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The Impact of Organic Selenium Supplementation on Rooster Semen Quality in Liquid Condition

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Abstract

This research was carried out to investigate the effects of various levels of dietary organic selenium supplementation (0, 0.1, 0.2 and 0.3 mg/Kg) on reproductive traits of rooster. Semen was collected using abdominal massage and samples were mixed together. Sperm characteristics including percentage of motile, viable and abnormal sperms were assessed. This experiment was carried out based on a completely randomized design. Results showed that the organic selenium supplementation had significant effects on sperm motility and viability in liquid condition ($P<0.05$). 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 in comparison with the control group. Birds were fed with organic selenium had lower abnormal sperm percentage in comparison with the control group ($P<0.05$). Supplementing diets with 0.1, 0.2 and 0.3 mg/Kg organic selenium had no effect on semen concentration, pH and volume ($P>0.05$). In conclusion, the use of organic selenium supplementation (0.3 mg/Kg) in diet of rooster could be recommended to improve semen quality.

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Introduction

Selenium (Se) is an essential element that has an important role in animal reproduction (Surai, 2002). There are at least 25 selenoproteins in human and animal body (Kryukov *et al*., 2003). Selenoproteins regulate various physiological functions such as antioxidant protection, redox regulation of gene expression, thyroid metabolism and sperm structure integrity maintenance. Selenium is an antioxidant, which may protect sperm membranes from toxic oxygen metabolites and therefore, enhances semen quality and fertilizing ability of chickens (Ebeid, 2009). Sperm cells have high concentration of long chain polyunsaturated fatty acids within the phospholipids and therefore, are prone to peroxidation damage (Poulos *et al*., 1973).

Spermatozoa and seminal leukocytes produce high amount of reactive oxygen species (ROS) that can reduce the viability and fertility of spermatozoa (Alvarez and Story, 1992). ROS changes the spermatozoon cytoskeleton and axoneme which leads to reduced sperm motility (De Lamirande and Gagnon, 1992), inhibited sperm-oocyte fusion (Aitken et al., 1989) and decreased fertility (Wishart, 1984). Free radicals can also attack the DNA within the sperm nucleus and enhance DNA fragmentations (Lopes *et al*., 1998) and such damages to the genome can reduce fertility (Roberts, 1998). Donoghue and Donoghue, (1997) reported that the addition of the antioxidants such as vitamin E, butylated hydroxyl toluene (BHT), and 4- hydroxy- 2, 2, 6, 6- tetra-methylpiperidine- 1-oxyl (Tempo) to extended turkey semen improves sperm survival, membrane integrity and reduces the loss of motility after 48 h storage. Ebeid (2009) found that supplementing dietary organic selenium improved semen quality characteristics such as sperm count and motility and reduced the percentage of dead sperm and enhanced the antioxidative status of seminal plasma of cockerel. The objective of the present study was to investigate the effect of supplemental dietary organic selenium on semen quality of rooster in liquid condition.

Materials and Methods

A total of 64 roosters (Ross 308) at 44 wks of age were selected and randomly assigned to 4 treatments. Each treatment was included 4 replicates of 4 roosters. The roosters were kept in wire cages (100×120 cm). The photoperiod was 16 h of light and 8 h of dark throughout the experiment. The experimental diets were formulated to meet or exceed the nutrient requirements recommended by National Research Council (NRC, 1994). Birds were fed the same basal diet but supplemented with organic selenium at 0, 0.1, 0.2 and 0.3 mg/Kg from 44 to 48 wk of age (Table 1). Organic selenium was supplied as Se-enriched yeast in the form of selenomethionine (Sel-Plx, AlltechInc, Nicholasvill, USA). The experimental diets were in mash form and drinking water provided *ad libitum*. Beginning at 42 wk of

age, all roosters were trained to the semen collection process using the abdominal massage method for two weeks (Donoghue and Wishart, 2000).

Table 1. Ingredients and main nutrients composition of the basal diet

Ingredients	Percent
Corn	75.47
Soybean meal (44% CP)	15.00
Wheat bran	6.00
Dicalcium phosphate	1.32
Limestone	1.30
Salt	0.41
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Calculated composition	
Metabolizable energy (Kcal/Kg)	2957
Crude protein (%)	13.45
Calcium (%)	0.85
Available phosphorus (%)	0.38
Sodium (%)	0.18
Lysine (%)	0.62
Methionine (%)	0.24
Methionine+ cysteine (%)	0.45

[1]Each kg of vitamin premix contained: Vitamin A, 3,500,000 IU; Vitamin D_3, 1,000,000 IU; Vitamin E, 9000 IU; Vitamin K_3, 1000 mg; Vitamin B_1, 900 mg; Vitamin B_2, 3,300 mg; Vitamin B_3, 5,000 mg; Vitamin B_5, 15,000 mg; Vitamin B_6, 150 mg; Vitamin B_9, 500 mg; Vitamin B_{12}, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg.

[2]Each kg of the mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg.

Commencing from 44 wks of age, semen was collected from roosters once weekly for 4 wks. Raw semen was diluted 1:1 with Beltsville Poultry Semen Extender (BPSE). After dilution, semen samples were transferred to culture plates and sperm characteristics including motile, viable, and abnormal percentages, semen pH, volume and concentration were assessed. Semen volume was determined using a scales glass and pH measured using of P731 pH meter (Elster Microcomputer, Germany). Sperm concentration was determined using the hemocytometer procedure (Bakst and Cecil, 1997). Total sperm cells produced/rooster were calculated as sperm cells/mL of semen volume. The percentage of motile spermatozoa was determined using compound microscope at 10 × magnification after placing a cover slip over 2-3 mm drop of semen on a warmed microscope slide (Biswas *et al*., 2009).

Sperm viability was determined using the eosin staining method as described by Ozkoca (1984). The staining solution was conducted by adding 2 g of eosin stain and 3 g of sodium citrate into distilled water. The solution was filtered with a paper filter (Whatman Inc, Clifton, NJ) before being used. The staining was performed with the addition of 1 drop of fresh semen onto 2 drops of staining solution on a microscope slide. Using another slide, a smear was made and allowed to dry. Unstained (intact) and red-colored (with damaged membranes) spermatozoa were counted as a counter stain. Dead spermatozoa retained more stain and appeared dark, whereas the viable ones appeared clear. The percentage of abnormal spermatozoa was also evaluated in the same sample by examining the morphology of a total count of 100 spermatozoa.

Statistical Analysis

The data obtained from the experiment were analyzed using SAS (SAS Institute, 1999) statistical programwith the ANOVA. Significantdifferences among treatment means were separated using Duncan's multiple range test with a 5% probability (Duncan, 1955).

Results

Effect of organic selenium on sperm motility is shown in Table 2. The organic selenium supplementation increased sperm motility during liquid storage ($P<0.05$). Using organic selenium at the level of 0.3 mg/Kg sperm motility significantly improved at the time of storage; 4, 8, 12 and 24 h in comparison with the control group. Effect of organic selenium on sperm viability is presented in Table 3. Organic selenium supplementation had a significant effect on sperm viability at the time of storage, 4, 8, 12 and 24 h ($P<0.05$). The highest and the lowest sperm viability at different storage time were obtained in the birds that received organic selenium and control diet, respectively. Effect of organic selenium on sperm abnormality is shown in Table 4. Supplementing the roosters diets with organic selenium had a significant effect on lowering the abnormal sperm percentage during storage time compared to the roosters in the control group ($P<0.05$). The highest abnormal sperm percentage was recorded in the control group. Effect of organic selenium on semen characteristics are shown in Table 5. Semen volume, pH and sperm concentration did not differ statistically between dietary treatment groups ($P>0.05$).

Table 2. Effect of different levels of organic selenium on sperm motility (%) at different storage times

Treatment	Storage times			
Se (mg/Kg)	4 h	8 h	12 h	24 h
0	77.17^{b}	69.22^{b}	37.11^{b}	15.28^{b}
0.1	77.74^{b}	69.90^{b}	37.02^{b}	18.04^{a}
0.2	78.15^{b}	69.01^{b}	39.18^{b}	19.32^{a}
0.3	81.25^{a}	73.70^{a}	42.13^{a}	20.70^{a}
SEM	0.71	0.54	0.60	0.39
P-value	0.04	0.04	0.03	0.03

^{a,b} Mean values in each column with different superscript letters were significantly different (P<0.05).

Table 3. Effect of different levels of organic selenium on sperm viability (%) at different storage times

Treatment	Storage times			
Se (mg/Kg)	4 h	8 h	12 h	24 h
0	78.93^{b}	71.12^{b}	39.09^{b}	18.23^{b}
0.1	80.02^{ab}	72.36^{b}	40.76^{b}	22.43^{a}
0.2	80.13^{ab}	71.20^{b}	41.21^{b}	23.13^{a}
0.3	82.11^{a}	75.74^{a}	44.01^{a}	23.79^{a}
SEM	0.76	0.74	0.34	0.92
P-value	0.03	0.03	0.02	0.04

^{a,b} Mean values in each column with different superscript letters were significantly different (P<0.05).

Table 4. Effect of different levels of organic selenium on sperm abnormal (%) at different storage times

Treatment	Storage times			
Se (mg/Kg)	4 h	8 h	12 h	24 h
0	11.76^{a}	18.21^{a}	29.87^{a}	39.56^{a}
0.1	8.18^{b}	16.11^{ab}	25.54^{b}	35.88^{b}
0.2	8.30^{b}	13.61^{b}	24.11^{b}	35.02^{b}
0.3	8.18^{b}	14.34^{b}	24.08^{b}	33.48^{b}
SEM	0.45	0.53	0.67	0.83
P-value	0.04	0.04	0.03	0.04

^{a,b} Mean values in each column with different superscript letters were significantly different (P<0.05).

Table 5. Effect of different levels of organic selenium on semen characteristics

Treatment	Volume (mL)	Concentration ($n \times 10^9$/mL)	pH
Se (mg/Kg)			
0	0.52	2.76	7.15
0.1	0.52	2.50	7.11
0.2	0.50	3.03	7.08
0.3	0.50	2.90	7.12
SEM	0.01	0.03	0.08
P-value	0.12	0.23	0.19

Discussion

Selenium is an important element for male fertility (Behne *et al*., 1986; Hansen and Deguchi, 1996). Mammalian (Kelso *et al*., 1997) and avian (Cerolini *et al*., 1997; Surai *et al*., 1997) spermatozoa have high amount of long chain polyunsaturated fatty acid (PUFA) within the membrane phospholipids. The high level of PUFA can enhance peroxidative damage to spermatozoa membranes and leads to decreased fertility (Wishart, 1984). The peroxidation causes undesirable changes in the structure of the acrosomal section of the spermatozoa and consequently reduces motility and viability of spermatozoa (Dimitrov *et al*., 2007). Lipid peroxidation produces various reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide which can cause oxidative damage to liver, kidney, brain, lung and are also responsible for sperm dysfunction (Hsu *et al*., 1998) in humans (Aitken *et al*., 1989), cattle (Beconi *et al*., 1991), rats (Shang *et al*., 1999), chickens and turkeys (Surai *et al*., 1998). As for the last one, lipid peroxides accumulate in the plasma membrane of the sperm cells and therefore, spermatozoa cannot penetrate into oocyte and this could be the reason for reduced fertility (Aitken *et al*., 1989). Consequently, an efficient antioxidant compounds is required to protect sperm membranes against peroxidative damage (Surai, 2002; Eid *et al*., 2006). Dietary Se deficiency decreases the number of normal sperm cells, motility and fertilizing capacity in chickens, turkeys and ducks (Surai *et al*., 1998). Edens (2002) found that, when 0.2 mg/kg organic Se was added in the cockerel's diet, the percentage of normal spermatozoa were increased. These results obtained in this study were also in agreement with those reported by Ebeid (2009) indicating that using organic Selenium enhanced sperm cell motility and reduced the percentage of dead sperm of a cockerel. Selenium has proven antioxidant properties and therefore, could reduce lipid peroxidation and preserve sperm cells durationstorage.

Conclusion

Present results showed that dietary organic selenium supplementation at the level of 0.3 mg/Kg significantly improved rooster sperm motility and viability during liquid storage. However, further work is essential to confirm and extend these findings.

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