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The effect of dietary organic selenium on reproductive performance of broiler breeder roosters under dexamethasone induced stress

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ABSTRACT

Stress has deleterious impact on semen quality and fertility of roosters. This study was investigated to know whether dietary supplementation of organic selenium (oSe) could improve semen quality and fertility of male broiler breeder under dexamethasone (Dexa) induced stress. Forty broiler breeder roosters (64 week of age) were randomly allotted to four groups (10 roosters/group) and fed a standard diet supplemented with different levels of oSe during 10 successive weeks of the experimental period. To induce stress, the birds received injections of 2 mg/kg BW of Dexa during weeks 5 and 6 of the experiment, in one-day-intervals manner. The roosters were not treated with Dexa and oSe (negative control; NC), or treated with Dexa and different levels of oSe including 0 (positive control; PC), 0.30 (Se30+Dexa) or 0.45 (Se45+Dexa) mg/kg diet. Body weight was measured weekly and semen quality parameters and fertility were evaluated every two weeks. Except for seminal volume and total sperm production which was not affected by the treatment, body weight, semen quality parameters, total antioxidant capacity (TAC) and malondialdehyde (MDA) in seminal plasma were influenced by interactive effect of treatment and time ($P < 0.05$). Dexamethasone injection adversely affected semen quality parameters (semen concentration, motility and plasma membrane integrity) in PC group compared to NC group ($P < 0.05$); however, dietary supplementation of oSe ameliorated these negative impacts in Dexa-treated roosters ($P < 0.05$). Fertility was also improved by dietary supplementation of oSe compared to control groups ($P < 0.05$). These results indicate that although induction of stress have negative effects on rooster semen quality parameters, dietary inclusion of oSe may exert beneficial impact on mitigating the harmful effects of stress on semen quality and fertility rate of broiler breeder roosters.

1. Introduction

Fertility is one of the main influential factors tightly associated with economic outcome in poultry flocks that is influenced by several variables such as breed, nutritional quality, age of the flock and the quality of inseminated semen [1]. Among factors involved in fertility rate, age-related variables such as increase in body weight and oxidative stress, low circulating testosterone, reduction in reproductive behavior, low sperm production and poor sperm quality have the highest impact on infertility commonly reported following the pick of egg production of the breeder roosters.

Fertility of roosters, however, is impressed by the level of the stress that they exposed and may improve by supplementation of anti-oxidative components. For instance, Eid et al. [2] showed that addition of 200 mg vitamin E/kg diet improved semen quality when cockerels injected by dexamethasone (Dexa), as the components commonly used for induction stress. In this line, there are several studies focused on supplementation of antioxidative components, both in diet and in sperm extender, to mitigate adverse effect of oxidative stress, and increase fertility rate in roosters [3–6]. Selenium (Se) is an essential element for poultry nutrition deeply influences productive and reproductive performance of breeder flocks [7]. By participating in the structure of several crucial enzymes and selenoproteins, Se exerts its anti-oxidative, anti-

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inflammatory, and anti-viral properties [8,9]. It has a direct contribution in the glutathione peroxidase (GSH-Px) structure, and hence, decreases oxidative damages [10] and improves hatchability of fertile eggs [11].

Although Se is implemented in both organic and inorganic forms, several undesirable characteristics of inorganic source, such as low bioavailability and efficiency, toxicity, poor retention, interactions with other minerals, and poor ability to maintain Se reserves in the body [12] has increased the welling for supplementation of organic Se (oSe) to diet. Usually, a 0.30 mg Se/Kg diet with at most 0.50 mg Se/kg diet is recommended to exert its beneficial effect and to prevent toxicity, respectively [13]. Dietary implementation of oSe instate of sodium selenite (an inorganic Se) has shown to increase egg production [14], fertility and hatchability [11]. Recently, a new stable oSe source which is a selenomethionine hydroxyanalogue (2-hydroxy-4-methylselenobutanoic acid) or HMSeBA (Selisseo®) has been developed to use instead of Selenomethionine (SeMet) that in purified form is unstable and easily oxidized. Higher bioavailability of the Seleno-amino acid speciation compared to other organic or mineral forms has been previously confirmed [12,15,16]. This lowers the amount of dietary Se needed to be supplemented in diet.

Based on the literatures, dietary implementation of Se may improve reproductive performance of avian flocks, mainly through its anti-oxidative properties and lowering oxidation level in spermatozoa. However, there is no conclusive information on the effect of long-term utilization of oSe on semen quality and fertility of the roosters under acute stress induction. Therefore, this study was conducted to clarify the role of a relatively long-term dietary oSe on semen quality attributes and fertility rate of broiler breeder rooster under Dexa induced stress.

2. Materials and methods

The experiment was carried out at Poultry Research Farm in the Animal Science Department of the Campus of Agriculture and Natural Resources, University of Tehran, Karaj, IRAN. All procedure conducted on the rooster was approved by Ethical Committee of Animal Science Department.

2.1. Chemicals

All chemicals, except for Selisseo® (Adisseo Co., France), were purchased from Merck Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Birds' management and experimental design

Forty mature Ross 308 broiler breeder roosters (weighting 5445.66 ± 92.99 g) were selected at 64 weeks of age from a commercial flock and were kept in individual pens (1.25 m × 1.25 m) under a controlled environment (15L:9D photoperiod regime and 20 ± 2 °C ambient temperature). Before starting the experiment, the roosters were habituated with the experimental condition and abdominal semen collection [17] for two weeks (64 and 65 weeks of age). The roosters were then divided randomly into four experimental groups (10 roosters/group) and either not treated with Dexa and Se (negative control; NC), or treated with Dexa and different levels of Se including 0 (positive control; PC), 0.30 (Se30+Dexa) or 0.45 (Se45+Dexa) mg/kg of diet, during 10 successive weeks of the experimental period (66–75 weeks of age). In this study, hydroxyselenomethionine, as the source of oSe, was added to a basal diet (Table 1). The birds were fed by a standard basal diet (150 g/bird/day) recommended by Ross 308 breeding guideline (Aviagen, USA). To induce acute stress, 2 mg Dexa/Kg of BW were administrated subcutaneously during week 5 and 6 of the experiment in one-day-intervals manner, while similar volume of saline was injected to the NC roosters. Body weight was measured weekly and semen quality characteristics were evaluated every two weeks, during the experimental period. Fig. 1 shows an outline of the experimental design including duration of Se supplementation, times of stress induction as well as times of semen sampling and artificial insemination.

Table 1
Ingredients and chemical composition of the diets fed to broiler breeder roosters and hens (As fed basis).

Ingredient	Amount (%)	
	Rooster	Hen
Corn	70.64	68.93
Soybean meal, 44% CP	6.50	17.88
Wheat bran	19.275	4.375
Dicalcium phosphate	0.78	0.73
Mineral oyster shall	1.45	6.98
Common salt	0.33	0.34
NaHCO3	0.10	0.10
Mineral premixes[a]	0.25	0.25
Vitamin premix[b]	0.25	0.25
DL-Met, 99%	0.10	0.16
L-Lysin	0.02	0
Phyzyme TPT	0.005	0.005
K2CO3, 56.5%	0.3	0
Total	100	100
Calculated nutrient content		
AME (kcal/kg)	2800	2800
CP (%)	11.5	14
Calcium (%)	0.9	3
Available phosphorus (%)	0.35	0.34
Sodium (%)	0.18	0.18
Digestible Lysine (%)	0.24	0.64
Digestible Methionine (%)	0.28	0.37
Digestible Met + cys (%)	0.47	0.57
Digestible Threonine (%)	0.36	0.47

[a] Provides (per kg of diet): Choline (C5 H14 N O), 300 mg; iron (FeSO4·7H2O), 50 mg; manganese (MnSO4·H2O), 120 mg; Zn (ZnO), 110 mg; copper (CuSO4·5H2O), 10 mg; selenium (Na2SeO3), 0 mg; iodine (KI), 2 mg.
[b] Provides (per kg of diet): vitamin A (retinyl acetate), 11,000 IU; vitamin D3 (cholecalciferol), 3500 IU; vitamin E (DL-α-tocopheryl acetate), 150 IU; vitamin K3 (menadione), 5.0 mg; vitamin B1 (thiamin), 3.0 mg; vitamin B2 (riboflavin), 12 mg; vitamin B3 (niacin), 55 mg; vitamin B5 (Dpantothenic acid), 15 mg; vitamin B6 (pyridoxine), 4 mg; vitamin B9 (folic acid), 2 mg; vitamin H2 (biotin), 0.25 mg; vitamin B12 (cobalamin), 0.03 mg.

2.3. Gross evaluation

During the 66–75 weeks of age, semen samples were collected from all the roosters in two weeks intervals. To measure seminal volume, a graduated microtube was used. Semen concentration was measured by adding diluted semen (1:200 in distilled water) to a Neubauer chamber using a light (Axiostar Plus; Zeiss, Germany) microscope (400 × magnification). Total sperm production was calculated by multiplying ejaculate volume and semen concentration. For measuring sperm motility, diluted semen (1:20 in Lake extender) was placed on a pre-warmed microscope slide (37 °C) and covered with a coverslip. Motile sperm were counted in five microscopic fields (400 × magnification) using light microscope (Axiostar Plus; Zeiss, Germany) and the average of them is reposted as total motility [18]. Sperm plasma membrane integrity was evaluated by an eosin-nigrosin staining method. For this test, 10 μL of stain was mixed with 10 μL of diluted semen (1:20 in Lake extender), and the mixture was then spread over the slide using a clean slide. After drying, the plasma membrane integrity was examined using light microscope (Axiostar Plus; Zeiss, Germany) at 1000 × magnification. The unstained sperms were considered as

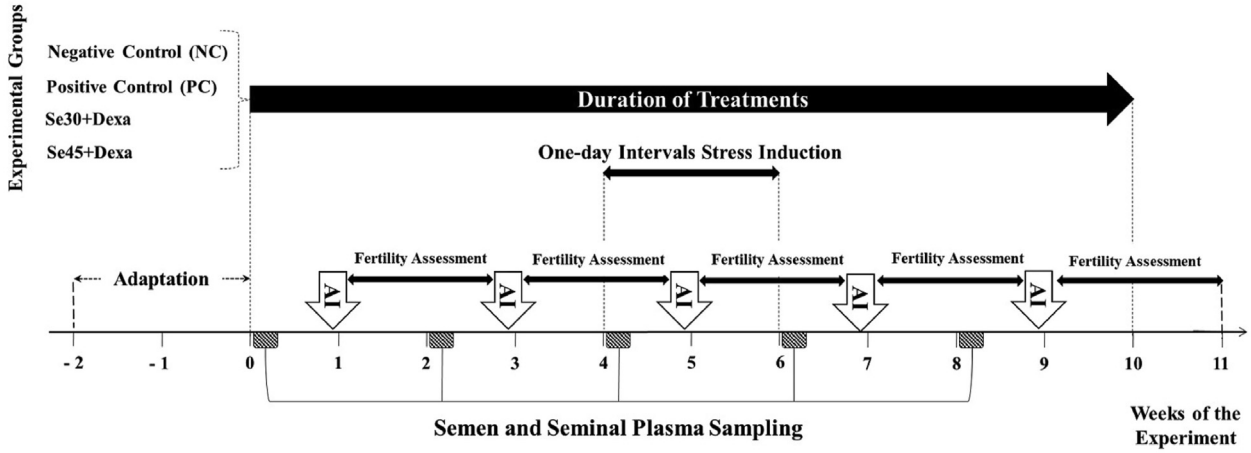

Fig. 1. An outline of the experimental design indicating initiation of dietary selenium (Se) supplementation and times of acute stress induction (subcutaneous injection of 2 mg dexamethasone (Dexa)/Kg BW).
Note: before starting the experiment, the roosters were habituated with the experimental condition and abdominal semen collection for two weeks (64 and 65 weeks of age). The roosters were then divided randomly into four experimental groups (n = 10 rooster per group kept in individually pens) and either not treated with Dexa and Se (negative control; NC), or treated with Dexa and different levels of Se including 0 (positive control; PC), 0.30 (Se30+Dexa) or 0.45 (Se45+Dexa) mg/kg of diet for 10 successive weeks (66–75 weeks of age).

sperm with integrated plasma membrane [5,19]. For evaluating plasma membrane functionality hypo-osmotic swelling (HOS) test was used [20]. Ten microliters of semen sample were mixed with 500 μL of hypo-osmotic solution (adding sodium citrate (1% w/v) to distilled water, Osmolarity: 100 mOsm/kg), and then incubated at 37 °C for 30 min. After incubation, the percentage of spermatozoa with coiled mid-pieces and tail segments were determined as spermatozoa with functional plasma membrane using light microscopy (Axiostar Plus; Zeiss, Germany) at a 1000 × magnification [6,21].

2.4. Total antioxidant capacity (TAC) and malondialdehyde (MDA) in seminal plasma

Following centrifugation of collected semen at 1500 × g (18 °C) for 15 min, seminal plasma samples were stored at −20 °C until analysis. Seminal plasma TAC was determined by a colorimetric enzymatic method [22] using a commercial kit (Randox, United Kingdom) and automated analyzer (Alcyon 300i Biochemistry Analyzer, USA). Concentration of MDA, as an index of lipid peroxidation, was measured using the thiobarbituric acid reactive substances (TBARS) as described previously [23]. The intra-assay coefficient variation (CV%) of TAC and MDA were 3.11% and 2.64%, respectively.

2.5. Artificial insemination

The semen samples were collected pooled and diluted by Lake extender in two weeks intervals from each experimental group [24,25]. Then, the diluted semen (0.2 mL, containing 200 × 10^6 spermatozoa/hen [25]) of each experimental group was artificially inseminated to Ross 308 broiler breeder hens (10 hens/group) in similar ages as the roosters. Each 10 inseminated hens were kept in floor pen (2.5 m × 2.5 m), bedded with wood shavings, and fed with a standard diet (Table 1). Eggs were collected from the day after the first artificial insemination for 13 consecutive days. The fertility was evaluated by virtual observing the development of the blastodisc to the blastoderm of a fresh unincubated egg [19] and expressed as fertility rate (the number of eggs with clearly developed blastoderm/the total number of eggs × 100).

2.6. Statistical analysis

Data were analyzed by SAS 9.4. Before analysis, normal distribution of the data was tested by Kolmogorov-Smirnov and Shapiro-Wilk test. Data that measured during time, such as sperm quality traits, were analyzed via Mixed procedure as repeated measurement data. The fertility rate, as a binary distributed data, was analyzed by GENMOD procedure using a logit odds ratio link function, and Tukey's multiple comparison tests was used to determine significant difference between experimental groups. Data are expressed as mean and standard error of the mean (SEM) and $P \leq 0.05$ and $0.05 \leq P \leq 10$ was considered as statistically significant difference and tendency, respectively.

3. Results

3.1. Body weight and sperm quality parameters

The effects of oSe on BW and semen quality attributes of roosters under dexamethasone induced stress are shown in Table 2. Body weight was not influenced by the treatments, but the effect of time and interaction effect of treatment and time was significant ($P < 0.05$). The interactive effect of treatmen×time on body weight (Fig. 2) showed that there was no significant effect of treatment on BW during the one to four weeks of the experiment. However, one-day intervals administration of Dexa decreased BW of roosters from week five up to the end of the experiment ($P < 0.05$). There was no significant effect of treatment or interaction effect of treatment and time on ejaculation volume and total produced spermatozoa per ejaculate, while they were affected by time ($P < 0.05$). The effects of treatment, time and interactive effect of treatment×time significantly influenced semen concentration, sperm motility, plasma membrane integrity and functionality (Table 2).

Semen concentration was decreased by injection of Dexa in PC compared to NC group ($P < 0.05$). However, feeding of oSe decreased the adverse effect of stress induced by Dexa, such that there was a significant difference between Se30+Dexa and Se45+Dexa groups compared to the NC group. Significant difference of semen concentration between oSe supplemented groups with PC and NC groups was first appeared at week four of the experiment, and it was remained up to the end of the trial (Fig. 3).

Table 2
Effect of dietary supplementation of different oSe levels under acute stress induced by dexamethasone (Dexa) injection on body weight and semen quality attributes.

Parameters	Experimental groups				SEM	*P* value		
	NC	PC	Se30+Dexa	Se45+Dexa		Treatment	Week	Treatment × Week
Body weight (Kg)	5.46	5.10	5.33	5.36	1.10	NS	<0.01	<0.01
Semen volume (mL)	0.35	0.28	0.21	0.30	0.06	NS	<0.01	NS
Semen concentration ($\times 10^9$/mL)	2.65^{a}	2.36^{b}	3.26^{a}	3.30^{a}	0.14	<0.01	<0.01	<0.01
Total spermatozoa per ejaculate ($\times 10^9$)	1.13	0.80	0.60	0.90	0.28	NS	<0.01	NS
Motility (%)	76.73^{ab}	73.66^{b}	82.73^{a}	83.31^{a}	2.41	<0.01	<0.05	<0.05
Plasma membrane integrity (%)	89.86^{a}	81.40^{b}	90.96^{a}	90.87^{a}	1.03	<0.01	<0.01	<0.01
Plasma membrane functionality (%)	56.99^{b}	57.67^{b}	64.61^{ab}	69.88^{a}	2.10	<0.01	<0.01	<0.01
Seminal TAC (mM/mL)	7.21	6.05	7.20	9.30	0.97	NS	<0.01	<0.05
Seminal MDA (mM/mL)	1.12^{a}	1.13^{a}	1.00^{ab}	0.96^{b}	0.04	<0.05	<0.01	<0.05

a, b: in each row, means with common superscripts are not statistically different (P > 0.05); NC: negative control; PC: positive control; NS: non-significant (P > 0.05).
Note: details of experimental design and treatments are represented in Fig. 1.

Dietary supplementation of oSe increased (P < 0.05) sperm motility in the oSe-treated roosters compared to the PC group (Table 2). The interaction effect of treatment and time on sperm motility (Fig. 4) showed that an improving effect of oSe was first appeared at week two of the experiment. Although administration of Dexa decreased sperm motility of the roosters, it showed a constant trend in the NC group during the experiment. Results revealed that oSe ameliorated the negative impact of stress induction on sperm motility, where following stress induction up to the end of the trial, the oSe-treated roosters had higher sperm motility compared to PC group (P < 0.05).

Administration of Dexa decreased sperm plasma membrane integrity in PC groups as compared to oSe-supplemented groups (Table 2). The significant difference between groups was first appeared four weeks after beginning the experiment up to the end of the trial (Fig. 5). During the last week of the trail, oSe-supplemented groups also had higher percentage of sperm with integrated plasma membrane than that of NC group (P < 0.05). Compared to the NC and PC groups, addition of 0.45 and 0.30 mg/kg diet oSe improved plasma membrane functionality two and four weeks after initial supplementation, respectively (P < 0.05; Fig. 6). Although, following stress-induction, plasma membrane functionality was decreased in Se30+Dexa and Se45+Dexa groups at week six, it showed a significant increasing trend during week eight of the experiment.

3.2. TAC and MDA

The effect of oSe on seminal plasma TAC and MDA of roosters under Dexa induced stress are shown in Table 2. Although the effect of treatment had no significant effect on TAC, it was influenced by the effects of time and interaction effect of treatment×time (P < 0.05). Dietary supplementation of oSe improved seminal TAC following four weeks of treatment compared with the both control groups (P < 0.05; Fig. 7). The significant difference was remained between oSe-treated groups and PC group from time of stress induction, onward. The effects of treatment, time and interactive effect of treatment and time significantly influenced seminal MDA (Table 2). During post stress induction period, concentration of MDA was lower in Se30+Dexa and Se45+Dexa groups compared to PC group (P < 0.05; Fig. 8).

3.3. Fertility

Supplementation of 0.30 or 0.45 mg oSe/kg of diet was associated with a higher fertility rate compared to the PC group (P < 0.05), even though during and after induction of stress (36.92%, 32.37%, 49.58%, 53.12% for total NC, PC, Se30+Dexa and Se45+Dexa fertility rates, respectively). Administration of Dexa during weeks five and six of the experiment caused a gradually decrease in fertility of PC roosters. However, in comparison to the control groups, the adverse

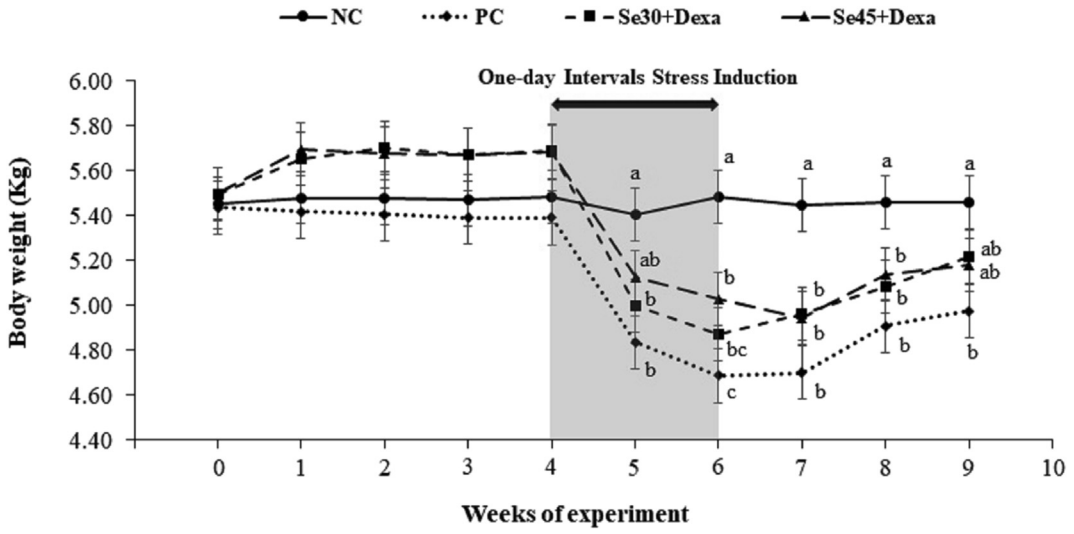

Fig. 2. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on body weight of roosters.
Note: details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

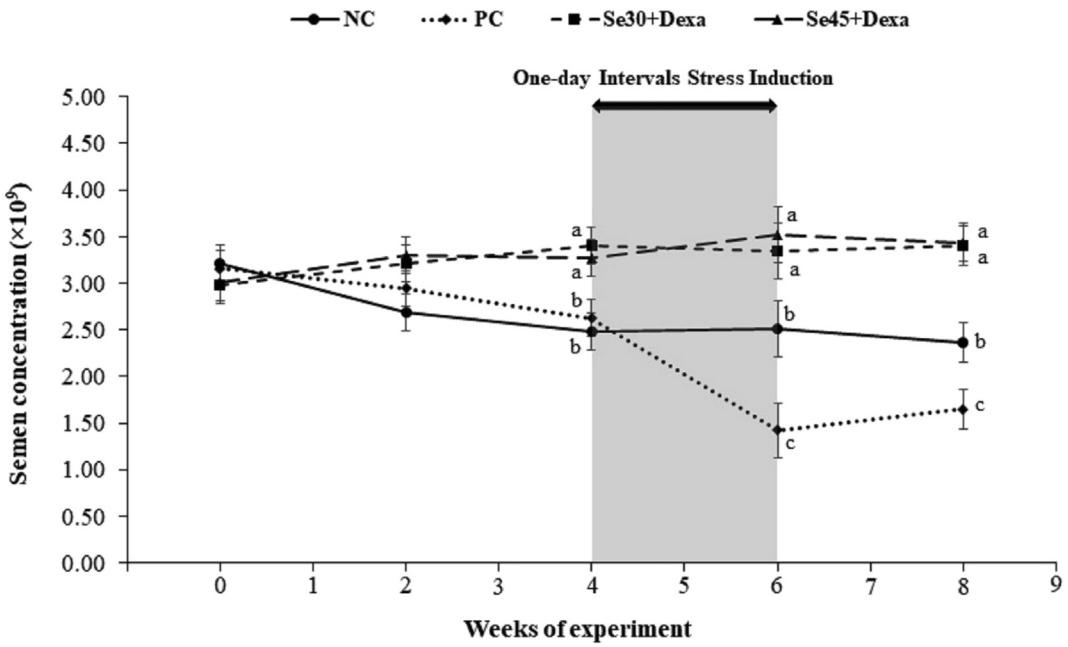

Fig. 3. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on semen concentration. Note: details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

effect of stress induction was decreased in Se30+Dexa and Se45+Dexa groups during and after the Dexa administration ($P < 0.05$; Fig. 9).

4. Discussion

Results of the present study indicated that following acute induction of oxidative stress, BW was decreased in the PC group. However, dietary supplementation of oSe alleviated the adverse effect of stress on BW. A great body of evidences have demonstrated that administration of oSe can influence body weight of broilers and breeder [26,27]. Several mechanisms such as stimulation of T_4 to T_3 conversion [28], higher blood circulation [29] and better feed conversion ratio [30] have been suggested for the effect of oSe on weight gain. Non-significant difference between experimental groups during first four weeks of the experiment show that a higher dosage or a longer period of treatment may require for illustrating the effect of dietary inclusion of oSe on BW. The ameliorating effect of oSe supplementation on BW loss observed in this study is probably explained by participating Se in the

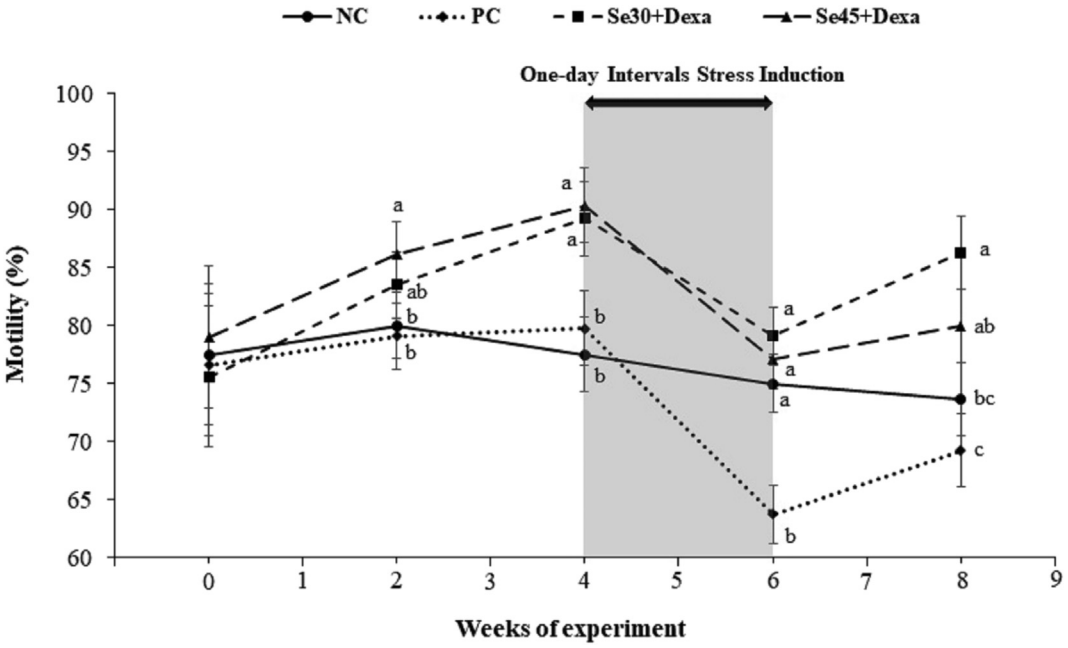

Fig. 4. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on sperm motility. Note: details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

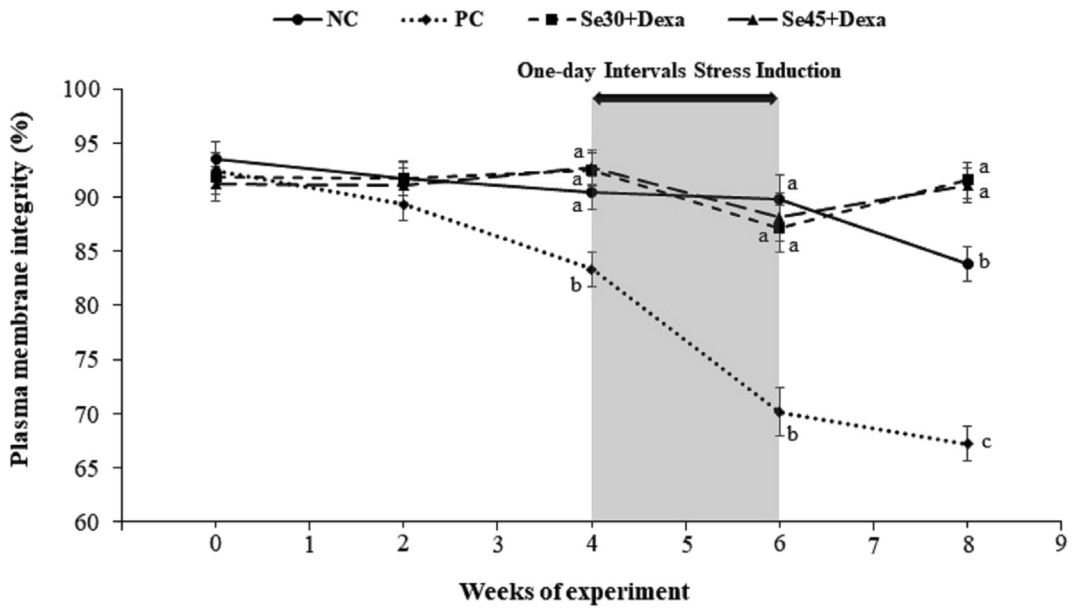

Fig. 5. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on sperm plasma membrane integrity. Not**e:** details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

antioxidant defense mechanisms [31].

Our results showed that dietary supplementation of oSe improved semen concentration in the treated groups and ameliorated adverse effect of stress induction observed in the PC group. The negative effect of stress on semen concentration is probably mediated by change in the number testicular germ cell and their differentiation [32–34]. The interval between feeding oSe and changes in semen concentration observed in this study is explained by time required for the experimental treatment to influence spermatogenesis process and the production of more sperm [13,35]. It is known that dietary supplementation of Se had a beneficial effect on seminiferous tubule development and promoted Sertoli cell viability [36] which in turn may improve sperm production [37]; whereas, Se deficiency induces testicular apoptosis. Over production of ROS during oxidative stress causes lipid peroxidation associated with an enhancement in apoptosis rate of germ cells [38]. It could be postulated that by participating in body antioxidant system and increase the Sertoli cell viability and regulation of apoptosis in testicular cells, dietary inclusion of Se mitigated the adverse effect of stress induction on semen concentration. However, treatments did not improve ejaculated volume and spermatozoa per ejaculated under stressful condition.

The results of the present study demonstrated beneficial effect of oSe on sperm motility after two weeks of dietary oSe

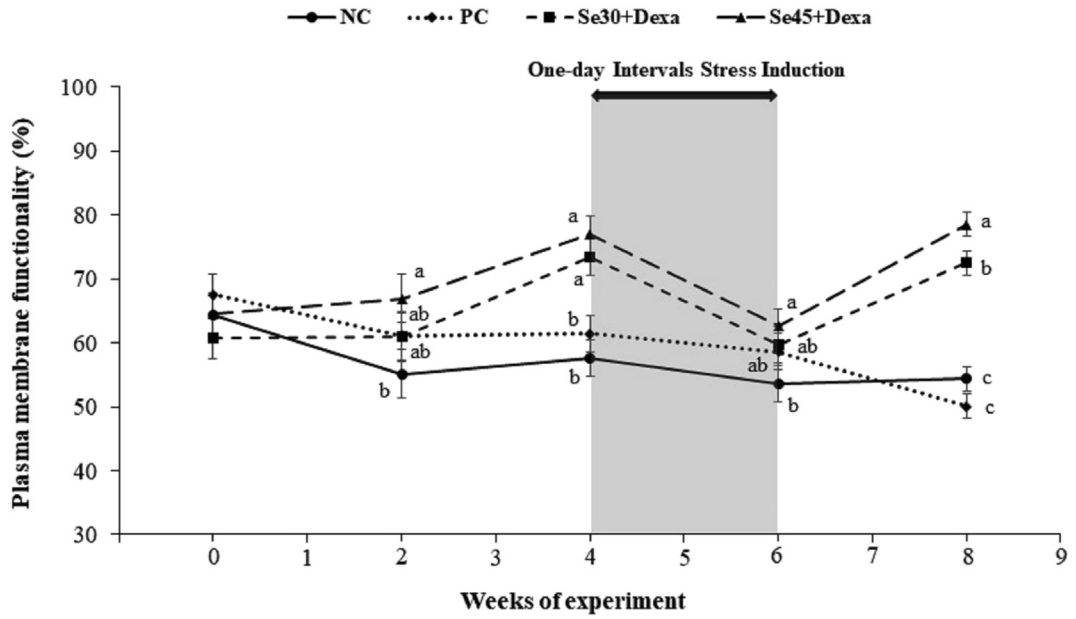

Fig. 6. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on sperm plasma membrane functionality. Not**e:** details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

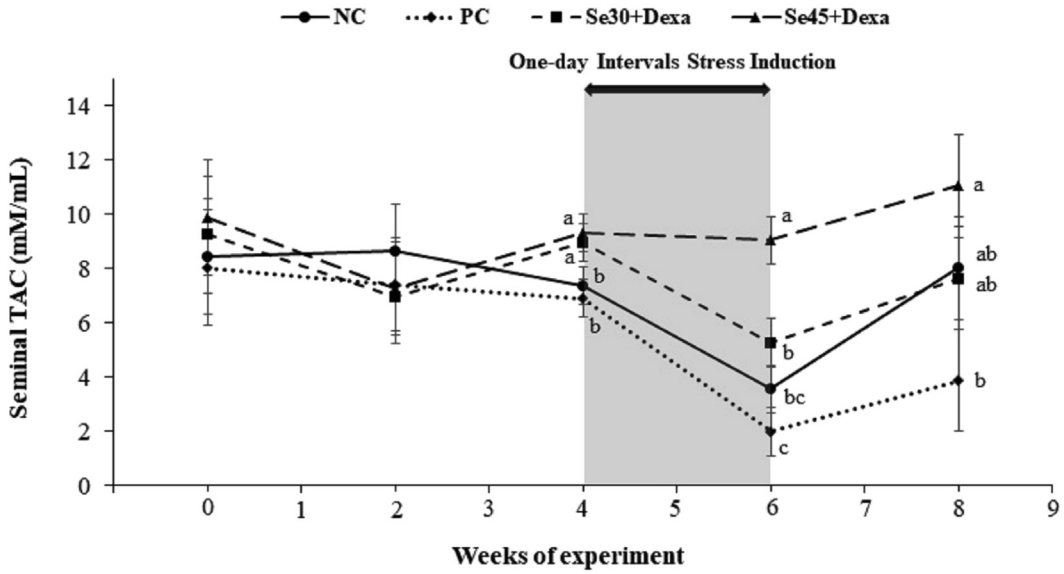

Fig. 7. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on total antioxidant capacity (TAC) in seminal plasma. Not**e:** details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

supplementation. However, stress induction significantly reduced sperm motility. Consistently, although five weeks of Se treatment did not significantly change sperm viability, it neutralized the adverse effect of stress induction, observed following Dexa administration. The higher sperm motility in oSe-treated roosters was also supported by the results of plasma membrane functionality, where the oSe-treated roosters showed a higher plasma membrane functionality following stress induction. These findings are explained by the potential of Se in protecting plasma membranes against ROS attack [3,13,39]. In agreement with the current results, Gallo et al. [40] demonstrated that dietary supplementation of 2.5 mg Se/kg of BW increased the rooster spermatozoa viability, concentration, and motility. A large volume of data indicating that oxidative stress can reduce sperm motility and supplementation of anti-oxidative component decrease harmful effect of oxidative stress [4,6,18,21,24]. Although a small amount of ROS is vital for normal sperm functionality [41], generation of high ROS levels following oxidative stress involved in infertility, mainly due to impairment of sperm motility. However, antioxidants have a significant role in protecting the sperm against peroxidative damage and they have ability to rescue sperm motility in *in vivo* and *in vitro* conditions [4,19,25,42,43]. Selenium appears to improve cellular antioxidant capacity and mitochondrial function, which plays an important role in sperm motility and viability. It is worth noting that, the higher percentage of viable sperm observed in oSe supplemented groups could involve in the higher percentage of motile sperm in these groups.

The results showed that four weeks after beginning the

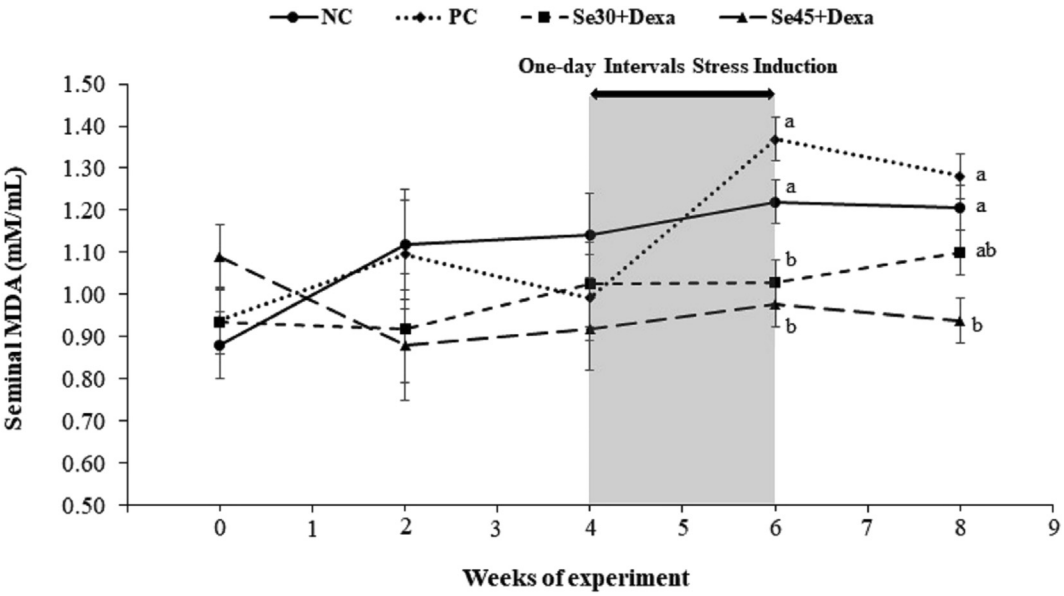

Fig. 8. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] and dexamethasone (Dexa) injection on (malondialdehyde) MDA in seminal plasma. Not**e:** details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

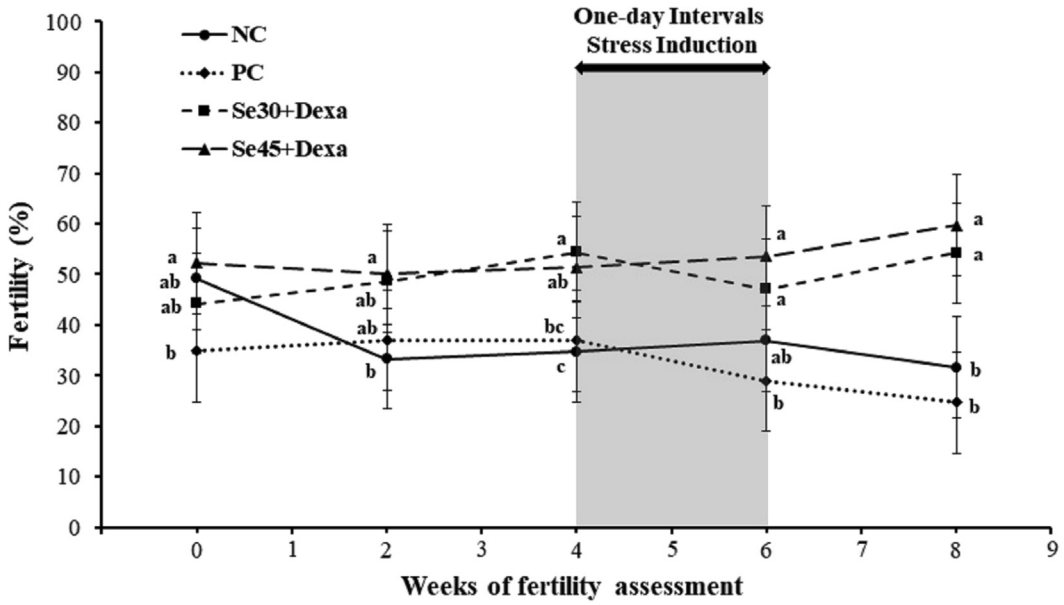

Fig. 9. Effect of dietary supplementation different levels of organic Se [0.30 (Se30) and 0.45 (Se45) mg/Kg of diet] on fertility rate of broiler breeder roosters and challenging by dexamethasone (Dexa). Not**e:** details of experimental design and treatments are represented in Fig. 1; NC: negative control; PC: positive control.

experiment, the seminal TAC was increased in oSe supplemented birds compared to control groups. Total antioxidant capacity assesses the cumulative effect of all antioxidants present within the semen, based on their ability to scavenge free radicals with any specific or nonspecific available mechanism(s). Selenium is a part of the glutathione peroxidase (GSH-Px) and other selenoenzymes or selenoproteins that are involved in the removal of hydrogen peroxide and prevention of lipid peroxides, produces during oxidative process in cells [44]. Wu et al. [45] reported that Se deficiency (0.0176 ± 0.002 mg Se/kg diet) lowers GSH-Px and SOD activity and the TAC in arterial walls, while dietary Se supplementation (1 μg Se/mL in drinking water) increased TAC in dose-depending manner. In the present study, with induction of stress, the seminal TAC decreased in NC, PC and Se30+Dexa groups; while the TAC level remained constant following stress induction in Se45+Dexa group as compared to the other group. Therefore, high levels of oSe are likely prevent the destructive effects of stress induction such as lipid peroxidation. The reason of decreased level of TAC during stress induction period in NC group is not fully understood; however, it may be due to several saline injections induced stress in this group. Results of the present study showed that during and after stress induction, seminal plasma concentration of MDA was lower in oSe supplemented roosters than that in the control groups. Peroxidation of polyunsaturated fatty acids (PUFAs) in sperm plasma membranes can give a rise the MDA that results in decreased plasma membrane integrity and functionality [46]. The positive effects of oSe observed in this study are explained by the crucial role of Se on increasing antioxidant capacity that protects the sperm plasma membrane against ROS, and lowers MDA.

In the present study, fertility of PC roosters was decreased as the stress was induced with Dexa. However, dietary oSe not only ameliorated the adverse effect of Dexa but also it improved the fertility capacity of the roosters as compared to NC group during and following stress induction. This improvement is explained by the ameliorating effect oSe on sperm quality attributes that were mostly decreased by administration of Dexa. It has been shown that sperm motility, acrosome reaction and implantation rate decreased by the effect of oxidative stress on human sperm [47]. Selenium localizes to the mid-piece of sperm [48] and its deficiency, depending on the severity, results in abnormal morphology, decreased motility, and infertility. Most likely, the higher fertility rate observed in the oSe-supplemented groups may be due to the better semen TAC that reflected in lower seminal MDA and higher sperm quality parameters. Both TAC and individual antioxidant levels have been shown to be lower in the semen of infertile men [49,50]. Eroglu et al. [51] reported a positive correlation between seminal plasma TAC levels and semen quality parameters such as concentration, motility, and morphology in men with idiopathic infertility. The number of spermatozoa stored in sperm storage tubules and their release and ability to join to and penetrate the perivitelline layer are considered as major factors influencing fertility in birds. Higher fertility rate observed in the Se-fed roosters probably is a result of improvement in quantity and quality of sperm stored in sperm storage tubules, which were subsequently involved in fertilization [5,21]. It is worth noting that the relatively lower fertility rate reported in this study is probably due to a two-weeks interval artificial insemination conducted in this study, while a one-week interval insemination is more common method.

5. Conclusion

Dietary supplementation of oSe lowered seminal MDA and improved seminal TAC and semen quality in broiler breeder roosters. Most improvements in the seminal quality indices were achieved after 2–4 weeks of daily administration of at least 0.30 mg Se/kg diet. Moreover, oSe supplementation ameliorated the negative effects of acute oxidative stress on semen quality parameters included by dexamethasone administration. The induced stress lowered the fertility rate of the roosters; however, dietary supplementation of oSe mitigated the observed adverse effect of the stress induction, mainly by improvement in antioxidant capacity of the semen.

CRediT authorship contribution statement

Ali Asghar Khalil-Khalili: Visualization, Investigation, Writing - original draft, Writing - review & editing. **Mahdi Zhandi:** Conceptualization, Methodology, Supervision, Project administration.

Mojtaba Zaghari: Conceptualization, Methodology. **Hassan Mehrabani-Yeganeh:** Conceptualization, Methodology. **Ali Reza Yousefi:** Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Meysam Tavakoli-Alamooti:** Resources.

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