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Effect of Dosing of Broiler Breeder Roosters (Ross) with different Levels of Nano-selenium Particles and Organic Selenium on Reproductive traits A Thesis Submitted

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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: Significantly excelled ($P<0.05$) 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. Significantly excelled ($P<0.05$) 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 aim of broiler breeder flocks because the number of fertilized eggs determines the profitability of laying hens (mothers 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 et al, 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 (Marmiroli & 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 over 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,

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the study aims to evaluate the use of selenium when administered to broiler breeder 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 was 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) m² 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, where 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, where 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 1 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%,**

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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
Semen Specific Traits
Bomber Size Measurement

A 1.5 mL micro-graduated plastic tube was used to measure the projectile volume.

Sperm Concentration

Sperm concentration was measured using a Haemocytometer, and after completing the count, we apply the following equation (Draji, 2007b):

$$(X / 80) \times 400 \times 400 \times 10 = X \times 20000$$

As:
X = number of sperms estimated in 5 mid-squares.
80 = the number of squares in which the sperms were counted (5 medium squares x 16 small squares).
400 = dilution ratio.
10 = The result is multiplied by this number to represent the number of sperms in 1 ml of semen, since the total area of the square of the number of cells = 1 mm^2 (the large square that contains 25 medium squares) and thus the volume of diluted semen inside this square = 1 mm^2 x 0.1 Ml (height of the solution above the square) = 0.1 mm^3, and this represents the volume of semen in which you counted the sperm, and therefore the total product is multiplied by 10 to represent the number of sperms in 1 mm^3.
400 = (Number of total squares on the slide) 25 medium squares x 16 small squares.
The resulting number will represent the number of sperms in 1 mm^3 of semen, and the result is then multiplied by 1000 to represent the number of sperms in 1 cm^3 (1 ml).

Sperm Motility Estimation

10 micro-liters of semen stored in the incubator at a temperature of 37 ° C are taken and mixed with 200 micro-liters of sodium citrate at a concentration of 2.9% and the mixture is mixed and then the mixture is placed on a glass slide (slide) and then the cover of the glass slide is placed over it and using an optical microscope equipped with a camera is done The individual sperm motility is measured under a force of 40X. As for the collective movement, 10 µL of the same semen is taken and placed on a glass slide (slide) and then placed on top of the glass slide cover and the measurement is done using the magnification force 10x in the microscope (Akhlaghi et al., 2014).

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Estimating the Percentage of Dead Sperm

One drop of semen is placed on a glass slide, and it is mixed with one drop of the eosin-nigrosine dye by the tip of another glass slide, then the mixture is gently pulled by the second end of the glass slide used for mixing to make a smear of the mixture, then the slide is left to dry for a minute One, then read (Lake & Sterwart, 1978). Then the number of dead sperms is calculated and the percentage of dead sperm is extracted according to the following equation:

Dead sperm percentage% = (number of dead sperms) / (total number of sperms) x 100

Salt Resistance Ratio Estimation

Take 10 micro-liters of semen and mix it with 200 microliters of sodium citrate solution at a concentration (2.9%) and place it on a glass slide (slide) and place the slide cover over it and leave for 20 minutes inside the incubator. Tail shattering or explosion (Ansari et al., 2017).

Then, the ratio of the resistance of the sperm wall to salt was determined according to the following equation (Al-Daraji et al., 2002):

Salt-resistant sperm resistance ratio = (number of salt-resistant sperms) / (total number of sperms) x 100

Estimating the Percentage of Deformed Sperm

A drop of semen is mixed with 10 drops of sodium citrate solution with a concentration of 2.9%, taking into account the concentration of semen in the ejaculate before dilution, then add one drop of Fast green fast - Eosin to the mixture, and leave for a minute at a temperature of 37 ºC. A swab of the mixture is taken on a glass slide, and the slide is left to dry. After that, the slide is read using the oil lens of the optical microscope with a magnification of 100 x. The deformed sperm appears in several forms, including pigmentation in the sperm in a transparent green color, the head color is bright red, and the color of the acrosome is light green (Darraji et al., 2002). According to the following equation:

The percentage of abnormal sperms% = (number of deformed sperms) / (total number of sperms) x 100

IVF and Fertility Rate

Females of Lohmann Brown chickens were vaccinated according to the method described by Al-Darraji (2007a) and Al-Darraji et al. (2011), which is summarized as follows: The first person carries the female between his left forearm and chest. He holds the legs of the female with both hands so that the head is under the arm. The second person presses on the abdomen and above the orifice of the collector, to turn the collector and protrude the vaginal opening. The second person inserts the insemination syringe deep (4 - 6 cm) inside the vagina, then the first person relieves pressure on the abdomen, to restore the vagina to its normal position, then injects the semen in the plastic insemination syringe into the vagina.

A dose (0.05 ml) of semen was used to inseminate each female, and the artificial insemination process was performed at seven in the evening, to ensure that all females had laid eggs, and to avoid the presence of an egg with a hard shell in the uterus when performing IVF.

The fertilized eggs were placed in the hatchery belonging to the Department of Animal Production / College of Agriculture / Al-Qasim Green University, where the fertilized eggs were collected, in the five days following the second day of the pollination process, and placed in the hatchery. And after the lapse of (7 days) after laying eggs in the hatchery, the eggs were examined by fracturing to calculate the fertility rate according to the following equation:

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Fertility percentage = (number of fertilized eggs) / (total number of eggs) x 100

Statistical Analysis

The data were analyzed using a completely random design (CRD) to study the effect of the studied parameters 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:

$$Yij = \mu + Ti + eij$$

As:
Yij: the view value j of transaction i.
μ: the general mean of the trait.
Ti: the effect of i treatment (as the study included the effect of 7 aforementioned treatments).
eij: the normally distributed random error with a mean equal to zero and a variance of σ2e.

Results and Discussion

1. Semen Quality Treats

Table (2) indicates the effect of the studied traits on the trait of the semen during the study period of (1-8) weeks after that the total average of it was taken, where it is noticed that there are no significant differences between the experimental treatments in the size of the Sperm, but in the sperm concentration a significantly excelled is observed (P <0.05) for treatment T5 compared to the rest of the coefficients, T3 and T2 excelled on treatment T1. There was no significant difference between the treatments T2, T3 and T4, T6, T7 on the one hand and the treatments T4, T6, T7 and the treatment T1 on the other hand, either in the ratio of in live sperm, significantly excelled (P<0.05) was observed for treatment T7 compared to the rest of the experimental treatments, and treatment T6 outperformed the two treatments T2 and T1. No significant difference was obtained between treatment T7 and treatment T6, as well as treatment T6 and treatments T4, T5, and T3 as well as between treatments T4, T5, and T3. And the two treatments T1, T2, and in the proportion of dead life, the two treatments T1, T2 were significantly excelled (P<0.05) on the two treatments T6, T7 and the treatments T3, T5, T4 on treatment T7. The statistical analysis showed no significant differences between the two treatments T1, T2 and the treatments T3, T4, T5, as well as between the treatments T3, T4, T5, and T6, and also between the two treatments T6, T7, as for the collective movement For life, a significantly excelled (P<0.05) was observed for treatment T5 compared to the rest of the study treatments, and a significantly excelled for treatment T2 on the treatments T3, T4, T6, T7 and T1, and for the two treatments T6, T7, on the treatments T3, T4, and T1. Control T1 did not show significant differences between the two treatments T3 and T4 as well as the two treatments T6 and T7, and the significantly excelled (P<0.05) of treatment T5 continued in the individual movement of the sperm compared to the rest of the experimental treatments, and the two treatments T2 and T7 excelled on the treatments T1, T3, T4, T6. Treatment T6 excelled the treatments T3, T4, and T1, and the two treatments T3 and T4 excelled on the first treatment T1. The table did not show significant differences between the two treatments T2, T7, and also the two treatments T3 and T4. As for the salt-resistant sperm ratio, a significant superiority was found (P<0.05). For all the treatments of nano-selenium and organic selenium compared to the control treatment T1, there were no significant differences between the treatments T3, T4, T5, T6, T7, and T2, and in the percentage of sperm abnormalities, no significant difference was obtained between the studied treatments. The treatment T5 excelled in sperm concentration and group and individual movement and the treatment T7 excelled in the proportion of live sperm and its improvement in the proportion of dead sperm, as well as the superiority of all treatments of nano selenium in the proportion of sperm resistance to salt maybe since nano-selenium, is similar to its role in the formation of steroids. Spermatogenesis and in rooster fertility where deficient selenium nutrition (0.02 ppm yeast) has significantly reduced the number of spermatogenic cell lines and sperm maturation (Kaur and Bansal, 2005), (Qazi et al., 2019) also confirmed these results that selenium may have a significant effect on the spermatogenesis process in roosters, and a fine balance in the regulation of oxidation and reduction is necessary for the optimal functioning of cells and it has been proven that changes in selenium levels may increase the oxidative state and can lead to oxidative stress, which negatively affects rooster fertility by affecting the biological processes

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and activity of antioxidant enzymes (Qazi et al., 2019). It should be noted that the main testicular selenoprotein is GPx4 (glutathione peroxidase 4) (Ahsan et al., 2014). Selenium is an essential component of the active site of GSH-Px (glutathione peroxidase) (Yoon et al., 2007), an enzyme involved in regulating hydrogen peroxide and lipid peroxide levels (Arthur, 2000). Selenium is also known as an essential component of testicular function and sperm motility in Chickens where selenium deficiency causes testicular dysfunction such as atrophy of the seminiferous tubules, abnormal sperm formation, immaturity of sperm, and decreased testicle size (Lin et al., 2005: Jerysz and Lukaszewicz, 2013), moreover, testicular selenium deposition may be related to testicular antioxidant system which has a protective effect on testicular spermatogenesis and dietary selenium works through enzyme (GSH-Px or phospholipid hydroperoxide glutathione peroxidase PHGPx) to protect germ cells and proteins and membranes from oxidative stress (Surai and Fisinin, 2014), and that sperms are more sensitive to oxidative damage due to the biochemical composition of sperm as they contain a higher percentage of polyunsaturated fatty acids and lower concentrations of antioxidant cytoplasmic enzymes compared to somatic cells (Sánchez- Gutiérrez et al., 2008), therefore, in conditions of increased oxidative stress induced by ROS (Reactive Oxygen Species) and with age of roosters, the integrity of the plasma membrane of sperm is affected as well as damage to sperm DNA by causing disturbances in chromatin condensation and the process of chromatin condensation is a step. Fundamental to both sperm maturation and fertilization capacity (Sánchez-Gutiérrez et al., 2008). In this regard, it has been demonstrated that selenium deficiency can lead to impaired chromatin intensification and reorganization processes by eliciting oxidative stress, and leads to poor sperm quality and reduced fertility in roosters (Qazi et al., 2019). The use of selenium significantly improved fluid characteristics. Seminal effects such as movement and focus, and reduced levels of free radicals and lipid peroxidation, and the rate of apoptosis in the testes (Kaur et al., 2018). As shown by Qazi et al. (2019), selenium affects reducing lipid peroxidation and improving the antioxidant state in the sperm plasma of roosters. Which may have translated into sperm that enhanced concentration and motility and reduced the proportion of dead sperm (Ebeid, 2012). This study agrees with (Rizk et al., 2017) who found a significant increase in sperm concentration, group and individual motility of sperm, significantly for roosters fed with nano selenium followed by organic selenium treatments compared with control treatment where nano selenium led to a significant decrease in dead sperms and animals. Distorted spermatids (Rizk et al., 2017). The exact mechanism of decreasing semen quality with age is not yet clear. The process of oxidation of polyunsaturated fatty acids due to aging may be responsible for vitality, motility, and reduced fertilization capacity in sperm (Rostato et al., 2006). In the proportions of different lipids, components (free fatty acids and cholesterol esters increase continuously with age) in spermatozoa can be associated with decreased fertility of roosters, and it has been proven that reproductive performance is expressed as (decreased sperm concentration, decreased motility, and vitality and increased sperm abnormalities) gradually decreased with Aging in broiler roosters (Alavi et al., 2020), and thus the use of nano selenium as an antioxidant plays a vital role in protecting cells from ROS by reducing free radicals and inhibiting peroxidation (Grazul-Bilska et al., 2009). Selenium has a pronounced effect on the reproductive performance of roosters (Behne et al., 1982), and can maintain normal testicular functions and cell structure (Ahsan et al., 2014), in addition to sperm motility and function (Ahsan et al., 2014), and Sertoli cell count and its involvement in improving reproductive performance of roosters not only by enhancing semen quality but also by suppressing free radicals (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 reproductive traits for the weeks of study

Treatments	Averages ± standard error							
	Semen volume	**Semen concentration X10^9**	**Live sperm %**	**dead sperm %**	**Mass motility %**	**Individual motility %**	**Salt resistant sperm %**	**Deformation spermatozoa ratio %**
T1	0.38 ± 0.02	26.85 ± 1.03 C	90.38 ± 0.61 C	9.16 ± 0.61 A	78.29 ± 0.58 E	79.50 ± 0.33 E	88.41 ± 1.35 B	8.95 ± 0.78
T2	0.48 ± 0.05	32.57 ± 2.53 B	90.78 ± 0.18 C	9.21 ± 0.18 A	90.83 ± 0.55 B	88.87 ± 0.69 B	91.58 ± 0.36 A	7.99 ± 0.28
T3	0.47 ± 0.01	32.83 ± 1.66 B	91.80 ± 0.95 BC	8.19 ± 0.95 AB	86.08 ± 0.37 D	84.95 ± 0.46 D	93.29 ± 0.36 A	7.12 ± 0.87
T4	0.40 ± 0.004	31.74 ± 1.85 BC	91.93 ±0.82 BC	8.06 ± 0.82 AB	85.25 ± 0.50 D	84.58 ± 0.46 D	91.66 ± 0.35 A	6.53 ± 0.96

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T5	0.50 ± 0.04	39.60 ± 1.77 A	92.01±0.13 BC	7.98 ± 0.13 AB	92.41 ± 0.29 A	92.16 ± 0.27 A	92.74 ± 0.23 A	7.19 ± 0.74
T6	0.42 ± 0.01	32.00 ± 0.96 BC	92.88 ±0.28 AB	7.11 ± 0.28 BC	88.41 ± 0.20 C	87.08 ± 0.56 C	91.74 ± 0.18 A	6.56 ± 0.46
T7	0.41 ± 0.04	29.52 ± 1.59 BC	94.10 ±0.62 A	5.89 ± 0.62 C	89.25 ± 0.33 C	89.50 ± 0.07 B	91.98 ± 0.55 A	6.43 ± 0.48
The level of morale	n.s	*	*	*	*	*	*	n.s

* 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.

Fertility Rate

Table (3) shows the effect of nano selenium and organic selenium on the fertility rate of the study treatments, where the nano selenium treatments were significantly superior (P <0.05) over the organic selenium and control treatments, as well as the organic selenium treatments significantly outperformed the control treatment. The significant increase in fertilization rate in the treatment of nano-selenium and organic selenium may be due to its role due to selenium, which improved reproductive characteristics, which increased the ability of sperm to fertilize, as it was found that the use of 0.60 mg nano selenium/kg of feed from roosters' diets improved its fertility without any side effects Where selenium plays an important role in antioxidants and protects different systems from damage caused by free radicals and oxidative stress of sperms. Broiler breeder from the age of 40 weeks onwards (Alavi et al., 2020). Adequate levels of selenium for the reproductive organs of roosters are required mainly for normal sperm synthesis, maturation, motility, and functions, in addition to its role in enhancing the antioxidant activity of glutathione peroxidase (Ahsan et al. 2014), as well. Continuous intake of dietary selenium has been included in enhancing the antioxidant activity of glutathione peroxidase, thus improving male fertility. (Irvine, 1996).

Table 3. Effect of dosing of broiler breeder roosters (Ross) with different levels of nano-selenium and organic selenium particles on fertility

Treatments	Averages ± standard error
	Fertility rate%
Control treatment	88.45 ± 0.02 C
Organic selenium treatments	93.84 ± 0.12 B
Treatments of nano-selenium	96.50 ± 0.28 A
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, 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.

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