozoa, spermatogonia and sperm cells themselves against the free oxygen radicals (Noblanc *et al.*, 2011 and Abdalla *et al.*, 2016)

Selenium (Se) is an essential trace element that is necessary for the maintenance of various physiological processes (Zhang *et al.*, 2006). In a cell culture system, selenium in the form of sodium selenite (SS) protects the cells from oxidative damage by reducing the production of free radicals and inhibits lipid peroxidation (Tatemoto *et al.*, 2004).

The health of the reproductive system of livestock has a vital role to achieve high reproductive performance. Reproduction is one of the most important production parameters in attaining profitability in a commercial farm operation. Selenium is essential for the maintenance of male fertility in human

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(Brown and Arthur, 2001) and rabbits (Kamel, 2012) also, is required for testosterone biosynthesis, and for formation and normal development of spermatozoa (Behne *et al.*, 1996). Both the testis and epididymis require exogenously supplied selenium in order to synthesize a variety of selenoproteins (Shalini and Bansal, 2007).

Selenium deficiency in the diet leads to the decline of ejaculate quality, which causes deteriorated fertilizing ability (Camejo *et al.*, 2011). Two selenium forms, organic and inorganic, are available, whereas it is assumed that organic form has more bioavailability and consequently, reduced toxicity compared to inorganic one (Brennan *et al.*, 2011).

The aim of the current work was to study the effect of different levels of organic selenium (0.0, 0.15, 0.30 and 0.45 mg/kg diet on semen quality, fertility and hatchability of local strain (Inshas) cockerels.

# MATERIAL AND METHODS

This study was carried out at the El-Sanafawy Farm, Sakha, Kafr El-Sheikh, Egypt.

#### Birds and experimental design:

A total number of 36 mature cocks of Inshas strain as a local strain at 28 weeks till 40 weeks of age were used in the present study. All birds were reared under similar management and housed individually in single cages in an open system house. Feed and water were offered *ad libitum* throughout the experimental period. The cockerels were randomly distributed into 4 experimental treatments, control and 3 supplemented treatments (9 cocks each).

#### **Experimental diets:**

The basal experimental diet was formulated to meet the nutrient requirements of local strain of chicken at laying period as recommended by Feed composition Tables for Animal & poultry feedstuffs used in Egypt (2001). Composition and calculated analysis of basal diet are shown in (Table 1). The 1<sup>st</sup> treatment (control) was given the basal laying diet without Selenomethionine (Sel-Plex). While, the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> treatment was fed the basal diet supplemental with 0.15, 0.30 and 0.45 mg Sel-Plex/kg diet, respectively.

#### Collected data:

#### Measurements:

Cocks were individually weighed at the beginning and end of the experimental period to the nearest 0.1 gram. Changes in live body weight were calculated as the differences between the two weights. Daily feed intake was recorded for each treatment. All data were calculated for each replicated and treatment throughout the experimental period.

#### **Blood** samples:

Blood samples were collected from brachial vein from each group after 10 days of treatment beginning and at the end of experiment. Blood serum was separated by centrifugation of blood at 3000 rpm for 15 min and stored at  $-20^{\circ}$ C until analysis.

#### Assays:

Alkaline phosphatase (ALP) was determined in serum by the method of Belfieid and Goldberg (1971), Total antioxidant capacity (TAOC) (Said *et al.*, 2003), Superoxid dismutase (SOD) (Nishikimi *et al.*, 1972), activity of aspartate (AST) and alanin (ALT) transaminases (Reitman and Frankel, 1957) and testosterone concentration assay by ELISA (Sauer *et al.*, 1982).

#### Semen quality:

Semen samples were collected from each cockerel twice weekly by the abdominal massage method (Lake and Stewart, 1978). Semen samples were examined for the following characteristics:

Ejaculate volume was determined to the nearest 0.01 ml. using 1.0 ml. tuberculin syringe. Sperm concentration was determined by using Thomaes–Zeis haemocytometer. While, total sperm output was calculated as (ejaculate volume x sperm concentration).

The progressive motility percentage was assessed according to (Tabatabaei *et al.*, 2009). Sperm livability percentage was determined using eosin/nigrosin stain according to (Lukaszewicz *et al.*, 2008), Sperm abnormalities percentage was determined during the examination of sperm livability at a high power magnification (400 x).

The percentage of acrosomal abnormalities, staining procedure for fixed samples have been developed to distinguish which spermatozoa have retained or lost the acrosome (Al- Daraji, 2001).

#### Morphometric of testis measurement:

At the end of the experiment, three cocks of each treatment were slaughtered, immediately testicular characteristics were recorded, in terms of weight, circumference, length and diameter of each testis. Testicular measurements were taken using a measuring tape rule.

#### Fertility trail:

At the 40<sup>th</sup> week of age, cocks divided each replicates by 1 cockerel and 7 hens each from the same strain. Each replicate was housed in each group cages (120x100x0.75 cm) with the same treatments. Eggs were daily collected from each treatment and incubated to determine fertility and hatchability percentage as fellow:-

Fertility percentage = (fertile eggs/total eggs) x 100.

Hatchability percentage = (hatched chick/fertile eggs) x 100.

#### Statistical analyses:

Data obtained were statistically analyzed as a one way design for change in weight, testis measurement, semen characteristics and fertility and two way design for enzymatic activity using analysis of variance (ANOVA) (SAS, 2001). A significant difference was used at 0.05 probability level and differences among treatments were tested using the Duncan's procedure (Duncan, 1955).

# **RESULTS AND DISCUSSION**

#### Growth performance:

Results in Table (2) reveal that final live body weight (LBW) and change in BW of cockerels treated with different doses of Se in groups (G4, G3 and G2) was significant higher (P<0.05) as compared to control group G1. Statistical analysis for data in Table 2 reveal that final live body weight (LBW) and change in BW of cockerels in group 4 (G4) was significantly the highest (P<0.05) than cockerels in groups G3, G2 and G1. These results is agree with Choct *et al.*, (2004) who found that live body weight of broiler chicks was significantly improved by supplementing diet with selenium. And revealed this enhancement due to that selenium is an essential for growth and performance.

Cocks fed dietary different levels of selenium did not show any significant differences among all groups in daily feed intake (Table 2). These results are harmony with El-Slamony *et al.*, (2015) who illustrated that supplementation of diet with 0.2 or 0.3 mg/ kg diet as Sel-Plex had no significant effect on feed intake of laying hens. Also, Abdalla *et al.*, (2015) reach to the same conclusion in cocks.

#### Morphometry of the testis:

Results presented in Table (2) reveal that cockerel testicular weight (absolute) was insignificantly higher (P<0.05) in groups (G4, G3 and G2) as compared to control group G1, also, testicular (length and circumferences) were not affected significantly (P<0.05) by level of Se supplemented to diet, being higher in G3, than other groups, while testicular diameters was similarity in (G3 and G1) and higher than G4 and G2. These findings reflected the importance of Se supplementation to diet which had a numeric effect on measures of testes (Table 2).

#### Semen quality, fertility and hatchability:

Results presented in Table (3) show that percentages of progressive motility and livability of cockerel spermatozoa were significantly (P<0.05) higher in groups G3 and G4 as compared to both groups G1 and G2, but the opposite trend in percentages of abnormality and acrosome damage of spermatozoa. The ejaculate volume and total sperm output were not significant affected by levels of Se supplemented. The present study is agreement with Jafari *et al.*, (2013) who found that the organic selenium supplementation had significant effects on sperm motility and viability in liquid condition.

The use of organic selenium at the level of 0.3 mg/Kg significantly improved sperm motility and viability at storage times of 4, 8, 12 and 24 h as well as had lower abnormal sperm percentage in comparison with the control group.

Alm El-Dein and Soliman (2015) showed that the highest values of ejaculate volume, sperm concentration, live sperm percentage, sperm motility, total sperm per ejaculate and total live sperm per ejaculate at 30 and 36 wks of age were recorded by Inshas cocks groups fed 0.6 and 0.8 mg Se/kg diet compared to cocks of control. On the opposite, abnormal sperm percentage and total abnormal sperm per ejaculate was higher in cocks of control group compared to selenomethionine groups. Also, Madkour *et al.*, (2015) indicated that the fertility and hatchability percentages were significantly increased by increasing dietary Se level in Japanase Quail. Jerysz and Lukaszewicz (2013) reported that the supplementation of selenium to diet of male significantly increased ejaculate volumes, sperm concentrations, and percentages of viable sperm while decreased percentages of immature sperm (spermatids). Also, Lipids peroxidation, expressed in terms of the malondialdehyde concentration, was lower in semen of the supplemented group as compared to the control.

Selenium is a trace element and frequently added to animal diet as a supplement for the maintenance of reproductive functions, and a deficiency in dietary selenium causes a decrease in sperm concentration, sperm motility, and sperm capacity in humans, lab animals, and farm animals, including poultry species (Hansen and Deguchi 1996). Selenium is a component of selenoproteins, such as glutathione peroxidase, which protect sperms against oxidative damage (Ahsan *et al.*, 2014).

A deficiency in dietary selenium can result in decreased numbers of normal spermatozoa per ejaculate, decreased motility and decreased fertilizing capacity. These phenomena have been demonstrated in rodents, humans and poultry such as chickens, turkeys and ducks (Surai, 2000 and Surai *et al.*, 2001).

Selenium deficiency has been linked to reproductive problems in rats, mice, chickens, pigs, sheep, and cattle (Combs and Combs, 1986) and supplementation with selenium has been reported to improve reproductive performance in sheep and mice (Tang *et al.*, 1991 and Van Ryssen *et al.*, 1992). Selenium is required for normal testicular development and spermatogenesis in rats (Behne *et al.*, 1996), mice, and pigs (Combs and Combs, 1986).

Effect of varying levels of organic selenium on cocks serum testosterone, fertility % and hatchability % are presented in Table (3). Group G3 (fed 0.3mg Sel-Plex/kg diet) recorded significantly the highest value for testosterone concentration comparing to other groups. In this respect, Abdalla *et al.*, (2015) found that concentration of plasma testosterone hormone increased gradually as the level of Se increased in the diet (0.2 and 0.3mg Sel-Plex/kg feed) of cocks but there was no significant difference among treatments. On the other hand, group G4 achieved the highest percentage of fertility and hatchability comparing to either control group or others fed dietary supplemented Se.

Vitamin E-Selenium supplementation had significant (P<0.05) effect on fertility and hatchability of quails. Highest fertility (88%) and hatchability (81%) were recorded in mating subgroup supplemented with vitamin E-Selenium. Mating group is recommended for better fertility and hatchability and economic perspective (Umar *et al.*, 2013 and Alm El-Dein and Soliman 2015).

#### Enzymatic activity:

Table (4) show that total antioxidant capacity (TAOC) in cocks serum was significantly influenced by supplementing different levels of selenium, which G3 (0.30mg) recorded significantly higher value (P<0.05) than in control G1, but no significant differences appeared among supplemental groups.

Values of serum TAOC of weaning pigs fed dietary 0.3 mg Se/kg diet as selenomethionine was significantly enhanced comparing to others fed the same level as sodium selenite (Cao, *et al.*, 2014)

It was widely accepted that proper Se intake could enhance the antioxidant status of the body. TAOC indicates the oxidation resistance capacity of the whole body. Wang *et al.* (2011) pointed out that 0.15 mg/kg of selenomethionine supplementation in broiler diet was more effective than sodium selenite to increase TAOC and decrease MDA concentration in serum and organs.

Table (4) show the SOD activity in serum of cockerel's was not influenced significantly by supplementing different levels of selenium.

Analysis of variance showed that the TAOC, mM/L and SOD MM/L activity was significantly higher at the end of experimental than initial (1.08 *vs.* 0.997 mM/L) and (175.79 *vs.* 164.05 mM/L), respectively, while, the interaction between treatment and time of sample were not affected significantly (Table 4).

Selenium (Se) is an essential trace element that is necessary for the maintenance of various physiological processes (Zhang *et al.*, 2006). In a cell culture system, selenium in the form of sodium selenite (SS) protects the cells from oxidative damage by reducing the production of free radical and inhibits lipid peroxidation (Tatemoto *et al.*, 2004).

Activity enzymes, aspartate (AST) and alanin (ALT) transaminases were significantly (P<0.01) higher in G1 than other groups, but in G2 were significantly (P<0.01) higher than in G3 and G4, while no significant differ between G2 and G3 or between G3 and G4 Table 4. Also, the enzymatic activity, aspartate (AST) and alanin (ALT) transaminases were significantly (P<0.01) higher in the final of experimental period than in initial. However the interaction between treatment and time of sample were not affected significantly (Table 4).

Several studies reported that the concentrations of ALT and AST in plasma were decreased by increasing level of Se in diets, Iqbal *et al.*, (2013), El-Slamony *et al.*, (2015) and Abdalla *et al.*, (2015)

Table (4) shows the alkaline phosphatase activity in serum of cockerel's was not influenced by supplementing different levels of selenium, but the general tendency was higher in group G3 (0.30 mg Se/kg diet) and lower in group G2 (0.15 mg Se/kg diet) than in other groups.

Oxygen species are very active on the cellular level resulting in various degrees of damage to the sperm cells. Sperm cells are very susceptible to lipid peroxidation by free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radical which could later lead to the structural damage of sperm membranes during the aerobic storage of sperm (Surai *et al.*, 1998).

Dietary Se could prevent lipid peroxidation of biological membranes. The results of increasing glutathione peroxidase activity in serum by organic Se in this experiment were in agreement with previous reports (Jiang *et al.*, 2009). These findings suggested that Se-enriched yeast improved antioxidative status of broilers by elevating activities of antioxidant enzymes, and also implicated that Se-enriched yeast supplementation may have a beneficial effect on oxidative stability and extended shelf life of fresh meat than sodium selenite.

# CONCLUSION

In conclusion, use of organic selenium supplementation (0.3 or 0.45 mg/Kg) in diet of cockerels could be recommended to improve semen quality, fertility, hatchability and oxidative status of roosters.

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# تأثير إضافة السيلينيوم في العلف على وزن الجسم والخصوبة و جودة السائل المنوى والحاله التأكسديه في الديوك

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الهدف من هذه الدراسة هو تحديد تأثير مستويات مختلفة من سلينوميثيونين (Sel-Plex) على صفات جودة السائل المنوي، الخصوبة، ونسب الخصوبه والفقس فى سلالة انشاص المحلية. تم تقسيم عدد ستة وثلاثين ديك بطريقه عشوائيه عند ٢٨ أسبوع من العمر إلى أربع مجموعات (٩ ديوك بكل مجموعه). تم تربيها تحت نفس الظروف البيئيه. المجموعه الاولى كنترول وتم تغذيتها على الغذاء الاساسى بدون اضافات ٠٠٠ والمجموعه الثانيه تم اضافه ١٠. ملليجر ام سلينيومثيونين/كجم علف و المجوعه الثالثه تم اضافة تم اضافة ٣.٠ ملليجر ام سلينيومثيونين/كجم علف والمجموعه الرابعة تم اضافه ١٠. ملليجر ام سلينيومثيونين/كجم علف و المجوعه الثالثه تم اضافة ويمكن تلخير ام سلينيومثيونين/كجم علف والمجموعه الرابعة تم اضافة ٤٠. ملليجر ام سلينيومثيونين/كجم علف و

سجل كل من معدل التغير اليومى فى وزن الجسم و وزن الجسم فى نهاية التجربه زياده معنويه (0.05) P)فى المجموعه الرابعه مقارنة بباقى المجموعات. كما لم يوجد فروق معنويه فى معدل استهلاك العلف اليومى للديوك لجميع المجموعات. سجل تركيز هرمون التستوستيرون زياده معنويه فى المجموعه الثالثه مقارنه بباقى المجموعات. فى حين كان معدل الخصوبه اعلى معنويا فى المجموعه الرابعه عنها فى باقى المجموعات. على الرغم من عدم وجود فروق ذات دلاله احصائيه فى نسبة الفقس بين المجموعات. سجل تركيز هرمون سجلت النتائج وجود فروق معنويه لكل من المقدره الحركيه ونسبة الحيوانات المنويه الحيه فى نسبة الفقس بين المجموعة سجلت النتائج وجود فروق معنويه لكل من المقدره الحركيه ونسبة الحيوانات المنويه الحيه فى المجموعتين الثالثه والرابعه معلم عنين الثانيه و الكنترول. وعلى العكس فى النسبه المئويه للشذوذ و الاسبر مات الميته. كما وجد زياده معنويه القدره الكليه لمضادات الاكسده فى المجموعه الثالثه مقارنة بالكنترول. سجل نشاط انزيمات الكبر (ALT) (AST) في معنويه بزيادة نشاطها فى مجموعة الكنترول مقارنة بالمجموعه الثالثه. وعموما يمكن التوصيه: باضافة السيلينيوم العضوي بمستويات (7. • أو ٤٠ • مليوما وي النظام الغذائي للموموم الثالثه. وعموما يمكن التوصيه: باضافة السيلينيوم العضوي بمستويات (7. • أو ٤٠ • مليوما م علف) فى النظام الغذائي للجموعة الثالثه. وعموما يمكن التوصيه: ونسب الفقس و الخصوبة وزيادة المقدره الكليه لمضادات المنوي بينوي معنويه بزيادة نشاطها فى مجموعة علف) فى النظام الغذائي لتحسين صفات السائل المنوي ونسب الفقس و الخصوبة وزيادة المقدره الكليه لمضادات الأكسدة للديوك.

Ingredients	Percentage (%)	Calculated analysis**	Percentage (%)
Yellow corn	66.00	Metabolizable energy (Kcal/Kg)	2747
Soybean Meal (44%)	23.00	Crude protein %	15.67
Wheat Bran	2.50	Crude fiber %	3.46
Di-Calcium Phosphate	1.50	Crude fat %	2.96
Limestone	6.20	Calcium %	3.34
Salt (Na CL)	0.40	Available Phosphorus %	0.42
DL-Methionine	0.10	Lysine %	0.89
Vit. & Min. Mixture*	0.30	Methionine %	0.39
Total	100.00	Methionine +cysteine %	0.66

Table (1): Composition and calculated analysis of experimental basal diets

\*Each Kg content: Vit. A, 100001U; Vit. D3, 20001U, Vit. K3, 1mg; Vit.E, 10mg; Vit. B1, 1mg, Vit. B2, 5mg, Vit. B6, 1.5mg, Vit. B12, 10mg, Niacin, 30mg, Pantothenic acid 10mg, Folic acid 1mg, Biotin, 50mcg, Choline, 260mg, Copper 4mg, Iron, 30mg, Manganese 60mg; Zinc 50 mg and Iodin 1.3mg, Selenium, 0.1mg, Cobalt, 0.1mg. \*\*According to feed composition Tables for animal & poultry feedstuffs used in Egypt (2001).

 Table (2): Live body weight and testes measurements of cockerel fed with different levels of selenium.

Parameters	Treatments (mg Se/kg diet)			
Farameters	G1 Control	G2 (0.15 mg)	G3 (0.30 mg)	G4(0.45 mg)
Live body weight (LBW)				
Initial LBW (g)	1828.3±0.83	1826.7±9.28	1823.3±10.9	1825.0±11.3
Final LBW (g)	1961.7±9.61 <sup>d</sup>	2128.3±7.41 <sup>c</sup>	$2210.0\pm5.0^{b}$	2273.3±3.73 <sup>a</sup>
Change BW (g/day)	$1.19{\pm}0.08^{d}$	$2.69 \pm 0.02^{\circ}$	$3.45 \pm 0.08^{b}$	$4.00{\pm}0.07^{a}$
Daily feed intake (g/cockerel/day)	121.9±0.12	$121.4 \pm 0.20$	121.2±0.42	121.2±0.15
Testes measurements				
Weight (g)	25.33±1.20	$26.97 \pm 2.94$	27.43±3.53	28.53±1.87
Length (cm)	$5.2 \pm 0.55$	$4.8 \pm 0.27$	$5.4 \pm 0.40$	4.1±0.32
Diameter (cm)	21.5±1.15	$19.5 \pm 2.57$	21.5±0.76	19.0±0.76
Circumferences (cm)	6.7±0.32	$6.2 \pm 0.30$	7.1±0.05	6.3±0.25

a, b and c: Means denoted within the same row with different superscripts are significantly different at P<0.01

 Table (3): Semen characteristics, serum testosterone, fertility % and hatchability % as affected by level of selenium

Parameters	Treatments mg Se/kg diet			
Farameters	G1 Control	G2 (0.15 mg)	G3 (0.30 mg)	G4 (0.45 mg)
Ejaculate volume (ml)	$0.553 \pm 0.077$	$0.589 \pm 0.069$	$0.644 \pm 0.095$	0.553±0.067
Sperm concentration ( $x10^6$ /ml)	$1.789 \pm 0.064^{b}$	$1.894{\pm}0.11^{ab}$	$1.925 \pm 0.082^{ab}$	$2.073 \pm 0.063^{a}$
Total sperm output (x10 <sup>6</sup> /ejac.)	$0.962 \pm 0.102$	$0.972 \pm 0.098$	$1.175 \pm 0.161$	1.189±0.13
Mass motility (%)	$63.75 \pm 1.5^{b}$	65.83±1.93 <sup>b</sup>	$80.00 \pm 1.23^{a}$	$84.17 \pm 1.0^{a}$
Live sperm (%)	$67.25 \pm 1.51^{\circ}$	$68.92 \pm 2.25^{\circ}$	$81.75 \pm 1.21^{b}$	$87.08 \pm 1.11^{a}$
Sperm abnormality (%)	$11.58 \pm 0.57^{a}$	$8.42 \pm 0.56^{b}$	$6.42 \pm 0.68^{\circ}$	$3.92 \pm 0.53^{d}$
Damaged acrosome (%)	4.58±0.31 <sup>a</sup>	$4.17 \pm 0.49^{ab}$	$3.25 \pm 0.35^{bc}$	$2.67 \pm 0.26^{\circ}$
Testosterone concentration	$0.49{\pm}0.02^{d}$	$1.87 \pm 0.04^{\circ}$	$2.45 \pm 0.03^{a}$	$2.02 \pm 0.05^{b}$
Fertility (%)	$82.5 \pm 0.58^{d}$	$89.4\pm0.70^{\circ}$	$92.0\pm0.30^{b}$	$94.3 \pm 0.46^{a}$
Hatchability (%) from fertile eggs	75.6±1.13 <sup>c</sup>	$86.4{\pm}0.78^{b}$	$89.3 \pm 0.58^{a}$	$92.1 \pm 0.70^{a}$

a, b, c and d: Means denoted within the same row with different superscripts are significantly different at P<0.01

	ALP	AST	ALT	TAOC	SOD	
Item					SOD	
	U/mL	U/L	U/L	mM/L	mM/L	
Treatment (mg Se/k	Treatment (mg Se/kg diet):					
(G1) Control	89.99±7.88	$25.18 \pm 1.47^{a}$	$49.93 \pm 2.10^{a}$	$0.99 {\pm} 0.02^{ m b}$	$166.02 \pm 8.39$	
(G2) 0.15 mg	83.27±8.65	$20.28 \pm 1.05^{b}$	44.38±1.69 <sup>b</sup>	$1.07 \pm 0.02^{ab}$	173.51±4.77	
(G3) 0.30 mg	97.06±5.96	$17.53 \pm 1.18^{bc}$	39.19±1.89 <sup>bc</sup>	$1.08 \pm 0.06^{a}$	$165.45 \pm 3.64$	
(G4) 0.45 mg	95.52±5.11	$18.22 \pm 2.03^{\circ}$	$40.38 \pm 2.67^{\circ}$	$1.01 \pm 0.01^{ab}$	$174.72 \pm 3.14$	
Time:						
Initial Time 1.T	$83.99 \pm 3.96^{b}$	$23.38 \pm 0.98^{a}$	$47.61 \pm 1.58^{a}$	$0.997 \pm 0.015^{b}$	$164.05 \pm 3.25^{b}$	
Finally Time F.T	$98.92 \pm 4.97^{a}$	$17.23 \pm 1.01^{b}$	39.33±1.33 <sup>b</sup>	$1.08\pm0.026^{a}$	175.79±3.56 <sup>a</sup>	
Interaction between	Interaction between Treatment and Time:					
(G1) X 1.T	73.8±6.79	$27.55 \pm 2.22$	$53.58 \pm 2.81$	$0.95 \pm 0.01$	151.16±1.82	
(G2) X I.T	86.8±11.24	$22.58 \pm 0.84$	48.35±0.79	$1.06\pm0.03$	168.17±7.5	
(G3) X 1.T	$86.8 \pm 6.98$	20.18±1.24	43.6±1.61	$0.99 \pm 0.015$	164.26±6	
(G4) X 1.T	88.6±7.22	23.20±1.55	$44.9 \pm 4.32$	0.991±0.014	$172.62 \pm 2.8$	
(G1) X F.T	106.2±0.92	22.80±1.19	46.28±1.89	$1.04 \pm 0.018$	180.87±11.31	
(G2) X F.T	79.7±15.33	$18.00 \pm 0.96$	40.42±1.51	$1.07 \pm 0.03$	$178.84 \pm 5.38$	
(G3) X F.T	$107.3 \pm 4.82$	$14.98 \pm 0.56$	$34.78 \pm 1.09$	$1.18 \pm 3.077$	$166.65 \pm 5.38$	
(G4) X F.T	$102.4 \pm 5.57$	13.24±0.46	35.87±1.00	$1.02 \pm 0.024$	176.81±6.1	

Table (4): Enzymatic activity in serum of cockerel fed with different levels of selenium.

a, b, and c: Means denoted within the same column with different superscripts are significantly different at P<0.01