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Supplementation of Sodium Selenite and Methionine on Concentration of Selenium in Egg and Serum, Antioxidant Enzymes Activity and Immune Response of Iranian Native Broiler Breeders

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Abstract

Effects of sodium selenite (SS) in offspring of broiler breeders has been reported, but the comparison between SS and different level of methionine on offspring has received limited, so this study was conducted to investigate sodium selenite and methionine effects on concentration of selenium in egg and serum of Iranian native broiler breeders. An experiment was conducted in 3 × 3 factorial experiment with three levels of sodium selenite (0.0, 0.3, and 0.5 mg/kg of diet) and three levels of methionine (0.23, 0.31 and 0.33 % of diet) to investigate reproductive performance and immune status of broiler breeders (64-74 wks). The higher methionine levels increased the egg weight until the dose of 0.31% of diet ($P < 0.05$). In this experiment, 0.5 mg/kg SS with 0.31 % methionine in the diet was found to increase egg weight ($P < 0.05$). The highest salable chicks were obtained by supplementing 0.3 mg/kg SS (P= 0.07). Increasing the level of methionine from 0.23 to 0.33% without SS and also, supplementation SS at 0.3 mg/kg with 0.33 % methionine exhibited higher serum selenium, although, no effect of methionine levels was observed on concentration of selenium in serum and transfer rate. Concentration of selenium in eggs and serum were significantly affected by interaction of SS and methionine levels in diet. An increment in glutathione peroxidase (GSH-Px) level tended to increase with SS (0.5 mg/kg) and methionine (0.33%) interaction (P=0.06). The results showed that increasing the level of methionine increases the immune response against influenza (AI) and Newcastle disease (ND) ($P = 0.0001$). These results suggest that higher level of methionine at 0.31% could improve egg weight and Influenza and ND titers, SS at dose of 0.5 could increase concentration of selenium in serum, whereas increasing of SS levels significantly raised embryonic mortality.

Introduction

Trace elements are very important to production, health and activities of major antioxidant enzymes. Selenium is an essential trace element that plays important role in many activities, including in the production, health and many antioxidant enzymes activity such as GSH-Px and thioredoxin reductase. This trace element also has fundamental roles in human health. Sodium selenite (SS) has been the major source of selenium in animals' diets to warrant a most favorable supply (Hoffmann *et al*., 2008). In the last few years many researchers found better replacement source for SS that have a high bioavailability and low toxicity such as seleno-yeast and seleno-methionine [Se]Met (Yoon *et al*., 2007; Reis *et al*., 2009). Organic selenium is found in the form of selenium yeast and [Se]Met. Organic selenium consumption is safe for humans and has a toxic effect at 0.5 mg/kg of diet.

Your body can metabolize and use all three forms of selenium, but the amount absorbed varies. About 90 % of [Se]Met and 50 % of selenite are absorbed. Selenium Methionine can replace methionine in the body tissue instead of methionine in case of need,

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however, the incorporation of seleno-methionine into tissue proteins and keratin in the cattle, birds and fish causes alkali disease. Two pools of reserve selenium have been detected in the body (Waschulewski and Sunde, 1988). The first is as [Se]Met that reportedly is different from methionine in terms of physiological function. The second reserve pool is the selenium found in liver GSH-Px. Following direct metabolism, the consumed selenite, selenate, and selenocysteine are all turned into selenide, which is the reduced form of selenium. According to Knight and Sunde (1988), selenium was less available for GSH-Px synthesis from dietary [Se]Met consumed less than 0.5 mg Se/kg in rats having a methionine-deficient diet. These researchers offered that [Se]Met had been preferentially entered into body proteins when methionine was limiting. Contrary to increased tissue selenium levels, The tissue GSH-Px activity decreased in a methionine deficient diet when the rate received methionine in a [Se]Met form (Waschulewski and Sunde. 1988)
Waschulewski and Sunde (1988) showed that methionine supplementation in rat for 8 wk resulted in a 1.8-fold increase in erythrocyte GSH-Px, suggesting that the additional methionine increased the availability of dietary [Se]Met or stored [Se]Met. Selenium absorption efficiency is relatively high (89±4) and depends on the dietary sources (Susanne *et al*., 2008). In contrast to inorganic forms [Se]Met is absorbed very rapidly and enhanced at low methionine intake in the intestinal (Vendeland *et al*., 1996).

According to above reports, we assume that perhaps the different levels of Methionine could affect availability of selenium in the inorganic resources. The one objective of this experiment was to compare GSH-Px plasma and egg selenium concentrations in naive broiler breeders that fed with different levels of methionine and selenium. We wanted to determine if the methionine that had been supplied in higher dose could be improved selenium transfer rate for egg and serum (reflected in antioxidants enzyme) when selenium intake is in different levels.

Therefore, the present experiment was designed to evaluate the antioxidant activity and selenium levels in serum and eggs of Iranian native broiler breeders that were fed diets supplemented with different doses of selenium as SS form and methionine during a 56-d period.

Material and Methods

Experimental Design and Diets

All the procedures used in this study were based on the approval of the Animal Rights Protection Committee of Sari Agricultural and Natural Resources University. A completely randomized design was applied in a 3 × 3 factorial arrangement, with three levels of SS (0.0, 0.3, and 0.5 mg/kg of diet) and three levels of methionine (0.23, 0.31, and 0.33 % of diet). At 64 wk of age, the birds were distributed among the pens based on body weight and egg production (2,215 ± 25.8 g). The experiment started two wks after adaptation period at 66 wk of age and lasted for 8 wks.

A total of 270 Iranian native broiler breeders were randomly allocated to nine groups with three replicates. Briefly, 10 females and 1 male were assigned to each of 27 pens. All birds were housed in breeder pens (120 × 80 cm; length × width) and had free access to feed and water. House temperature tried to keep at 24 ± 3ºC and the length of lighting was16 h per day. The experimental diets were based on corn and soybean meal formulated according to Iranian native broiler breeder's guide to meet all nutrients requirement except selenium and methionine. Sodium selenite was supplemented to corn basal diet (According to table 1) at the levels of 0, 0.3, 0.5 mg/kg. Methionine was added at the level of 0.0027 and 0.0057 % (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) to the basal diets to provide 0.23, 0.31, and 0.33 % (Table 1).

Data collection

Productive Performance

Egg production and settable eggs were recorded daily. Percentage of settable eggs, egg production, and egg weight were measured as indicated by Kazemi-Fard *et al*. (2013). Archimedes method (Hempe *et al*., 1988) was used for specific gravity assay. Egg shell thickness, egg quality and egg shell surface area (SA) were measured as indicated by Tahmasbi *et al*. (2012) and Ousterhout (1980), respectively. The shell weight per unit of surface area (SWUSA) was calculated by dividing the dried shell weight by the surface area of each egg. Haugh unit calculated based on albumen height and egg weight using the formula: $HU = 100 \log10 (H - 1.7 W^{0.37} + 7.56)$, (Carter, 1975). Where: HU= Haugh unit, H= height of the albumen (mm) and W= egg weight (g).

Hatch Characteristics and Embryonic Mortality

Eggs were gathered for six successive d on a weekly basis for all replicates; they were weighed separately. Thirty-six settable eggs per pen were set for incubation on 66 and 74 wk of age. Eggs were incubated in James-way model Micro Pt- 100 commercial incubator. The eggs were put for incubation at 37.15℃ dry bulb and 29.62℃ wet bulb temperatures (0 - 19 d). After 10 days of incubation, the eggs were checked for infertility. All infertile eggs were broken and tested with the naked eye to prove embryonic mortality. The remaining unhatched eggs were put for analysis of developmental stage of dead embryos. The time of embryonic death was allocated to one of four groups: early dead (≤ 7 d),

mid-dead (8-16 d), late dead (17-21 d), and pips. The rate of fertile eggs to total eggs set was set to explain fertility. After 19 days, the eggs were put into baskets; then, these baskets were put into the hatchery cabinets and the hatcher was adjusted to 36.44℃ dry bulb and 32.18 ˚C wet bulb temperatures. After 21.5 days of incubation, the number of hatched eggs was logged. The number of hatched chicks to fertile eggs was set to explain the hatchability of fertile eggs and the percentage of hatched chicks to the total eggs set was used to explain the cumulative hatchability. As explained by Dziaczkowska (1980), chick quality was determined based on normal and abnormal chick.

Table 1. Feed ingredients and chemical composition of experimental diets

item	sodium selenite (0 mg/kg)			sodium selenite (0.3 mg/kg)			sodium selenite (0.5 mg/kg)		
	Methionine level (%)			methionine level (%)			Methionine level (%)		
Ingredients (%)	0.21	0.31	0.33	0.21	0.31	0.33	0.21	0.31	0.33
Corn	69.87	69.86	69.86	69.57	69.56	69.56	69.37	69.36	69.36
Soybean meal	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22	22.22
Calcium carbonate	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
Di-calciu phosphate	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Sodium chloride	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
L-Lysine	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
DL-MET	-	0.0027	0.0055	-	0.0027	0.0055	-	0.0027	0.0055
Sodium selenite (mg/kg)	-	-	-	0.3	0.3	0.3	0.5	0.5	0.5
Vitamin premix‡	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix#	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chemical analysis for selenium (mg/kg)	0.20	0.20	0.02	0.401	0.401	0.401	0.468	0.468	0.468
Calculated Feed analysis									
ME (kg/kcal)	2850	2850	2850	2850	2850	2850	2850	2850	2850
CP (%)	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
P_a (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ca (%)	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Met (%)	0. 21	0. 31	0. 33	0. 21	0. 31	0. 33	0. 21	0. 31	0. 33
SAA (%)	0.45	0.60	0.64	0.45	0.60	0.64	0.45	0.60	0.64

‡Vitamin premix (per kg of diet) supplied: vitamin A: 12000 IU; vitamin E: 100 IU; vitamin K3: 7 mg; vitamin B1: 3 mg; vitamin B2: 12 mg; vitamin B3: 12 mg; Nicotinic acid: 40 mg; vitamin B6: 4 mg; vitamin B9: 1.5 mg; vitamin B12: 0.04 mg; vitamin B19: 0.25 mg; Choline chloride: 200 mg.
Vitamin premix: vitamin D3 was added at the rate of 0, 3500, and 4200 IU/kg of diet to provide three vitamin D3 diets.
#Mineral premix provided (mg/kg of diet): Mn: 60; Fe: 60; Zn: 100; Cu: 10; Co: 0.2; I: 0.5 and Se: 0.4.

Selenium Concentrating in Diet, Egg and Serum Content

Total selenium concentrations in diet, egg, and serum samples were determined by inductively coupled plasma–mass spectrometry (ICP–MP, AES 4100; Australia). We added the 1g homogenized sample to the PTFE vessel, then added 5 mL nitric acid 65% and 2.5 mL Hydrogen peroxide 30% to allow the sample to predigest for at least 2h at 120 ºC. Then sample was diluted up to 25 mL, 10 mL of sample was transferred to a 25 mL volume flask and 5 mL concentrated HCl was added. The solution was taken at water bath at 80 ºC for 30 minutes. Transfer percentage of Se from the feed to the eggs and sera was considered as a transfer rate (Delezie *et al.*, 2014).

Antioxidant Enzymes Activities in Plasma

To specify GSH-Px and SOD activity, six blood samples were taken from each treatment in the middle and end of experiment (at 7:00 am). Plastic tubes containing EDTA were used to hold the blood taken from the left brachial vein. Samples were kept on ice up to the time when plasma was divided by centrifuging (1500 × g for 20 minutes) within 60 min of collection. For further analysis, plasma was put for harvesting and was kept at -20°C until. The commercial RANSEL and RANSOD kit was used to specify the concentrations of GSH-Px and SOD (RANDOX Laboratories Ltd., London, UK) according to the manufacturer's instructions. In brief, the oxidation of glutathione is catalyzed by GSH-Px through cumene hydroperoxide. The conversion of the oxidized glutathione the reduced form takes place in the presence of glutathione reductase and nicotine amid di-nuecleotide phosphate (NADPH), which is accompanied with the oxidation of NADPH to $NADP^+$. Superoxide dismutase speeds up the dismutation of the toxic superoxide radical, produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This technique uses xanthine and xanthine oxidase to produce superoxide radicals that contribute to the formation of a red formazan dye by reacting with 2-(4-iodophenyl-3-(4-

nitrophenol)-5-phenyltetrazolium chloride. The degree of inhibition of this reaction determines the superoxide dismutase activity.

Newcastle and Influenza Immunity

Following the administration of the dead vaccine, two blood samples (2 to 3 mL) were gathered as two birds from the left brachial vein in each pen at 4, 6, and 8 wk. All blood samples underwent centrifuging at 1000 × g for 5 min to isolate sera; then the sera was put for harvesting and kept at -20 ℃ until analysis. The hemagglutination inhibition (HI) method was applied to determine the presence of Newcastle and Influenza antibodies in sera samples.

Statistical Analysis

Data were analyzed according to a completely randomized design with a 3 × 3 factorial arrangement using the GLM procedure of SAS (2003) software. Newcastle and Influenza antibody titers data were analyzed using the repeated measurement procedure of the SAS in PROC MIXED procedure. Differences among treatment means were measured by Duncan's multiple range test and considered significant at $P < 0.05$.

Results and Discussion

Analyzed selenium contents in the experimental diets were slightly greater than calculated values. No significant effect was found for treatments on the egg shell thickness, shell weight unit per surface area, shape index, yolk and albumin weight, thin shell and Haugh unit (data not shown).

Table 2. The effect of sodium selenite, methionine level and their interaction on productive performance, eggshell quality and settable egg of Iranian native broiler breeder

Item		Productive performance			Egg shell quality		Settable egg (%)
		Egg production (%)	Egg weight (g)	Egg mass (%)	Egg shell (%)	Specific gravity	
Sodium selenite (mg/kg)							
0.0		32.76	55.04	19.82	8.62	1.08	92.25
0.3		35.11	57.20	21.31	8.50	1.08	93.62
0.5		32.31	56.23	19.56	8.38	1.08	92.47
Methionine level (%)							
0.23		32.06	55.47^{ab}	19.30	8.56	1.07	91.77
0.31		34.22	58.23^{a}	21.07	8.51	1.08	91.65
0.33		33.90	54.78^{b}	20.31	8.42	1.08	94.92
Sodium Selenite × Methionine level							
0.0	0.23	31.81	56.78^{ab}	19.84	8.95	1.07	91.22
0.0	0.31	33.47	56.99^{ab}	20.79	8.52	1.08	89.85
0.0	0.33	33.00	51.35^{b}	18.38	8.39	1.08	95.67
0.3	0.23	34.81	57.93^{a}	21.07	8.50	1.08	93.85
0.3	0.31	34.47	56.68^{ab}	20.73	8.50	1.08	92.09
0.3	0.33	36.04	56.98^{ab}	22.11	8.48	1.07	94.92
0.5	0.23	29.57	51.68^{b}	16.99	81.23	1.07	90.23
0.5	0.31	34.71	61.01^{a}	21.70	8.51	1.08	93.02
0.5	0.33	32.66	56.00^{ab}	19.99	8.40	1.08	94.15
P-value							
Sodium selenite		0.67	0.31	0.61	0.67	0.63	0.71
Methionine		0.78	0.05	0.64	0.86	0.51	0.14
Sodium selenite × Methionine		0.97	0.017	0.76	0.78	0.51	0.71
SEM		1.18	0.72	0.69	0.097	0.001	2.19

Means in a same column with different superscripts significantly differ (P < 0.05).

Productive Performance

Performance of Iranian native broiler breeders fed diets supplemental with different levels of SS and methionine during the whole trial period is showed in Table 2. Selenium and methionine supplementation did not influence ($P > 0.05$) egg production, egg mass, shell weight, specific gravity, and settable egg. This results for egg production agree with Payne and southern (2005) indicated no difference in percentage hen-day production when breeders were fed different levels of SS. Jing *et al.* (2015) showed that the addition of different levels of selenium to the feed did not significantly affect egg production of laying hens. The higher methionine added levels increased the egg weight until the dose of 0.31% of diet ($P < 0.05$). In this experiment, 0.5 mg/kg SS with 0.31% methionine in the diet was found to increase egg weight ($P < 0.05$) compare to 0.5 mg/kg SS with 0.23% Met and 0 mg/kg SS with 33% Met. However, the results disagree with those of Utterback *et al.* (2005) who reported no significant differences ($P > 0.05$) in egg weight (Utterback *et al.*, 2005). Some

researchers showed that increased dietary methionine intake couldn't improve egg production, specific gravity and egg mass (Bunchasak and Silapasorn, 2005; Nassiri-Moghaddam *et al*., 2012). Petersen *et al*. (1983) found that increasing dietary methionine increased egg weight without affecting egg production (Petersen *et al*., 1983). Safaa *et al*. (2008) showed that an increase in methionine content of the diet from 0.31 to 0.36% did not affect hen performance at any age. Similar to our experiment Jackson *et al*. (1987) observed that egg weight increased by increasing dietary methionine. Also, Petersen *et al*. (1983) reported that reducing dietary methionine declined egg weight without any effect on the egg production.

Table 3. Effect of sodium selenite, methionine level and their interaction on the hatch, embryonic mortality and salable chicks in the Iranina native broiler breeders (%)

Item		Hatch. of set egg	Hatch. of fertile egg	Embryonic mortality (%)			Salable chicks
				1 to 9 d	10 to 17 d	18 to 21.5 d	
Sodium selenite (mg/kg)							
0		75.534	81.652	0.00	0.28	24.18	92.59[b]
0.3		78.293	81.008	0.89	1.16	19.66	96.24[a]
0.5		71.771	80.779	0.53	0.26	31.91	92.89[ab]
Methionine level (%)							
0.23		71.425	78.034	0.53	0.64	27.40	92.37
0.31		79.144	83.430	0.46	0.75	23.12	95.03
0.33		75.029	81.974	0.42	0.31	25.23	94.32
Sodium selenite × Methionine level							
0	0.23	71.425	78.034	0.00	0.00	1.11	89.55
0	0.31	79.144	83.430	0.00	0.85	0.85	94.73
0	0.33	75.029	81.974	0.00	0.00	0.79	93.50
0.3	0.23	74.00	74.00	0.00	1.14	3.09	94.39
0.3	0.31	85.71	86.54	1.39	1.39	0.69	96.63
0.3	0.33	81.98	85.08	1.29	0.94	2.38	97.68
0.5	0.23	78.10	78.75	1.59	0.79	0.79	93.16
0.5	0.31	71.38	71.38	0.00	0.00	0.00	93.72
0.5	0.33	75.84	75.84	0.00	0.00	0.84	91.78
P-value							
Selenite sodium		0.136	0.972	0.42	0.32	0.08	0.07
Methionine		0.069	0.367	0.98	0.77	0.72	0.27
Sodium selenite × Methionine		0.630	0.843	0.40	0.88	0.43	0.62
SEM		3.794	4.000	0.26	0.24	2.23	0.71

Means in a same row with different superscripts significantly differ ($P < 0.05$).

Hatchability and Embryonic Mortality

The results of hatchability, embryonic mortality and salable chicks are shown in Table 3. Different levels of SS and methionine had not significant effect on the hatchability and embryonic mortality in early and mid-phase of incubation. Our findings were in agreement with the results of Lesson et al. (2008) who did not find any positive effect of concentration and source of selenium hatchability in broiler breeders. It has been reported that supplementation of selenium at 1 mg/kg had not any significant effect on hatchability (Pappas *et al.*, 2006). Some researchers have shown that, when SS levels rose in the diet, hatchability percentage decreased (Ort and Latshaw, 1978). Xue *et al*. (2017) showed that methionine has a positive effect on the hatchability.

They found this effect may be due to the presence of methionine and cysteine in the GSH-Px enzyme structure. Abdalla *et al* (2005) and Abd-El-Samee *et al*. (2007) Showed that, when DL-methionine supplemented to broiler breeder diet, hatchability increased. The findings of these researchers showed that increased methionine supplementation led to significant increased hatchability. In agreement with the results of these studies, it has been reported that the use of methionine supplementation has led to an increase in hatching (Naulia and Singh, 2002). These results are not consistent with the reports by Abdalla *et al.* (2005); they found that increased methionine levels in layer diets led to decreased fertility percentage. There are contradictory results about the effect of methionine on hatchability, which is partly referred to type of setter, the number of eggs placed in the incubator, and that the incubated device used in this experiment was not of industrial type.

The amount of selenium in the diet plays a decisive role in the concentration of selenium in the egg (Kenyon and Spring, 2003). Similar to our results (Table-4), Boruta *et al*., 2007) also showed that increasing the amount of selenium in the diet led to increase of selenium concentration in eggs and blood sera. Selenium plays immunostimulatory effects on the lymphoid tissue of the immune system in chickens (Szeleszczuk *et al*., 2004). High levels of selenium or deficiency have a negative effect on reproductive traits. Excess selenium is considered embryo toxic (Boruta *et al.,* 2007), which is also visible (late embryonic mortality) in our experiment results (Boruta *et al*., 2007). The results of Table 4 showed that high levels of selenium or its deficiency increased embryonic mortality in the late phase of incubation. In pheasants, excess selenium caused abnormal behavior in its female sex, decreased embryonic development, hatchability and chick quality (Latshaw *et al*., 2004).

Table 4. The effect of sodium selenite, methionine level and their interaction on concentration of selenium on egg, serum and transfer rate of Iraninan native broiler breeders

Item		Egg		Serum	
		Concentration of Selenium in egg(µg/g)	Transfer rate (%)	Concentration of Selenium in erum(µg/ml)	Transfer rate (%)
Sodium Selenite (mg/kg)					
0		0.11	57.22^{A}	0.11^{B}	59.78^{a}
0.3		0.15	38.31^{B}	0.11^{B}	28.38^{b}
0.5		0.14	29.87^{B}	0.13^{A}	29.04^{b}
Methionine (%)					
0.23		0.12	35.35^{a}	0.12	38.21
0.31		0.13	37.98^{a}	0.12	41.98
0.33		0.15	52.07^{b}	0.12	37.57
Sodium selenite × Methionine level					
0	0.23	0.083^{b}	41.67^{b}	0.118^{bc}	59.10^{ab}
0	0.31	0.083^{b}	41.67^{b}	0.113^{bc}	56.56^{b}
0	0.33	0.176^{a}	88.33^{a}	0.131^{b}	65.65^{a}
0.3	0.23	0.113^{ab}	28.12^{b}	0.109^{bc}	27.27^{dce}
0.3	0.31	0.176^{a}	43.83^{b}	0.093^{c}	23.29^{e}
0.3	0.33	0.173^{a}	43.00^{b}	0.125^{b}	31.19^{dc}
0.5	0.23	0.170^{a}	36.27^{b}	0.132^{b}	28.26^{dce}
0.5	0.31	0.133^{ab}	28.45^{b}	0.157^{a}	33.62^{c}
0.5	0.33	0.116^{ab}	24.89^{b}	0.118^{bc}	25.24^{de}
P-value					
Sodium selenite		0.17	0.001	0.002	0.0001
Methionine		0.26	0.033	0.68	0.26
Sodium selenite × Methionine		0.038	0.006	0.003	0.008
SEM		0.024	7.68	0.006	2.017

Means in a same column with different superscripts significantly differ (P < 0.05).

Selenium Content in Egg and blood serum

The influence of SS, methionine and their interaction on the selenium concentration in eggs, blood serum, and their transfer rate are shown in Table 4. A significant interaction between SS and methionine was found on concentration of selenium in egg ($P < 0.05$). The concentration of selenium increased to 0.176 (µg/g) in the group with 0.0 mg SS/kg and 0.33% Methionine compared with the control group (0.083; 0.0 mg SS/kg and 0.23% Methionine), the transfer rate of Se for egg was affected ($P < 0.05$) by SS, methionine, and their interaction. Supplementing the diet with SS reduced its transmission in eggs and serum, also adding of methionine more than 0.23 % increased egg transfer rate. The results of this table showed adding methionine at dose 0.33 % increased egg transfer rate when the concentration of SS was lowest (0.0 mg/kg). The selenium concentration for serum and serum transfer rate ($P < 0.001$) was affected in hens that were fed higher dose (0.05 mg/kg) of selenium. In fact, the higher selenium content of the feed (SS) manifested itself in the form of plasma selenium of native broiler breeders. As well as, this effect was visible for SS and methionine (SS × methionine interaction). Increasing the level of methionine from 0.23 to 0.33% exhibited a higher serum transfer rate from hens supplied with higher levels of SS (0.3 and 0.5 mg/kg). Highest selenium serum mean levels were found when SS and methionine was added at dose of 0.5 mg/kg and 0.31% in the diet. No effect was observed on the selenium concentration and serum transfer rate for methionine levels. In the experiment of Delezie et *al*. (2014) highest transfer rates were obtained by

supplementing the lowest level of selenium independent. Selenium absorption efficiency is relatively high (50-95%) and depends on the dietary sources (Robinson and Thomson, 1983). In contrast to inorganic forms [Se]Met absorbed very rapidly and enhanced at low methionine intake in the intestinal (Vendeland *et al*., 1992). There is a dynamic mechanism comparable to that of its S-analogue methionine (Wolffram *et al*., 1989). The concentration of selenium in the plasma was higher in the birds that received 0.3 mg/kg diet than the other group without selenium supplementation (Jing et al., 2015). Serum selenium concentrations was decreased in broilers received the corn soybean meal diet without selenium supplementation in compare to hens fed corn soybean meal with supplemental selenium in diets, regardless of source (Payne *et al*., 2005). Previous researchers have showed that selenium supplementation in to diets for broiler breeders or laying hens enhanced the selenium content of eggs. These finding are in agreement of our experiment. One of main dietary factor influencing availability of selenium includes methionine, (Fairweather-Tait and Hurrell, 1996). The quantity of selenium intake determines its resorption from the gastrointestinal tract, its retention, and metabolism in the body. The liver does not store selenium. Insufficient selenium decreases its serum level significantly. The resorbed selenium is transferred by the bloodstream that ultimately binds to plasmatic proteins and integrates to all tissues (Cousins and Cairney, 1961). Liver have central role in the selenium metabolism and homeostasis, and protein plasma mediated it for absorption and metabolize. According to this document methionine is one of the main components to synthesis of protein that bound to selenium and transport selenium to tissue such as eggs. Skrivan *et al*. (2010) indicated that supplementation of either form ([Se] Met, SS) of selenium significantly increased the selenium concentration in eggs.

Table 5. The effect of sodium selenite, methionine level and their interaction on antioxidant enzymes in plasma of Iraninan native broiler breeders

Item		Glutathione Peroxidase (U/g of Hb)			Superoxide Dismutase (U/mL)		
		GSH-Px $_1$	GSH-Px $_2$	GSH-Px $_{overall}$	SOD_1	SOD_2	$SOD_{overall}$
Sodium selenite (mg/kg)							
0		218.2	282.36	250.30	151.14	154.93	153.04
0.3		245.5	279.55	262.53	149.13	144.56	146.84
0.5		400.7	264.79	332.74	150.98	145.27	147.81
Methionine level (%)							
0.23		193.4	289.93	241.64	150.20	149.01	149.52
0.31		295.1	276.56	285.82	149.33	149.00	149.17
0.33		376.0	260.21	318.11	151.72	147.39	149.36
Sodium selenite × Methionine level							
0	0.23	102.9	436.0	269.5^{ab}	149.29	150.53	149.91
0	0.31	285.7	264.4	275.1^{ab}	146.08	168.19	157.13
0	0.33	266.1	146.6	206.4^{b}	158.06	146.08	152.07
0.3	0.23	190.9	243.1	217.0^{b}	154.23	147.94	151.08
0.3	0.31	385.5	390.0	387.8^{ab}	149.17	139.17	144.17
0.3	0.33	160.1	205.5	182.8^{b}	143.98	146.58	145.28
0.5	0.23	286.3	190.7	238.5^{b}	147.07	148.37	146.61
0.5	0.31	213.9	175.2	194.6^{b}	152.75	139.66	146.20
0.5	0.33	701.8	428.5	565.1^{a}	153.12	150.59	151.42
P-value							
Sodium selenite		0.21	0.97	0.54	0.92	0.19	0.33
Methionine		0.25	0.93	0.63	0.91	0.97	0.99
Sodium selenite × Methionine		0.13	0.07	0.06	0.56	0.16	0.64
SEM		131.09	100.17	97.60	6.87	7.02	5.17

Means in a same column with different superscripts significantly differ (P < 0.05)
GSH-Px = glutathione peroxidase; SOD = superoxide dismutase
GSH-Px1= at 70 wk GSH-Px2= at 74 wk SOD_1= at 70 wk SOD_1= at 74 wk

Glutathione Peroxidase and Superoxide Dismutase Activity

As can be seen from Table 5, different levels of SS and methionine did not significant effect on GSH-Px and SOD activity ($P > 0.05$). Moreover, the results of this study revealed that interaction between SS and methionine on the GSH-Px in whole of experiment tended to be significance ($P= 0.06$). Supplementation of SS at 0.5 mg/kg with methionine at 0.33 % significantly increased plasma GSH-Px compared to hens fed SS at doses 0.0, 0.3 with methionine at doses 0.23, 0.33%. Glutathione peroxidase is the general

name belonging to the enzyme family performing peroxidase activity. It plays a biological role by guarding an organism against all oxidative damages. They also contribute to the decreased free hydrogen peroxide to water (Brigelius-Flohé and Medicine, 1999). One of the reasons why it is possible to justify this conclusion is that methionine which is an analog to the [Se]Met compounds plays important roles to support cysteine for GSH synthesis. In opposition to the results obtained in this experiment, Leeson *et al*. (2008) showed GSH-Px activity in plasma was affected by selenium concentration.

In consist of our experiment Waschulewski and Sunde (1988) found that when rats fed 0.5 mg selenium as [Se]Met/kg tissue selenium increased significantly. The concentration of selenium in the erythrocyte and muscle in rats when fed with methionine deficiency was higher than methionine-supplemented to diet, while the concentration of enzyme was increased when methionine was added to the diet.

Immune Response

The data showed that the antibody titer against influenza didn't change during wks 70 to 74 at 0.3 mg / kg and 0.31% methionine (Table-7), Also this trend was observed for 0.0 mg / kg SS with 0.33 % methionine ($P < 0.05$), but during the time, this effect was meaningful. The results indicate that GSH-Px levels were highest when 0.5 mg / kg SS with 0.31% methionine and 0.3 mg / kg / mL with 0.31% methionine were placed in the diet. In treatments of 0.5 mg / kg SS and 23% of methionine, concentrations of selenium in eggs and serum as well as GSH-Px enzyme were higher. The results of this study showed that, increasing the SS content in the range of 0.3 to 0.5 mg / kg with added levels of methionine in the range of 0.23 to 0.31% to diet can be increased amount of selenium in eggs and serum. In addition, increase in the amount of GSH-Px in plasma was observed which can be led to strengthening the immune system and antioxidants. Spallholz (1990) reported that with balanced of dietary selenium concentration in broiler chickens, increasing of peroxidase concentration was observed in the blood. In some studies, the effect of selenium on the immune system with enhancement of both cell-mediated and humoral immune has been demonstrated.

It has been reported that in some viral diseases, the effect of selenium is not due to virus removal, but prevent genetic adaptations in the viral genomic RNA. It has been reported that over 20 types of seleno-proteins have been identified in the human body, which expressed by 25 genes in the human genome (Hatfield and Gladyshev., 2002; Lescure *et al*., 2002). Except for GSH-Px, other enzymes such as thioredoxin reductases and three iodothyronine deiodinases are also part of the selenoenzyme's family. Seleno-proteins P and W are other seleno-proteins that involved in antioxidant functions. Due to the immune function, there is also a specific seleno-protein in T cells called 15-kDa seleno-protein. Seleno-phosphate synthetase is another protein having selenium that speeds up the production of seleno-phosphate. This seleno-phosphate is involved as the main inorganic element to produce selenocysteine from the serine during seleno-protein synthesis. The afore-mentioned seleno-proteins could be suitable options for the involvement of selenium in the immune system. (Arthur, 2001; McKenzie *et al*., 2002). Lymphocytes that have selenium deficiency are less able to proliferate in response to mitogen, leukotriene B_4 is produced in macrophages which is essential for the activity of neutrophils, and the deficiency of selenium causes destruction of its production (McKenzie *et al*., 2002; Spallholz, 1990). It has been argued that selenium increases the production of IFN gamma and other cytokines, proliferation of T cell and increased the number of T helper cells (Broome *et al.*, 2004). Reffett *et al*. (1988) Reported that adding selenium may increase IgM level in calves. The influenza virus can be hampered by nutritional elements such as lysine, proline, ascorbic acid, green tea extract, N-acetyl cysteine, and selenium through the activity of neuraminidase (Willemsen *et al*., 2011).

Table 6. The effect of treatment (sodium selenite and methionine level interaction) during the time on Antibody titer against Newcastle of Iranian native broiler breeders (Anti log_2)

Item		Time		
Sodium selenite × Methionine level		70 wk	72 wk	74 wk
0	0.23	17.3^{d}	384^{abc}	106^{cd}
0	0.31	18.7^{d}	512^{bc}	341^{bcd}
0	0.33	3.3^{d}	426^{bcd}	298^{bcd}
0.3	0.23	8.7^{d}	298^{bcd}	47.7^{cd}
0.3	0.31	13.3^{d}	1066^{a}	192^{bcd}
0.3	0.33	16.0^{d}	298^{bcd}	106^{cd}
0.5	0.23	$4·00^{d}$	106^{cd}	53.3^{d}
0.5	0.31	12.0^{d}	597^{b}	128^{cd}
0.5	0.33	8.70^{d}	298^{bcd}	106^{cd}
P-value				
Treatment × Time		0.0001	0.0001	0.0001

Means with different superscripts significantly differ ($P < 0.05$).

Table 7. The effect of treatment (sodium selenite and methionine level interaction) during the time on Antibody titer against Influenza of Iranian native broiler breeders (Anti log_2)

Item		Time		
Sodium selenite × Methionine level		70 wk	72 wk	74 wk
0	0.23	18.7^{d}	170.7^{cd}	128^{cd}
0	0.31	26.7^{d}	682.7^{a}	256^{cd}
0	0.33	10.7^{d}	85.3^{d}	96.0^{d}
0.3	0.23	18.7^{d}	384^{bc}	64.0^{d}
0.3	0.31	18.7^{d}	256^{cd}	176^{cd}
0.3	0.33	64^{d}	170.7^{cd}	106^{d}
0.5	0.23	16^{d}	64^{d}	64.0^{d}
0.5	0.31	21.3^{d}	512^{ab}	213^{cd}
0.5	0.33	50.7^{d}	213.3^{cd}	74.7^{d}
P-value				
Treatment × Time		0.0001	0.0001	0.0001

Means with different superscripts significantly differ ($P < 0.05$)

It is concluded that 0.3 SS mg/kg with 0.33% methionine can be effectively used to increase salable chick without detrimental effects on performance and embryonic mortality. The results from this experiment showed that SS with methionine result in greater deposition of selenium in serum. High selenium and methionine contents of diet have been correlated with higher activity of GSH-PX in plasma. The current study demonstrated that supplementation of methionine to inorganic source of selenium could improve bioavailability of selenium and transfer rate of it.

References

Abdalla A, Yakout HM & Khalifah MM. 2005. Determination of lysine and methionine requirements of gimmizah strain during egg production stage. Proc. 3rd Inter. Poultry Conference Egypt.

Abd-EL-Samee MO, EL-Sheikh TM & Ali AM. 2007. An attempt to alleviate heat stress and improve laying hens performance and egg quality during summer season by using some amino acids or vitamins. Egypt. Poultry Science, 27: 875- 891.

Arthur JR. The glutathione peroxidases. 2000. Cellular and Molecular Life Sciences, 57: 1825–1835.

Boruta A, Oewierczewska E & Roszkowski T. 2007. Effect of organic and inorganic forms of selenium on the morphological composition of eggs and selenium content in egg mass. Medycyna Weterynaryjna (In Polish), 63: 238-241.

Brigelius-Flohe R.1999. Tissue-specific functions of individual glutathione peroxidases. Free Radical Biology and Medicine, 27: 951–965. DOI: 10.1016/S0891-5849(99)00173-2

Broome CS, McArdle F, Kyle JA, Andrews F, Lowe NM & Hart CA. 2004. An increase in selenium intake improves immune function and poliovirus handing in adults with marginal selenium status. The American Journal of Clinical Nutrition, 80: 154-62. DOI: 10.1093/ajcn/80.1.154

Bunchasak C & Silapasorn T. 2005. Effects of adding methionine in low-protein diet on production performance, reproductive organs and chemical liver composition of laying hens under tropical conditions. International Journal of Poultry Science, 4: 301-308. DOI:10.3923/ ijps.2005. 301.308

Carter TC. 1975. The hen's egg: Estimation of shell superficial area and egg volume, using measurements of fresh egg weight and shell length and breadth alone or in combination. British Poultry Science, 16: 541-543. DOI: 10.1080/00071667508416224

Cousins FB & Cairney M. 1961. Some aspects of selenium metabolism in sheep. Australian Journal of Agricultural Research, 12: 927–943. DOI: 10.4141/cjas68-045

Delezie E, Rovers M, Van der Aa A, Ruttens A, Wittocx S & Segers L. 2014. Comparing responses to different selenium sources and dosages in laying hens. Poultry Science, 93: 3083–3090. DOI: 10.3382/ps.2014-04301

Dziaczkowska L. 1980. The pattern for the assessment of turkey chicks for fattening. In polish.; Centre for Poultry Research and Development, Center for Water Fowl. Poznan.

Fairweather-Tait S & Hurrell RF. 1996. Bioavailability of minerals and trace elements. Nutation Research Review, 9: 295–324. DOI: 10.1079/NRR19960016

Hoffmann PR & Marla JB. 2008. The influence of selenium on immune responses. Molecular Nutrition and Food Research, 52: 1273–1280. DOI: 10.1002/mnfr.200700330

Hatfield DL & Gladyshev VN. 2002. How selenium has altered our understanding of the genetic code. Molecular and Cell Biology, 22: 3565–3576. DOI: 10.1002/mnfr.200700330

Hempe JM & Laukxen RC. 1988. Savage JE. Rapid determination of egg weight and specific gravity using a computerized data collection system. Poultry Science, 67: 902-907. DOI: 10.3382/ps.0670902

Jackson ME, Hellwig HM & Waldroup WP. 1987. Shell quality: Potential for improvement by dietary means and relationship with egg size. Poultry Science, 66: 1702–1713. DOI: 10.3382/ps.0661702

Jing CL, Dong XF, Wang ZM, Liu S & Tong JM. 2015. Comparative study of DL-[Se] Met vs SS and seleno-yeast on antioxidant activity and selenium status in laying hens. Poultry Science, 94:965–975. DOI: 10.3382/ps/pev045

Kazemi□Fard M, Kermanshahi H, Rezaei M & Golian A. 2013. Effect of different levels of fennel extract and vitamin D3 on performance, hatchability and immunity in post molted broiler breeders. Iranian Journal of Animal Science, 3: 735-745.

Kenyon S & Spring P. 2003. Egg selenium concentrations in breeder's hens fed Na – selenite or Sel Plex® supplemented diets. XthEuropean Symp. On the quality of eggs and egg poducts, September 23-26, Saint – Brieuc – Ploufragan, Cotes d' Armor France, pp. 182.

Knight SAB & Sunde RA. 1988. Effect of selenium repletion on glutathione peroxidase protein in rat liver. Journal of Nutrition, 118: 853-58

Kurz B, Jost B & Sch¨unke M. 2002. Dietary vitamins and selenium diminish the development of mechanically induced osteoarthritis and increase the expression of antioxidative enzymes in the knee joint of STR/1N mice. Osteoarthritis and cartilage, 10: 119–126. DOI: 10.1053/joca.2001.0489

Latshaw JD, Morishita TY, Sarver CF & Thilsted J. 2004. Selenium toxicity in breeding Ring-Necked pheasants (*Phasianus colchicus*). Avian Diseases, 48: 935-939. DOI: 10.1637/7177-030904R

Leeson S, Namkung H, Caston L, Durosoy S & Schlegel P. 2008. Comparison of selenium levels and sources and dietary fat quality in diets for broiler breeders and layer hens. Poultry Science, 87: 2605–2612. DOI: 10.3382/ps.2008-00174

Lescure A, Gautheret D & Krol A. 2002. Novel selenoproteins identified from genomic sequence data. In: Methods in Enzymology 347 (Sies, H. and Packer, L., eds.), pp.; 57–70. Academic Press, San Diego, CA.

McConnell KP & Cho GJ. 1965. Transmucosal movement of selenium. American Journal of Physiology, 208: 1191–1195. DOI: 10.1152/ajplegacy.1965.208.6.1191

McKenzie RC, Arthur JR & Beckett GJ. 2002. Selenium and the regulation of cell signaling, growth, and survival: molecular and mechanistic aspects. Antioxidants and Redox. Signaling, 4: 339–351. DOI: 10.1089/152308602753666398

Nassiri-Moghaddam H, Kazemi-Fard M, Agah MJ, Hosseini SJ & Mirakzehi MT. 2012. Effect of different levels of methionine, protein and tallow on the productive performance and egg quality of laying hens in the late-phase production. Brazilian Journal of Poultry Science, 14: 149-158. DOI: 10.1590/S1516-635X2012000200010

Naulia AH & Singh MA. 2002. Effect of dietary protein and supplemental fat on performance of laying hens. International Journal of Poultry Science, 4: 1986-1989. DOI: 10.3923/ijps.2005.986.989

Ort JF & Latshaw JD.1978. The toxic level of SS in the diet of laying hens. Journal of Nutrition, 108: 1114–1120.http:// doi:10.1093/jn/108.7.1114

Ousterhout LE. 1980. Effects of calcium and phosphorus levels on egg weight and egg shell quality in laying hens. Poultry Science, 59: 1480-1484. DOI: 10.3382/ps.0591480

Pappas AC, Acmovic T, Sparks NHC, Surai PF & McDevitt RM. 2006. Effects of supplementing broiler breeders diets with organoselenium compounds and polyunsaturated fatty acids on hatchability. Poultry Science, 85: 1584–1593. DOI: 10.3382/ps.0591480

Payne RL & Southern LL. 2005. Changes in GSH-Px and tissue selenium concentrations of broilers after consuming a diet adequate in selenium. Poultry Science, 84: 1268–1276. DOI: 10.1093/ps/84.8.1268

Payne RL, Lavergne TK & Southern LL. 2005. Effect of inorganic versus organic selenium on hen production and egg selenium concentration. Poultry Science, 84: 232–237. DOI: 10.1093/ps/84.2.232

Petersen CF, Sauter EA, Steele EE & Parkinson JF. 1983. Use of methionine intake restriction to improve egg shell quality by control of egg weight. Poultry Science, 62: 2044–2047. DOI: 10.3382/ps.0622044

Reffett JK, Spears JW & Brown TT. 1988. Effect of dietary selenium on the primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus. Journal of Nutrition, 118: 229–235. DOI: 10.1093/jn/118.2.229

Reis RN, Vieira SL, Nascimento PC, Pena JE, Barros R & Torres CA. 2009. Selenium contents of eggs from broiler breeders supplemented with SS or zinc-l-selenium methionine. Journal of Applied Poultry Research, 18: 151–157. DOI: 10.3382/japr.2008-00069

Robinson MF & Thomson CD. 1983. The role of selenium in the diet. Nutrition Abstracts and Reviews, 53. 3-26.

Safaa HM, Serrano MP, Valencia DG, Arbe X, Jime´nez-Moreno E, La´zaro R & Mateos GG. 2008. Effects of the levels of methionine, linoleic acid, and added fat in the diet on productive performance and egg quality of brown laying hens in the late phase of production. Poultry Science, 87: 1595–1602. DOI: 10.3382/ps.2008-00005

SAS Institute. 2003. SAS/STAT 9.1.3 User's Guide. SAS Inst. Inc., Cary, NC.

Susanne B, Erik HL, Jens JS, Knut F, Kim O, Lars CS & Sven M. 2008. Absorption, excretion, and retention of selenium from a high selenium yeast in men with a high intake of selenium. Food Nutrition Research, 52: 10.3402.

Skrivan M, Bubancova I, Marounek M & Dlouha G. 2010. Selenium and alpha-tocopherol content in eggs produced by hens that were fed diets supplemented with selenomethionine, SS and vitamin E. Czech. Journal of Animal Science, 55: 388–397. DOI: 10.17221/92/2010-CJAS

Spallholz JE. 1990. Selenium and glutathione peroxidase: essential nutrient and antioxidant component of the immune system. Advances in Experimental Medicine and Biology, 262: 145–158.

Szeleszczuk P, Karpinska E, Bielecki W, Kosowska G &Borzemska W. 2004. Evaluation of lithium and selenium toxicity for chicken embryos and one-day-old chicks. Medycyna Weterynaryjna, 60: 492-495.

Tahmasbi AM, Mirakzehi MT, Hosseini SJ, Agah MJ & Kazemi-Fard M. 2012. The effects of phytase and root hydroalcoholic extract of Withania somnifera on productive performance and bone mineralization of laying hens in the late phase of production. British Poultry Science, 53: 204-214. DOI: 10.1080/00071668.2012.662628

Utterback PL, Parsons CM, Yoon I & Butler J. 2005. Effect of supplementing selenium east in diets of laying hens on egg selenium content. Poultry Science, 84: 900–1901. DOI: 10.1093/ps/84.12.1900

Vendeland SC, Butler JA & Whanger PD. 1992. Intestinal absorption of selenite, selenate, and selenomethionine in the rat. Journal of Nutrition Biochemistry, 3: 359-365. DOI: 10.1016/0955-2863(92)90028-H

Vendeland SC, Deagen JT & Whanger PD. 1996. Uptake of selenotrisulfides of glutathione and cysteine by brush border membranes from rat intestines. Journal of Inorganic Biochemistry, 47: 131-140.

Waschulewski H & Sunde RA. 1988. Effect of dietary methionine on utilization of tissue selenium from dietary selenomethionine for glutathione peroxidase in the rat. Journal of Nutrition, 118: 367-374. DOI:10.1093/jn/118.3.367

Waschulewski H & Sunde RA. 1988. Effect of dietary methionine on tissue selenium and glutathione peroxidase (EC 1.11.1.9) activity in rats given selenomethionine. British Journal of Nutrition, 60: 57-68. DOI: 10.1093/jn/118.3.367

Willemsen H, Swennen Q & Everaert N. 2011. Effects of dietary supplementation of methionine and its hydroxy analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperatures. Poultry Science, 90: 2311-20. DOI: 10.3382/ps.2011-01353

Wolffram S, Berger B, Grenacher B & Scharrer E. 1989. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. Journal of Nutrition, 119: 706-712. DOI: 10.1093/jn/119.5.706

Xue X, Wang Y, Liu W, Ju T & Zhan X. 2017. Effects of different methionine sources on production and reproduction performance, egg quality and serum biochemical indices of broiler breeders. Asian-Australian Journal of Animal Science, 30: 828–833. DOI: 10.5713/ajas.16.0404

Yoon I, Werner T & Butler J. 2007. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poultry Science, 86: 727–730. DOI: 10.1093/ps/86.4.727