

## **Effect of Dosing of Broiler Breeder Roosters (Ross) with different Levels of Nano-selenium Particles and Organic Selenium on Physiological and Histological Traits A Thesis Submitted**

**Aws Alaa Hassan Al-Kaabi<sup>1\*</sup>, Dr. Emad Abdul-Jabbar Ali<sup>2</sup>**

<sup>1\*</sup>College of Agriculture, Al-Qasim Green University, Iraq. E-mail: aws.P400119@agre.uoqasim.edu.iq

<sup>2</sup>College of Agriculture, Al-Qasim Green University, Iraq. E-mail:Emadali76@agre.uoqasim.edu.iq

### **ABSTRACT**

This experiment was conducted in the poultry farm of the Animal Production Department at the College of Agriculture - Al-Qasim Green University for 8 weeks from 1/10/2020 - 1/12/2020, preceded by two weeks to train roosters to give semen, and the experiment aimed to study the effect of Ross broiler breeder were dosed with different levels of nano-selenium and organic selenium particles in reproductive and biochemical characteristics. The stoke of the breeder (Ross 308) was used at the age of (64) weeks, and the roosters were divided randomly into 7 treatments with 5 replicates for each treatment and each one rooster included, and the treatments were as follows (0, 1 mg/kg organic selenium feed, 1.5 mg/kg organic selenium feed, 2 mg/kg organic selenium feed, 0.5 mg/kg nano selenium feed, 0.75 mg/kg nano selenium feed., 1 mg/kg nano-selenium feed) for the treatments (T1, T2, T3, T4, T5, T6, T7), respectively, and obtained the following results: Significantlyexcelled ( $P<0.05$ ) for treatment T5 in sperm concentration, group, and individual motility. The concentration of FSH, LH, and Glutathione Peroxidase compared to the rest of the treatments. Significant ( $P<0.05$ ) for treatment T7 in the proportion of live sperm and concentration of testosterone hormone, and its significant improvement in the percentage of dead sperm compared to the rest of the treatments. Significant superiority ( $P<0.05$ ) for all treatments of nano-selenium and organic selenium in the ratio of sperm resistance to salt compared with treatment Control, significant superiority ( $P<0.05$ ) for treatment T2 in the absolute weight of the testis, significant superiority ( $P<0.05$ ) for treatment T1 in estrogen concentration compared with the rest of the treatments.

### **KEYWORDS**

Nano Selenium, Organic Selenium, Reproductive Traits, Broiler Broilers.

### **Introduction**

The production of fertilized eggs is the main goal of broiler breeders flocks because the number of fertilized eggs determines the profitability of laying hens (breeders flocks). Therefore, fertility decline is considered one of the most important economic losses related to production in the poultry industry for broiler breeder stoke, and the sharp decrease in fertility A big problem, especially after the age of 50 weeks (Remero-Sanches et al., 2008). Also, hatching eggs at high fertility rates increases the production of chicks and this is the main goal of breeders (Avital-Cohen et al., 2015), as in the poultry industry the fertility of roosters is a factor. Important and major and represents the means of preserving the species, the continuation of reproduction, and the permanence of production. Therefore, attention must be paid to reproductive capacity, and the treatments affecting it because of its importance in improving production. (Al-Darraji, 2008; Al-Rawi and others, 2011). Selenium is an important trace mineral that has many essential roles at the cellular and organic levels in poultry health. The biological effects of selenium are studied mainly by selenite proteins. Selenium plays both structural and enzymatic roles. It is well known for its catalytic and antioxidant functions (Qazi et al., 2019). A unique element because it is made up of some amino acids (selenomethionine and selenocysteine) and thus participates in specific biological roles and includes protection from the harmful action of oxidation, improving immunity, and improving growth and development so the main source of selenium is through nutrition (Marmioli&Maestri, 2008; Kabata-Pendias, 2011). As between Surai and Fisinin, (2014) selenium is an essential component of poultry nutrition and a great deal of information has accumulated on the past twenty years indicating that the dietary form of selenium is one of the main determinants of its efficiency and in general, selenium is an important factor in ensuring fertility in roosters and is considered important for the production of Hatching eggs to maintain the antioxidant system of the developing embryo (Surai and Fisinin, 2014), which is an essential component of the enzyme glutathione peroxidase (GSH-Px) and is present in all reproductive organs and sperm tissues where its availability is necessary for protection against oxidative stress (Surai and Fisinin, 2014), and when selenium is deficient in the diet, the concentrations of selenium and glutathione peroxidase decreased, while lipid peroxidation increases in the testicles of roosters (Shi et al., 2014). Based on the above data, the study aims to the following: To evaluate the use of selenium when administered to broiler broilers from different sources (nano selenium and organic selenium) and to know its effect on the reproductive, physiological, and

histological performance of these birds, to compare the effect of nano selenium and organic selenium and to determine the optimal source, to determine Optimum concentrate from two sources of selenium, which improves the reproductive traits of broiler breeder.

## Material and Methods

This experiment was conducted in the poultry farm of the Animal Production Department at the College of Agriculture - Al-Qasim Green University for 8 weeks from 1/10/2020 - 1/12/2020, and before the start of the experiment the roosters were trained to give semen for two weeks only, where they used In this experiment, 35 roosters from the strain of ROSS 308 are broiler breeder stock. The roosters were prepared from the Modern Poultry Company - Al Kut, with an average weight of 5.850 kg and at the age of (64) weeks. Roosters were raised in the farm of the Animal Production Department prepared and divided into reservations, the dimensions of each reservation (1.5 x 1) m<sup>2</sup> and each room were divided into three sections according to the ground breeding system, and the lighting system was followed (14 hours/day) for the duration of the experiment with 10 daily giving Dark hours. And organic selenium and nano-selenium were given to roosters in the form of capsules, which were dosed to roosters at the rate of one capsule/day for each rooster. The roosters were distributed randomly, as the treatments are shown below: The first treatment (T1): - a control treatment. The second treatment (T2): - Dosing of organic selenium at a concentration of 1 mg/kg feed. The third treatment (T3): - Dosing of organic selenium at a concentration of 1.5 mg/kg of feed. Fourth treatment (T4): - Dosing of organic selenium at a concentration of 2 mg/kg feed. Fifth treatment (T5): - Dosing of nano-selenium at a concentration of 0.5 mg/kg feed. The sixth treatment (T6): - Dosing of nano-selenium at a concentration of 0.75 mg/kg of feed. Seventh treatment (T7): - Dosing of nano-selenium at a concentration of 1 mg/kg feed.

## Food Treatment

The roosters were fed on a diet of male broiler breeder, containing raw protein 16.53% and representative energy of 2788.44 kcal/kg of feed, as they were prepared and prepared in the Al-Baraka Crusher - Babylon, where the feed was provided at 146 g of feed/bird/day. Feed and capsules were provided. At fixed times throughout the experiment, Table 2 shows the components of the diet.

**Table 1.** Shows the feed used in the experiment and its chemical composition

Components	Utilization ratio
Yellow corn	37
Wheat	14
Barley	16.2
Soybean cake (44% protein)	20
Wheat bran	6.8
Mixtures of vitamins and minerals * Premix	2
Limestone Powder	3
Vegetable oil	1
Total	100%
Calculated chemical composition **	
Representative Energy (kcal/kg feed)	2788.44
Crude protein	16.53
Crude fiber	3.45
Calcium	1.26
Available phosphorous	0.74
Methionine + cysteine	0.69
Lysine (%)	0.85

\* Premix Maxcare of Belgian origin, each 1 kg contains: crude protein 7.9%, lysine 2.4%, methionine 7.7%, methionine + cysteine 7.7%, calcium 23.1%, phosphorous 3.3%, sodium 5.5%, representative energy 2903 kcal/kg, vitamin A 400,000 IU, Vitamin D3 300,000 IU, Vitamin Hy.D 20,000 IU, Vitamin E 800 IU, Vitamin K 80 ppm, Vitamin B1 40 ppm, Vitamin B2 160 ppm, Calcium pantothenate 320 ppm, Niacin 600 ppm, Biotin 1600 ppb, Vitamin B12 1000 ppb, Folic acid 40 ppm, Vitamin B6 160 ppm, Iron 2800 ppm, Copper 600 ppm, Zinc 2400 ppm, Magnesium 4000 ppm, Iodine 80 ppm, Selenium 8 ppm.

\*\* Chemical analysis computed according to NRC (1994).

Dicalcium phosphate has a concentration of calcium of 24%, phosphorus 18%.

Oil 9000 kcal / kg.

## **Preparation of Nano-selenium and Organic Selenium Materials**

Gray-to-black nano-selenium material with a purity of 99.9% and particle size nm80, origin India, produced by (NANOSHEL) company, and organic selenium material (Se-Yeast) from one of the scientific offices / Bab Al-Muzam / Baghdad.

## **Studied Traits**

### **Hormonal Traits**

Blood samples were drawn from the pterygoid vein. Using a 5 ml syringe equipped with a Needle, and according to Al-Darajiet al., (2008), the blood is drawn by inducing pressure release and then the blood is emptied after removing the needle from the plastic syringe into 10 ml plastic tubes, and these tubes are placed in a centrifuge at a speed of 6000 A precise 15-minute course to separate the serum from the cellular part. After separation, the serum samples are transferred to other plastic tubes, and stored at -20 ° C. Hormone tests were performed in the analytical laboratory (Faculty of Science / University of Babylon) as follows:

### **Measuring Sex Hormones**

The level of follicle-stimulating hormone (FSH), ovulation hormone (LH), testosterone, and estrogen hormone in the blood serum was measured using a special detection kit (Kit) from the Chinese company (Bioassay Technology Laboratory) using ELISA technology and according to the method of work followed by the manufacturer.

Measurement of glutathione peroxidase (GPx) enzyme activity, Glutathione peroxidase enzyme activity was determined according to a method by Hafemann et al., (1974).

### **Blood Cell Traits**

Blood tests were performed in the analytical laboratory (Faculty of the Science / University of Babylon), where the image of blood was measured using the (Mythic 18 ret) device from the French company (Orphee) by taking (50 microns) of blood automatically and using the detection kit of the device. Blood standards are according to the mechanism of the device, and the following tests were performed:- RBC ,Hemoglobin blood HB, and The volume of PCV cells.

### **Histological Qualities**

At the end of the experiment, 14 roosters of these treatments were slaughtered at a rate of 2 roosters from each treatment randomly, and the testes and liver were extracted, after which a tissue segmentation of the testes and liver was performed after being soaked with a 10% formalin solution (Almansour, 2009) to study the histological changes. The thickness of the interstitial tissue, the diameter of the seminal tubules, the height of the epithelial tissue, the diameter of the blood vessels, the number of interstitial cells (Lydic cells) in the testis were measured.

### **The Weight of the Testicles**

After the roosters were slaughtered, the testicles were extracted from the body cavity and weighed with a sensitive scale, after which they were washed with running water and kept in plastic boxes containing formalin at a concentration of 10%, and placed in the refrigerator until the tissue segmentation was performed, and the absolute weight of it was extracted.

### **Fabric Chopping**

1. A sample with a thickness of 1 square cm was taken and fixed with formalin 10% for a period of 2-7 days, and after fixation, the sample was washed with running water to get rid of the remnants of formalin, as the tissue preparation was done with the technique of embedding with paraffin and the following:
  - a) Ophthalmic drying: DEHYDRATION: by drawing water from the cells by a series of upward passes with ethyl alcohol (70, 80, 90, and 100%) at two hours per concentration.

- b) CLEARING tissue: by xylene for one and a half hours - two hours until the tissue becomes transparent and light brown in color.
  - c) Embedding: EMBEDDING by placing the sample with a 57 ° C paraffin bath for two hours.
  - d) Make a paraffin block for the sample BLOCKING and let it cool down and then put it in the refrigerator.
  - e) SECTIONING with a microtome 5-7 microns in thickness.
  - f) Place the tissue strips in a 44m water bath for textile brushes.
  - g) MOUNTING Hold the tissue on a glass slide and leave it to dry.
2. Staining with hematoxylin, eosin, and my late:
  - a) Put the slides in xylene for two hours to remove the paraffin from the tissue.
  - b) Return the water to the tissue cells bypassing the slides at decreasing concentrations of alcohol (100, 90, 80, and 70%) for 2-3 minutes, and then placing the slides with distilled water for 5 minutes.
  - c) Placing the slides with hematoxylin dye for 5 minutes to stain the nuclei, and then immersing them in water for 5 seconds.
  - d) Applying the slides with eosin stain for one minute only.
  - e) Withdraw water from the cells by immersing the slides in ascending alcohol concentrations (70, 80, 90, and 100%) for 5 seconds for each concentration.
  - f) Putting slides in xylene to purify tissue from alcohol residue.
  - g) Developing DPX (Bancroft & Marilyn, 2008).
3. The measurement was done by using an optical microscope with a (4x) magnifying glass. Five readings were collected within five microscopic fields. The tissue sections were photographed using a Future Win Joe microscopic camera.

## Statistical Analysis

The data were analyzed using a completely random design (CRD) to study the effect of the studied treatments on the different traits. The significant differences between the averages were compared using Duncan's test, (1955) polynomial.

The program used SAS (2012) in the statistical analysis according to the following mathematical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

As:

$Y_{ij}$ : the view value  $j$  of transaction  $i$ .

$\mu$ : the general mean of the trait.

$T_i$ : the effect of  $I$  treatment (as the study included the effect of 7 aforementioned treatments).

$e_{ij}$ : the normally distributed random error with a mean equal to zero and a variance of  $\sigma^2_e$ .

## Results and Discussion

### Bodyweight and Testicle Absolute Weight

Table (2) shows the effect of the studied treatments on the body weight and the absolute weight of the testis during the study period, where it is noticed that there are no significant differences between the experimental treatments in the body weight. As for the absolute weight of the testis, we note a significantly excelled ( $P < 0.05$ ) for the T2 treatment compared to the rest of the experiment treatments. The treatment T5 was excelled the treatments T1, T3, T6, T7, and the two treatments T4 and T5 excelled on the treatments T3, T6, and T1, and there was no significant difference between treatment T4 and treatment T5, as well as between treatment T4 and T7 as well between treatments T1, T3, T6, T7. The significant improvement in the absolute weight of the testis is due to the role of nano-selenium and organic selenium in improving reproductive traits, as Li et al. (2020) showed that the testis is the most important male reproductive gland and that testicular growth is critical for fertility and testicular weight is a traditional quantitative indicator of the organ Closely related to the number of germ cells. Testicular size closely follows changes in FSH levels, increases in mass with increased FSH, and decreases in mass with decreased FSH. Testicular weight in birds is closely related to daily sperm production and thus there is a direct relationship between testicle size and FSH concentration being the catalyst for synthesis. Sperm (Vizcarra et al., 2010), as indicated by Lukusa and Lehloeny, (2017) the role of selenium in the development of germ cells and Sertoli cells thus increasing the size and weight of the testicles, and testicular weights in birds are closely related to daily sperm production

(Vizcarra et al., 2004) As high concentrations of selenium lead to low testicular weight (Shi et al., 2010).

**Table 2.**Effect of dosing of broiler breeder roosters (Ross)with different levels of nano-selenium and organic selenium particles on body weight and absolute testis weight

Treatments	Averages $\pm$ standard error	
	body weight kg	testicle absolute weight(gm)
<b>T1</b>	<b>6.16 <math>\pm</math> 0.45</b>	<b>21.01 <math>\pm</math> 0.75</b> <b>D</b>
<b>T2</b>	<b>6.31 <math>\pm</math> 0.09</b>	<b>42.67 <math>\pm</math> 1.09</b> <b>A</b>
<b>T3</b>	<b>6.19 <math>\pm</math> 0.17</b>	<b>20.90 <math>\pm</math> 1.63</b> <b>D</b>
<b>T4</b>	<b>6.28 <math>\pm</math> 0.13</b>	<b>25.19 <math>\pm</math> 0.18</b> <b>BC</b>
<b>T5</b>	<b>6.20 <math>\pm</math> 0.17</b>	<b>28.69 <math>\pm</math> 1.58</b> <b>B</b>
<b>T6</b>	<b>5.87 <math>\pm</math> 0.06</b>	<b>21.23 <math>\pm</math> 0.61</b> <b>D</b>
<b>T7</b>	<b>5.88 <math>\pm</math> 0.13</b>	<b>21.61 <math>\pm</math> 1.77</b> <b>CD</b>
<b>The level of morale</b>	<b>n.s</b>	<b>*</b>

\* The averages carrying different letters within the same column differ significantly between them at a level of ( $P < 0.05$ ). Treatments T1, T2, T3, T4, T5, T6, T7 are control treatment, a Dosing of 1 mg/kg organic selenium feed, a Dosing of 1.5 mg/kg organic selenium feed, a Dosing of 2 mg/kg organic selenium feed, a Dosing of 0.5 mg/kg Nano selenium feed, a Dosing of 0.75 mg/kg nano selenium feed, a Dosing of 1 mg/kg nano selenium feed respectively.

### Sex Hormones and Glutathione Peroxidase Enzyme

Table (3) shows the effect of the studied treatments on sex hormones and the enzyme glutathione peroxidase during the trial period, and showed a significantly excelled ( $P < 0.05$ ) in testosterone for treatment T7 on the treatments T1, T3, T5, T6 and the excelled of the treatments T2, T4, T7 on treatment T5 and not There were significant differences between the treatments T4, T7, and T2, as well as between the treatments T2, T3, T4, and T1, as well as between the two treatments T5, T6. As for the estrogen hormone, the treatment T1 significantly excelled ( $P < 0.05$ ) on the treatments T4, T6, T7, and the two treatments T2 excelled the two treatments. T1 on the two treatments T4, T6, and there were no significant differences between the treatments T1, T2, T3, T5, as well as between the treatments T3, T5, T7, and T2, as well as between the two treatments T4, T6. As for the FSH hormone, the treatment T5 significantly excelled ( $P < 0.05$ ). Treatments T2, T3, T4, T6, T7 and the two treatments T1, T5 excelled on the treatments T2, T3, T4 as well as the treatments T1, T6, T7 on the two treatments T2, T3 and there were no significant differences between the two treatments T1, T5 and the treatments T1, T6, T7 and between the treatments T4, T6, T7, as well as the absence of a significant difference between the two treatments, T2 and T3. As for the LH hormone, the significant superiority ( $P < 0.05$ ) of treatment T5 continued on the rest of the experiment treatments, and the results were excelled. The tendency of T7 on the treatments T1, T2, T3, T4 and the absence of significant difference between the two treatments T5, T7 and between the two treatments T6, T7 and the treatments T1, T2, T3, T4, T6 and the continuity of significantly excelled ( $P < 0.05$ ) for treatment T5 in the glutathione peroxidase enzyme on the rest of the experimental treatments, treatment T2 was excelled on treatment T7, and there was no significant difference between the two treatments T2, T5, as well as between the treatments T1, T2, T3, T4, T6.

The increase in testosterone concentration in treatment T7 may be due to the role of nano selenium, which is important for testosterone biosynthesis, as it was found that blood testosterone concentrations have a positive

relationship with selenium concentrations (Shi et al. 2010). Likewise, selenium supplements have been shown to act on Improving testosterone level and semen quality in different animal species (Oluboyo et al., 2012), however, the exact underlying mechanisms that regulate testosterone production are still unclear and require further understanding. Since testosterone is produced by the cells of your hand, it needs further study. In the modifying mechanisms associated with selenium for testosterone synthesis (Ren et al., 2012), selenium has a potential regulatory role in some basic cellular functions and this role is modulated by activating signal-regulated kinases (ERK) signaling pathways. However, the precise role of selenium in testosterone biosynthesis by modulating the kinase signaling pathway and the fate of your hands' cells during the organism development process. Semen wate is not fully understood (Ren et al., 2012), and studies have shown that selenium can modulate the cellular proliferative processes related to apoptosis in the cells of your hands and activate this mechanism mainly in the regulation of oxidative stress and apoptosis (Shi et al., 2017; Qazi et al., 2019). Optimum testosterone concentrations are essential for the normal development of sperm cells. During testosterone biosynthesis, reactive oxygen species are produced and their excessive production contributes to poor fertility in roosters or sometimes sterility (Nishimura et al., 2001), in addition to its role as a plasma selenoprotein, it may play a role as an antioxidant within cells (in LADC cells) in addition to glutathione peroxidase in the face of H<sub>2</sub>O<sub>2</sub> resulting from testosterone biosynthesis (Nishimura et al., 2001) as well as concerning the effect of selenoprotein on steroid biosynthesis in cells Lydec, in addition to its role as an antioxidant inside cells, it may act as an extracellular antioxidant that also protects the cells of your hands from the harmful oxidative action, indicating the physiological function of the selenoprotein in the production of testosterone in the cells of Lydec (Nishimura et al., 2001; Qazi et al., 2019). As for the significant increase in the concentration of FSH and LH hormones in treatment T5, the results of the studies also showed an increase in the concentrations of these hormones in the plasma (Hezarjaribi et al., 2016) as the testicular function is controlled by the secretion of hormones releasing the gonadotropin (GnRH) responsible. on stimulating the gonads of the pituitary gland to secrete the hormones LH and FSH (Griswold, 1998), it is known that selenium accumulates in the anterior lobe of the pituitary gland (Lukusa and Lehloenya, 2017). An increase in the concentration of selenium in the plasma may have led to the activation of the GnRH receptors. The anterior pituitary gland leading to increased production of LH and FSH (Ottinger et al., 2004). It is worth noting that testicular size closely follows changes in FSH levels, increases in mass with increased FSH, and decreases in mass with decreased FSH, as testicular weight in birds is closely related to daily sperm production, and therefore there is a direct relationship between testicular size and FSH concentration. Being the catalyst for spermatogenesis (Vizcarra et al., 2010), These improvements can also be due mainly to selenium in enhancing antioxidant activity (Hosny et al., 2020). Selenium participates in the synthesis of selenoproteins that transport selenium from the blood to various organs, including the testis, which reduces the production of unnecessary free radicals in the testicle, which protects cells. Testis from the harmful action of oxidation and thus improvement of sperm and hormone concentrations (Hosny et al., 2020). The high concentration of the enzyme glutathione peroxidase in treatment T5 compared with the rest of the experimental treatments may be due to the role of the antioxidant nano selenium Levander (1986) indicated that the deficiency of nano selenium causes an increase in the formation of free radicals and a decrease in the body's defense against oxidative processes, usually, This condition is defined as oxidative stress, where the addition of selenium to roosters' diets significantly increased glutathione peroxidase activity in the liver, testicles, sperm and seminal plasma (Surai and Fisnin, 2014), which significantly reduced sperm and tissue sensitivity to lipid peroxide. It is critically important that the inducible form of GSH-Px (glutathione peroxidase) accounts for more than 75% of total enzyme activity in chicken sperm and on 60% in the testes and liver of young roosters. Indeed, selenium is an important component of the antioxidant system in Semen (Surai and Fisnin, 2014). Glutathione peroxidase also protects the developing sperm from DNA damage caused by oxidative stress. Subsequent stage By cross-linking with proteins in the midsection region, the glutathione peroxidase provides midsection integrity by becoming the structural component of the mitochondrial sheath which is an essential component of sperm stability and motility (Beckett and Arthur, 2005). Roosters' reproductive functions as it acts as a selenium transport protein and is expressed in vesicle-like forms in the basal region of Sertoli cells (Olson et al., 2007) The relationship between increased selenium and increased GPx4 (4 glutathione peroxidase) expression in the cock testicles where glutathione peroxidase increases with increasing levels of selenium in the feed. (Jafarzadeh et al., 2020) This explains the significant improvement in semen characteristics in treatment T5.

**Table 3.**Effect of dosing of broiler breeder roosters (Ross)with different levels of nano-selenium and organic selenium particles on sex hormones and the enzyme glutathione peroxidase

Averages ± standard error
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Treatments	Testosterone ng/ml	Estrogen pg/ml	FSH mIU/ml	LH mIU/ml	Glutathione peroxidase U/L
T1	1.09 ± 0.12 BC	4.83 ± 0.90 A	5.45 ± 0.45 AB	1.61 ± 0.45 C	1130.21 ± 4.81 B
T2	1.35 ± 0.11 AB	3.63 ± 1.59 AB	0.79 ± 0.01 D	1.01 ± 0.05 C	1177.08 ± 21.65 AB
T3	1.08 ± 0.07 BC	2.99 ± 1.38 ABC	1.11 ± 0.09 D	1.39 ± 0.03 C	1123.44 ± 3.90 B
T4	1.41 ± 0.25 AB	0.69 ± 0.12 C	1.98 ± 0.37 CD	1.21 ± 0.14 C	1135.42 ± 0.14 B
T5	0.62 ± 0.05 D	2.38 ± 0.57 ABC	7.47 ± 1.29 A	4.08 ± 1.22 A	1207.81 ± 39.39 A
T6	0.71 ± 0.16 CD	0.40 ± 0.02 C	3.66 ± 0.01 BC	2.18 ± 0.13 BC	1131.25 ± 0.00 B
T7	1.51 ± 0.05 A	1.46 ± 0.008 BC	3.88 ± 1.23 BC	3.35 ± 0.54 AB	1015.10 ± 12.93 C
The level of morale	*	*	*	*	*

\* The averages carrying different letters within the same column differ significantly between them at a level of ( $P < 0.05$ ). Treatments T1, T2, T3, T4, T5, T6, T7 are control treatment, the Dosing of 1 mg/kg organic selenium feed, the Dosing of 1.5 mg/kg organic selenium feed, the Dosing of 2 mg/kg organic selenium feed, the Dosing of 0.5 mg/kg Nano selenium feed, the Dosing of 0.75 mg/kg nano selenium feed, the Dosing of 1 mg/kg nano-selenium feed respectively.

### Blood Cell Traits

Table (4) shows the effect of the treatments for cellular blood traits, where an increase in the level of significance ( $P < 0.05$ ) for treatment T2 in the number of red blood cells was observed on the rest of the study treatments, and there was no significant difference with treatment T1, as well as an increase in the level of significance for the two treatments T7 and T1 on treatment T5. There was no significant difference between the treatments T1, T3, T4, T6, T7 and the treatments T3, T4, T5, T6 and in hemoglobin concentration the significant increase ( $P < 0.05$ ) for treatment T2 continued on all traits, followed by treatment T7 in terms of height with a significant level of concentration Hemoclopin on the treatments T1, T3, T4, T5, T6 while the treatments T4, T6 they excelled on the treatments T5, and there was no significant difference between the treatments T1, T3, T4, T6 and between the treatments T1, T3, T5, with the continuation of the significant increase ( $P < 0.05$ ) for treatment T2 in the ratio of the PCV to the rest of the treatments, and at the time when treatment T4 was similar to treatment T2, we notice an increase in the level of significance on treatment T5, followed by treatment T7, which also excelled treatment T5, and no significant difference appeared between the treatments T3, T4, T6, T7., T1, and among the treatments T3, T5, T6, and T1, the improvement in the blood traits of the treatment T2 may be one is caused by organic selenium, as selenium is the functional component of GPx4, which depends on selenium, protects blood cell traits and components from oxidative damage to membranes, and adding 1 mg of nano-selenium / kg of feed has a positive effect on the consistency of biological membranes and the performance of immune cells (Pelyhe and Mézes, 2013). And the addition of various sources of organic selenium or nano-selenium to roosters' diets led to a significant increase in the number of red blood cells and hemoglobin concentration, and selenium improves the activity of the hematopoietic organs in order to increase the number of red blood cells, hemoglobin content and the volume of accumulated cells (Ihsan and Qader, 2012), also these results are in agreement with those obtained by Selim et al., (2015) who found that the blood test showed the highest number of erythrocytes, PCV and hemoglobin values by adding organic or nanoscale forms of selenium compared to inorganic selenium, ( Hanafy et al., 2009, EI-Sheikh et al., 2010) reported that organic selenium supplementation at 0.2 and 0.3 ppm significantly increased hemoclobin concentrations, among others Mohapatra et al., (2014) that nano-selenium is more effective ( $P < 0.05$ ) in increasing hematological treatments different from those of inorganic sodium selenite at 0.3 ppm and that selenium has an antioxidant effect on the red blood cell membrane and prevents degradation of red blood cells Maturing due to condensation of erythropoiesis as the red blood cell count and hemoglobin value increase (Rizk et al., 2017). Also, increasing the level of the hormone testosterone leads to an increase in the red blood cell formation promoter (Eric et al., 2014), as the enhancer stimulates the production of red blood cells (RBC), and an increase in the level of the hormone testosterone leads to an increase in the sensitivity of the red blood cells to an increase in the enhancer of

formation. Red cells, leading to an increase in red blood cell production (Jelkmann, 2007).

**Table 4.**Effect of dosing of broiler breeder roosters (Ross)with different levels of nano-selenium and organic selenium particles on blood cell traits

Treatments	Averages $\pm$ standard error		
	RBC Cell / mm <sup>3</sup> / blood	HB mg / mm <sup>3</sup> / blood	PCV %
T1	3.28 $\pm$ 0.16 AB	14.15 $\pm$ 0.89 CD	43.00 $\pm$ 1.15 BC
T2	3.91 $\pm$ 0.47 A	20.80 $\pm$ 0.63 A	49.50 $\pm$ 0.28 A
T3	2.72 $\pm$ 0.06 BC	14.60 $\pm$ 0.46 CD	44.00 $\pm$ 1.15 BC
T4	2.73 $\pm$ 0.06 BC	15.40 $\pm$ 0.17 C	46.00 $\pm$ 1.73 AB
T5	2.46 $\pm$ 0.17 C	12.65 $\pm$ 0.66 D	41.00 $\pm$ 1.15 C
T6	3.08 $\pm$ 0.12 BC	15.75 $\pm$ 0.66 C	44.00 $\pm$ 1.73 BC
T7	3.13 $\pm$ 0.07 B	18.35 $\pm$ 1.12 B	45.00 $\pm$ 1.15 B
<b>The level of morale</b>	*	*	*

\* The averages carrying different letters within the same column differ significantly between them at a level of (P <0.05). Treatments T1, T2, T3, T4, T5, T6, T7 are control treatment, the Dosing of 1 mg/kg organic selenium feed, the Dosing of 1.5 mg/kg organic selenium feed, the Dosing of 2 mg/kg organic selenium feed, the Dosing of 0.5 mg/kg Nano selenium feed, the Dosing of 0.75 mg/kg nano selenium feed, the Dosing of 1 mg/kg nano-selenium feed respectively.

### The Histological Features of the Testicle

Table (5) indicates the effect of the studied treatments on the histological characteristics of the testis during the study period, where a significantly excelled (P <0.05) of treatment T3 on the rest of the treatments was observed in the thickness of the interstitial tissue of the seminal tubules, and the superiority of treatment T2 on the treatments T4, T6, T7, and T1 and the excelled of the two treatments. T2, T5 on treatment T1, while there were no significant differences between the two treatments T2, T3, as well as between treatments T1, T4, T6, T7, and in the diameter of the seminiferous tubules significantly (P <0.05), treatment T2 exceeded the study treatments, and treatment T3 excelled on the treatments T1, T4, T6, T7, and the two treatments T4 and T5 excelled on treatment T1. The table shows that there is no significant difference between the two treatments T2, T3 as well as between the two treatments T4 and T5, as well as between the treatments T1, T6, T7, and in the height of the germinal epithelium, the table shows a significant superiority (P < 0.05) for treatment T4 over all the study coefficients and significant superiority for treatment T5 over the two treatments T1, T6 and for treatment T2, T3, T4, T5 over treatment T1 and there was no significant difference between the treatments T2, T3, T5, T7 as well as between the treatments T3, T6, T7. Also, there was no significant difference between T6, T7, and T1 treatments, and in the diameter of blood vessels, it was significantly higher (P <0.05). T2, T5, T6, T7 on the two treatments T3, T4 and the treatment T1 over the treatment T4, and there was no significant difference between the treatments T1, T2, T5, T6, T7 and the two factors T1, T3 as well as the two treatments T3, T4. Significant (P <0.05) for the transaction T2 on the treatments T1, T5, T6, T7 and there were no significant differences between the treatments T2, T3, T4 as well as between the treatments T1, T3, T4, T5, T6, T7.

The improvement in the histological characteristics of the T3, T5, and T2 treatments may be due to the role of organic nano selenium and selenium, which increases the size of the testicles and seminal tubes and increases the treatments of the seminal tubules and the daily sperm production by increasing the secretion of testosterone and thus increasing the efficiency of Sertoli cells and Leyden cells and the diameter of the lumen (Okpi and Udoumoh, 2016) also found that changes in selenium levels (deficiency or excess) can lead to increased apoptosis in the sperm generating cells and thus reduce the fertility of roosters (Qazi et al., 2019). Seminal tubules and daily sperm



production are the results of testosterone enhancement and the efficiency of Sertoli cells (Safaa et al., 2019).

**Table 5.**Effect of dosing of broiler breeder roosters (Ross)with different levels of nano-selenium and organic selenium particles on the histological traits of the testis

Treatments	Averages $\pm$ standard error				
	Thickness of interstitial tissue $\mu$ m	Diameter of seminiferous tubule $\mu$ m	Height of germinal epithelium $\mu$ m	Diameter of blood vessels $\mu$ m	Number of interstitial cells
<b>T1</b>	8.16 $\pm$ 0.90 D	136.84 $\pm$ 3.38 D	64.62 $\pm$ 3.24 D	35.05 $\pm$ 1.39 AB	4.20 $\pm$ 0.37 B
<b>T2</b>	11.41 $\pm$ 0.66 AB	208.79 $\pm$ 3.69 A	76.50 $\pm$ 1.94 BC	35.86 $\pm$ 1.72 A	6.60 $\pm$ 0.50 A
<b>T3</b>	12.58 $\pm$ 0.58 A	202.60 $\pm$ 7.39 AB	77.08 $\pm$ 1.95 BC	30.92 $\pm$ 0.60 BC	5.00 $\pm$ 0.63 AB
<b>T4</b>	9.51 $\pm$ 0.30 CD	177.70 $\pm$ 9.61 C	88.630 $\pm$ 3.32 A	30.39 $\pm$ 1.65 C	5.00 $\pm$ 0.63 AB
<b>T5</b>	10.19 $\pm$ 0.59 BC	185.45 $\pm$ 8.39 BC	78.55 $\pm$ 4.04 B	36.23 $\pm$ 1.59 A	4.40 $\pm$ 0.58 B
<b>T6</b>	9.37 $\pm$ 0.44 CD	129.33 $\pm$ 2.70 D	69.13 $\pm$ 2.84 CD	36.50 $\pm$ 1.68 A	3.80 $\pm$ 0.58 B
<b>T7</b>	8.58 $\pm$ 0.37 CD	134.37 $\pm$ 3.06 D	72.71 $\pm$ 2.46 BCD	38.30 $\pm$ 1.66 A	3.80 $\pm$ 0.48 B
مستوى المعنوية	*	*	*	*	*

\* The averages carrying different letters within the same column differ significantly between them at a level of (P <0.05). Treatments T1, T2, T3, T4, T5, T6, T7 are control treatment, the Dosing of 1 mg/kg organic selenium feed, the Dosing of 1.5 mg/kg organic selenium feed, the Dosing of 2 mg/kg organic selenium feed, the Dosing of 0.5 mg/kg Nano selenium feed, the Dosing of 0.75 mg/kg nano selenium feed, the Dosing of 1 mg/kg nano-selenium feed respectively.

### Liver Histological Test

The histological sections of the liver were studied at the end of the experiment, and it is observed in the first treatment T1, where it showed a normal appearance of the liver, and the histological sections of the liver in treatment T2 were similar to those in the control group as they showed a normal appearance of the central vein, hepatocytes, and sinuses. In treatment T3, it was found that there was slight congestion of the central vein with clear expansion and congestion of the sinusoid, which led to slight compressive atrophy of the hepatocyte cords compared with the control treatment, and in treatment T4, slight congestion of the central vein was found with the development of minor diffuse degeneration of hepatocytes. compared to the control treatment.

As for the nano-selenium treatments, the treatment T5 showed a normal appearance of the central vein, hepatocytes, and sinuses as in the control treatment, but treatment T6 showed congestion of central veins with clear expansion and congestion of the sinuses that led to compressive atrophy. Simple hepatocyte cords and in treatment T7, the liver tissue sections of this group showed central venous congestion and sinusoidal congestion with moderate severity diffuse degeneration of the hepatocellular cords compared to treatment T1, the cytotoxic effect of increasing the concentration may be Selenium in T3, T4, T6, T7 coefficients on cell proliferation is that excessive selenium can lead to cell cycle interruption indicating that selenium deficiency may also restrict cell proliferation by mediating the expression of the cell cycle (Shi et al., 2017). It was also found that the highest concentrations of selenium in chickens were in the blood serum, liver and chest muscles, and they increased with an increase in the level of dietary selenium (0.03 to 1.3 mg/kg feed) and the size of the increase was significantly greater when feeding nano selenium compared to sodium selenite and Animal studies have shown that the liver is the main target organ for selenium toxicity due to the excessive accumulation of absorbed selenium in the liver and the fact that the formation of reactive oxygen species generated by selenium (ROS) are the main mechanisms of selenium toxicity (Mézes and Balogh, 2009), however Addition of 1.20 mg / kg of selenium did not cause signs of toxicity indicating that the optimum range of toxic dietary levels of nano selenium is higher than

that of sodium selenite and the possible cause of higher tolerance to selenium in the form of nano selenium is its high muscle retention rate which may effectively reduce Available selenite to stimulate toxicity and this hypothesis was supported by the study using nanoselenium or intravenous sodium selenate which showed that the percentages of nanoselenium in the whole body were significantly higher than those of selenite (Hu et al., 2012; Pelyhe and Mézes, 2013), and it should be noted that the deposition of selenium in testicular tissue will be different according to its chemical forms, feeding period, animal type, age and physiological conditions. The cause of testicular and hepatic degeneration may be a high concentration of selenium deposited in the liver and testis (Shi et al., 2017). Also, suitable selenium has a specific antioxidant effect while an excess of selenium can halt the cell cycle by increasing the level of ROS (Shi et al., 2017).

## Note

The research is based on the thesis of the first researcher.

## References

- [1] Al-Darraj, Hazem Jabbar and Al-Hayani, Walid Khaled and Al-Hussaini, Ali Sabah. 2008. *Bird's blood cell, Ministry of Higher Education and Scientific Research, College of Agriculture, University of Baghdad.*
- [2] Almansour, M.I., 2009. Histoiological Alterations Induced by Lead in the Testes of Quail Coturnix. *King Saud University, Res.J of Env. Tox* 3:24-30.
- [3] Al-Rawi, Abdul-Jabbar Abdul-Karim and Al-Baghdadi, Muhammad Fawzi Abdul-Ghani and Darraj, Hazem Jabbar. 2011. Genetic and phenotypic features of the penetration of the sperm into the ovum and some characteristics of the semen in white cockerels. *Diyala Journal of Agricultural Sciences*, 100-88: 3.
- [4] Avital-Cohen, N., R. Heiblum, A. Rosenstrauch, Y. Chaiseha, N. Mobarkey, M. Gumulka, and I. Rozenboim. 2015. Role of the serotonergic axis in the reproductive failure associated with aging broiler breeder roosters. *Domest. Aanim. Endocrinol.*, 53:42-51.
- [5] Bancroft, JD., and Marilyn G., (2008). *Theory and practice of histological techniques*. 1st Edn., London, Elsevier Limited. PP: 168-173.
- [6] Beckett, G.J.; Arthur, J.R., 2005. Selenium and endocrine systems. *J. Endocrinol.*, 184, 455–465.
- [7] Duncan, D.B., 1955. Multiple Rang and Multiple F-test. *Biometrics*.11:4-42.
- [8] EI-Sheikh, A.M.H., E.A. Abdalla and M.M. Hanafy., 2010. The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. 2-Immune system and some physiological aspects in bandarh chicks affected by organic selenium. *Egypt. Poult. Sci.*, 30: 517-533.
- [9] Eric B., Thomas G. T., S. Basaria, M. N. Davda, W. Guo; M. Li, J. C. Westfall, H. Bae, V. Gordeuk and S. Bhasin., 2014. Testosterone Induces Erythrocytosis via Increased Erythropoietin and Suppressed Hecpidin: Evidence for a New Erythropoietin/Hemoglobin Set Point. *J GerontolA Biol Sci Med Sci.*, 69(6):725–735.
- [10] Griswold, M. D., 1998. The central role of sertoli cells in spermatogenesis, *Semin. Cell Dev.Biol.*, 9, 411-416.
- [11] Hafemann DG, Sunde RA, Houestra WG. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J Nutr.*,104:580–584.
- [12] Hanafy, M.M., A.M.H. EI-Sheikh and E.A. Abdalla., 2009. The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. 1-The effect of organic selenium (sel-plex P') on productive, reproductive and physiological traits of bandarh local strain. *Egypt. Poult. Sci.*, 29: 1061-1084.
- [13] Hezarjaribi, A., Rezaeipour, V. and Abdollahpour, R., 2016. Effects of intramuscular injections of vitamin E-selenium and a gonadotropin releasing hormone analogue (GnRHa) on reproductive performance and blood metabolites of post-molt male broiler breeders. *As. Pac. J. Reprod.*, 5, 156-160.
- [14] Hosny. N. S., Hashem. N. M., Morsy. A.S., and Abo-elezz. Z. R. 2020. Effects of Organic Selenium on the Physiological Response, Blood Metabolites, Redox Status, Semen Quality, and Fertility of Rabbit Bucks

- Kept Under Natural Heat Stress Conditions. *Front Vet Sci.* 2020; 7: 290. Published online 2020 Jun 12. 10.3389/fvets.2020.00290
- [15] Hu C., Li Y., Xiong L., Zhang H., Song J., Xia M., (2012). Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. *Animal Feed Science and Technology* 177, 204–210. Doi:10.1016/j.anifeedsci.2012.08.010.
- [16] Ihsan, T.T. and G.K. Qader, 2012. Effect of feed supplementation of selenium and vitamin E on production performance and some hematological treatments of broiler. *KSU J. Nat. Sci.*, 15: 46-56.
- [17] Jafarzadeh. H., Allymehr. M., Talebi. A., Asri Rezaei. S. and Soleimanzadeh. A. 2020. Effects of Nano-Selenium and Sodium Selenite on SelP, Gpx4 and SelW Genes Expression in Testes of Broiler Breeder Roosters. *Bulgarian Journal of Veterinary Medicine*, 23, No 2, 218-228.
- [18] Jelkmann, W., 2007. Erythropoietin after a century of research: younger than ever". *Eur. J. Haematol.*, 78: 183–205.
- [19] Kabata-Pendias E., (2011). Trace elements in soils and plants, 4th edn. *CRC Press, Taylor & Francis, Boca Raton*.
- [20] Levander, O. A., 1986. Selenium. Page 209 in Trace Elements in Human and Animal Nutrition. Vol. 2, 5th ed. W. Mertz, ed. *Academic Press, Inc., New York, NY*.
- [21] Li. M., Zhang. Y. & Li. S., (2020). Effects of selenium deficiency on testis development and autophagy in chicks, *Italian Journal of Animal Science*, 19:1, 753-761, DOI: 10.1080/1828051X.2020.1786739.
- [22] Lukusa, K., and Lehloeny, K.C., 2017. Selenium supplementation improves testicular characteristics and semen quality of Saanen bucks. *Small Ruminant Research* <http://dx.doi.org/10.1016/j.smallrumres.2017.04.016>
- [23] Marmiroli N., and Maestri E., (2008). Health implications of trace elements in the environment and the food chain. In: Prasad MNV (ed) Trace elements as contaminants and nutrients: consequences in ecosystems and human health. *Wiley, Hoboken*, pp 23–53.
- [24] Mézes, M., and Balogh K., 2009. Prooxidant mechanisms of selenium toxicity – a review, *Acta Biol. Szegediensis*, 53 (Suppl.1) 15-18.
- [25] Mohapatra, P., RK. Swain, S.K. Mishra, T. Behera, P. Swain, S.S. Mishra; N.C. Behura, S.C. Sabat, K. Sethy, K. Dhama and P. Jayasankar, 2014. Effects of dietary nano- selenium on tissue selenium deposition, antioxidant status and immune functions in layer chicks. *Int. J. Pharmacol.*, 10: 160-167.
- [26] Nishimura, K.; Matsumiya, K.; Tsujimura, A.; Koga, M.; Kitamura, M.; Okuyama, A., 2001. Association of selenoprotein P with testosterone production in cultured Leydig cells. *Arch. Androl.*, 47, 67–76.
- [27] NRC, 1994. Nutrient Requirements of Poultry, 9th rev. ed. National Academy Press, Washington, DC.
- [28] Okpe G.C., and Udoumoh A.F., 2016. Effect of season on structural composition of drake testes and daily spermatozoa production as determined by quantitative testicular histology. *Med. Weter*, 72(10), 627-631.
- [29] Olson, G.E., Winfrey, V.P., Nagdas, S.K., Hill, K.E., Burk, R.F., 2007. Apolipoprotein E receptor-2 (apoER2) mediates selenium uptake from selenoprotein P by the mouse testis. *J. Biol. Chem.*, 282, 12290–12297.
- [30] Oluboyo, A.; Adijeh, R.; Onyenekwe, C.; Oluboyo, B.; Mbaeri, T.; Odiegwu, C.; Chukwuma, G.; Onwuasoanya, U., 2012. Relationship between serum levels of testosterone, zinc and selenium in infertile males attending fertility clinic in Nnewi, south east Nigeria. *Afr. J. Med. Med. Sci.*, 41, 51–54.
- [31] Ottinger, M. A., Abdelnabi, M., Li, Q., Chen, K., Thompson, M., Harada, N., Viglietti-Panzica, and Panzica, G. C., 2004. The Japanese quail: a model for studying reproductive aging of hypothalamic system. *Exp. Gerontol.*, 39, 1679.
- [32] Pelyhe. Cand Mézes. M., 2013. Myths and Facts About the effects of Nano-Selenium in Farm Animals – Mini-Review. *Eur. Chem. Bull.*, 2(12), 1049-1052.
- [33] Qazi I. H., C. Angel., Haoxuan Yang, Evangelos Zoidis, Bo Pan, Zhenzheng Wu, Zhang Ming, Chang-Jun

- Zeng, Qingyong Meng, Hongbing Han, and Guangbin Zhou.2019. Role of Selenium and Selenoproteins in Male Reproductive Function: A Review of Past and Present Evidences. *Antioxidants*, 8(8), 268; <https://doi.org/10.3390/antiox8080268>.
- [34] Remero-Sanchez H, Plumstead P, Leksrisonpong N, Brannan K, Brake J., 2008. Feeding broiler breeder males. 4. Deficient feed allocation reduces fertility and broiler progeny body weight. *Poultry Science*; 87:805-11.
- [35] Ren X-m, Wang G-g, Xu D-q, Luo K, Liu Y-x, Zhong Y-h, CaiY-q. 2012. The protection of selenium on cadmiuminduced inhibition of spermatogenesis via activating testosterone synthesis in mice. *Food Chem Toxicol.*, 50(10): 3521–3529.
- [36] Rizk. Y. S.; Ibrahim. A. F.; Mansour. M. K.; Mohamed. H. S.; El-Slamony. A. E.; and Soliman. A. A. M.2017. Effect of Dietary Source of Selenium on Productiv and Reproductive Performance of Sinai Laying Hens Under Heat Strees Conditions. *Egypt. Poult. Sci. Vol: 37-II*:461-489.
- [37] Safaa. A.M. Ali, El-Wardany. I., Hassan. A.M., and Amal M. H., 2019. Effect of Vitamin E-Selenium Supplementation on some Semen Quality traits of muscovy Drakes. *Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt* 27(2),1627-1636.
- [38] SAS., 2012. Statistical Analysis System, Users Guide. Statistical. Version 9.1 th ed. SAS.Inst. Inc.Cary.N.C. USA.
- [39] Selim, N.A., N.L. Radwan, S.F. Youssef, T.A. Salah Eldin and S. Abo Elwata. (2015). Effect of inclusion inorganic, organic or nano selenium forms in broiler diets on: 2-Physiological, immunological and toxicity statuses of broiler chicks. *International Journal of Poultry Science* 14 (3): 144-155.
- [40] Shi L, Song R, Yao X, Ren Y. 2017. Effects of selenium on the proliferation, apoptosis and testosterone production of sheep Leydig cells in vitro. *Theriogenology*. 93:24–32.
- [41] Shi, L.; Zhang, C.; Yue, W.; Shi, L.; Zhu, X.; Lei, F., 2010. Short-term effect of dietary selenium-enriched yeast on semen treatments, antioxidant status and Se concentration in goat seminal plasma. *Anim. Feed Sci. Technol.*, 157, 104–108.
- [42] Shi.L., Zhao.H., Ren.Y., Yao. X., Song. R., Yue. W., 2014. Effects of different levels of dietary selenium on the proliferation of spermatogonial stem cells and antioxidant status in testis of roosters.*Anim. Reprod. Sci.*, 149, pp. 266-272.
- [43] Surai, P. F., and V. I. Fisinin., 2014. Selenium in poultry breeder nutrition: an update. *Anim. Feed Sci. Technol.*, 191:1–15.
- [44] Vizcarra JA, Kreider DL., and Kirby JD., 2004. Episodic gonadotropin secretion in the mature fowl: serial blood sampling from unrestrained male broiler breeders (*Gallus domesticus*). *Biol Reprod.*, v.70, p.1798-1805.
- [45] Vizcarra JA., Kirby JD., and Kreider DL., 2010. Testis development and gonadotropin secretion in broiler breeder males. *Poult Sci*, v.89, p.328-334.