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Effects of selenium and flaxseed on selenium content and antioxidant properties of eggs and immune response in hens

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Abstract

This study aimed to evaluate effects of supplementing the diets of laying hens with different sources of Se and different levels of flaxseed on egg Se concentration, yolk antioxidant properties and immune response. In a completely randomized design, 384 Hy-Line W-36 hens (aged 50 weeks) were assigned to a 3 × 4 factorial arrangement, comprising four replicates of eight hens. For 10 weeks, the birds were fed one of three basal diets containing 0, 50, and 100 g/kg of flaxseed and regular Se content from mineral premix, supplemented with no additional Se (SN) and 1 mg/kg of Se from sodium selenite (SS), Se-enriched yeast (SY), and hydroxy selenomethionine (SOH). The greatest Se concentration in the yolk was observed in diets containing organic Se sources. The SOH diet produced the greatest albumen Se concentration, followed by SY, and then SS. Using any Se supplementation improved glutathione peroxidase activity and total antioxidant capacity, compared with SN. However, superoxide dismutase activity and malondialdehyde were not affected by Se supplementation. Cell-mediated immune response was improved by Se supplementation, especially SS. Antibody response against sheep red blood cell (SRBC) was not affected by Se source, except that IgM was greater in the hens that received SS and SY. Neither flaxseed supplementation, nor its interaction with Se source was significant for any trait. Thus, supplementation of diets with 1 mg/kg Se, especially from organic sources, enriched the egg and improved some yolk antioxidant properties and immune system function. Supplemental flaxseed did not influence any measured traits.

Keywords: egg albumin, egg yolk, Immune function, inorganic selenium, organic selenium
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Introduction

Eggs are an important foodstuff in basic nutrition, serving as a source of protein, fat, and micronutrients. Manipulation of egg yolk composition through improving its polyunsaturated fatty acid (PUFA) content, particularly omega-3 fatty acids, is considered a common goal in developing eggs as a functional food (Miranda *et al*., 2015). Flaxseed oil, which contains about 70% PUFA, mostly alpha-linolenic acid (ALA), and has the highest PUFA/SFA ratio among oilseeds, is known to be an appropriate source to increase omega-3 in diets (Zambiazi *et al*., 2007).

Enrichment of egg yolk with omega-3 PUFA is often associated with increased susceptibility to lipid peroxidation, owing to increased free radical production, especially in high stress conditions. To protect the yolk, tissues and cells from oxidative damage, dietary supplementation can be implemented, with suitable doses of natural antioxidants, including vitamin E, carotenoids, and Se. Antioxidants may be transferred from the feed into the egg yolk, thus producing antioxidant-enriched eggs (Surai & Fisinin, 2012).

Supplementing animal feeds with Se can provide a means to increase human Se intake. As an antioxidant, Se is likely to contribute to better shelf-life in animal products. The most important contributor to its protective antioxidant function is glutathione peroxidase (GSH-Px), which acts against harmful reactive oxygen species at various sites in the body, including the gastrointestinal tract, plasma, and in biological membranes (Fasiangova & Borilova, 2017).

Sodium selenite (SS) is the most common inorganic form of Se supplementation because of its low cost, but it has limited biological utilization. Organic Se compounds such as selenomethionine (Se-Met) and selenocysteine (Se-Cys) are part of proteins, and have higher bioavailability and lower toxicity (Surai &

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Fisinin, 2014; Jing *et al*., 2015). Some new sources of organic selenium supplements are currently being used in poultry nutrition (Suchy *et al*., 2014), such as Se-enriched yeast (SY), Se-enriched unicellular alga chlorella, and Se chelates such as Zn-L-Se-Met (zinc selenomethionine), with Se-Met as the major component. Recently, a new organic Se source, known as 2-hydroxy-4 methylselenobutanoic acid (HMSeBA or hydroxyl Se-Met or Selisseo) (SOH), has been developed and is reported to have increased bioavailability compared with SY and SS (Jlali *et al*., 2014).

Numerous studies have found that the level and form of Se can variously affect the egg Se reserves, antioxidant stability, and the immunity response of birds. According to European regulations for feed additives, the suitable amount of Se supplementation is 0.3 mg/kg of complete feed. However, other studies have found that a higher level of this element can be used in diets for poultry, resulting in better antioxidant properties and higher Se content in eggs (Suchy *et al*., 2014; Fasiangova & Borilova, 2017; Fasiangova *et al.*, 2017). Supplementation with SY increased the Se content in eggs, especially in albumen, by up to twice the level that was achieved using SS (Aljamal, 2011).

It has been suggested that the thiobarbituric acid reactive substance (TBARS) content in eggs is lower in hens that were supplemented with extra Se as a result of the increased glutathione (GSH) content and GSH-Px activity. However, the ameliorative effects of inorganic Se supplementation were mitigated during long periods of storage (Skrivan *et al*., 2010; Wang *et al*., 2010). Antioxidant stability of chicken was improved in the birds that received SY compared with those that received SS, again through higher GSH-Px, SOD, and total antioxidant capacity (TAC) (Suchy *et al.*, 2014). Selenium is also essential for the activity of human and animal immune systems (Alhidary *et al*., 2015). It is necessary for the metabolism of prostaglandins, stimulation of neutrophil function, and proliferation of T and B lymphocytes (Pappas *et al*., 2005; Fasiangova & Borilova, 2017). A deficiency of Se affects humoral immunity response, resulting in reduced levels of the IgG and IgM antibodies. Organic forms of Se are said to be more effective than the inorganic forms in improving immune functions in broilers (Suchy *et al.*, 2014).

In most studies that assess Se deposition in egg yolk and albumen, Se has been supplemented at 0.3 to 0.5 mg/kg of the diet for three to eight weeks (Fasiangova *et al*., 2017). In this study, Se was added to the diet at 1 mg/kg for a 10-week trial period to assess the effects of supplementing the diet of laying hens with various sources of Se, together with flaxseed, on egg Se deposition, yolk antioxidant status, and immune response of the birds. Haug *et al*. (2007) reported that a high dietary selenium dosage resulted in a higher concentration of the long-chain omega-3 fatty acids in eggs, and suggested that a high selenium intake might increase the synthesis of long-chain omega-3 fatty acids from alpha-linolenic acid (ALA) or decrease their rate of degradation. Pappas *et al*. (2004) found an increase in the selenium concentration of fertile eggs from broiler breeders fed fish oil. They suggested that omega-3 fatty acids may affect selenium metabolism and deposition in the embryo rather than selenium absorption from the diet. The authors could not find any reports on the effect of dietary omega-3 fatty acid on the selenium enrichment of eggs. This study was designed to investigate how diets that contained various levels of flaxseed as an omega-3 source might affect the selenium enrichment of eggs produced by layers that were fed selenium from sodium selenite, Se-enriched yeast, and hydroxy selenomethionine.

Materials and Methods

The care and use of animals for this study was approved by the Animal Welfare Committee of the University of Mohaghegh-Ardabili, Iran.

A total of 384 Hyline-W36 laying hens, aged 50 weeks, were assigned to a 3×4 factorial arrangement of treatments, comprising four replicates of eight birds for a 10-week trial. Birds were randomly placed in wire cages, so that each replicate consisted of two adjacent cages with separated feeder and four hens in each. The chemical composition of flaxseed in this experiment is presented in Table 1. Three iso-energetic and iso-nitrogenous basal diets, containing 0 g/kg, 50 g/kg and 100 g/kg of flaxseed, were formulated to meet the nutritional requirements of hens based on Hyline-W36 commercial management guides (Table 2). The basal diets, which contained 0.2 mg/kg Se from a mineral premix, were augmented with no additional Se, with 1 mg/kg of Se from SS, with 1 mg/kg of Se from SY (Sel-Plex, Alltech Int.) and with 1 mg/kg of Se from HMSeBA or hydroxy-selenomethionine (SOH) (Selisseo, Adisseo Co. Ltd., Beijing, PRC). Feed and water were provided ad libitum. The temperature and relative humidity of the house were approximately 24–26 °C and 50–60%, respectively. A lighting schedule of 16 hours light and 8 hours dark was implemented.

Table 1 Chemical composition of flaxseed

Items	g/kg
Fat	342
Crude Protein	221
Fibre	268
Ash	55
Water	114
Fatty acids (% of total fatty acids)	
Palmitic acid, 16:0	6.4
Stearic acid , C18:0	4.5
Oleic acid , C18:1	21.8
Linoleic acid , C18:2	15.2
α-Linolenic acid, C18:3	52.1
Saturated fatty acids	10.9
Mono-unsaturated fatty acids	21.8
Poly-unsaturated fatty acids	67.3

To assess the chemical characteristics, three eggs were collected randomly from each replicate at the end of the fifth and tenth weeks of the experiment to determine their Se concentration and in the tenth week for other characteristics. Egg yolk and egg albumen of the samples were separated using a manual egg yolk separator. The yolks and the albumen from the same replicate were pooled separately, homogenized well using a blender, and stored in plastic micro-tubes at -20 °C until analysed. To determine the Se concentration in the eggs, the samples were dried at 65 °C for 12 hours and ground. Briefly, 0.5 g of egg yolk or egg albumen was digested in a mixture of 1 ml HNO3 and 9 ml deionized water until the solution was clear. Then, the solution was diluted with deionized water to a final volume of 25 ml. Se concentration of the sample was determined using an inductively coupled plasma-mass spectrometer (ICP/MS, Agilent 7500cx, Tokyo, Japan).

The homogenized yolks that had been stored were analysed at the end of the experiment to assess lipid peroxidation and antioxidant status. TBARS analysis was performed with some modification to determine MDA level. TBA levels were expressed as milligrams of MDA per kilogram of yolk (Salih *et al*., 1987). 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity as inhibition concentration (IC%) was determined to evaluate the TAC of yolks (Brand-Williams *et al*., 1995). The assay of SOD activity was performed using the method of inhibition of epinephrine auto-oxidation in alkaline medium and measuring the absorbance of the resulting product at 340 nm. The result was expressed as a value of unit per gram of extracted protein (U/g) (Wawrzykowski & Kankofer, 2011). Glutathione peroxidase activity in the yolk was measured indirectly by a coupled reaction with glutathione reductase. Glutathione peroxidase catalyses oxidation of glutathione (GSH) in the presence of tert-Butyl hydroperoxide as a substrate. Oxidized glutathione is then reduced in the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), and NADPH is oxidized to create NADP. The oxidation process is accompanied by a decrease in light absorbance at 340 nm. This GSH-Px activity was expressed as unit per gram of extracted protein (U/g) (Paglia & Valentine, 1967; Aljamal, 2011).

SRBC were used as an antigen to assess humoral immune response. At the end of the experiment, two hens from each replicate were randomly selected and injected intravenously (brachial vein) with 0.2 ml 9% SRBC diluted in PBC. After one week, 2 ml of blood samples were taken from each bird. The blood was then centrifuged (2500 × g, 10 min, 17 °C). The serum was used to determine the antibody level against SRBC by the hemagglutination test (Ambrose & Donner, 1973; Heckert *et al*., 2002). IgG and IgM were also measured according to the SRBC method and microtiter was done using mercaptoethanol for agglutination (Delhanty & Solomon, 1966; Isakov *et al*., 2005).

Table 2 Ingredients and nutrient composition of iso-energetic and iso-nitrogenous basal diets with different amounts of flaxseed (as-fed)

Ingredients (g/kg)	Diet 1	Diet 2	Diet 3
Corn	489.5	475.3	461.0
Soybean meal (44% CP)	243.5	220.0	196.6
Wheat	100.0	100.0	100.0
Flaxseed	0.0	50.0	100.0
Oyster shell	109.5	109.4	109.3
Dicalcium phosphate	18.8	18.7	18.5
Poultry oil	27.0	15.0	3.1
Sodium chloride	3.9	3.8	1.7
DL-Methionine	1.8	1.8	1.7
Vitamin premix[1]	3.0	3.0	3.0
Mineral premix[2]	3.0	3.0	3.0
Calculated analysis			
Metabolizable energy (Kcal/kg)	2750	2750	2750
Crude protein	154.5	154.5	154.5
Calcium	44.2	44.2	44.2
Available phosphorus	4.6	4.6	4.6
Sodium	1.9	1.9	1.9
Arginine	11.2	11.4	11.6
Lysine	8.4	8.2	8.0
Methionine	4.3	4.3	4.4
Methionine + Cysteine	6.7	6.7	6.8
Threonine	6.0	6.0	5.9

[1] Provided per kg of diet: vitamin A: 8,800 IU; vitamin D3: 2,500 IU; vitamin E: 11 IU; vitamin K3: 2.2 mg; thiamine: 1.5 mg; riboflavin: 4 mg; pyridoxine 2.5 mg; pantothenic acid: 8 mg; nicotinic acid: 35 mg; folate: 0.48 mg; cyanocobalamin, 0.01 mg; choline chloride: 200 mg
[2] Provided per kg of diet: Mn (MnSO4, H2O): 75 mg; Zn (ZnO): 64 mg; Fe (FeSO4, H2O): 75 mg; Cu (CuSO4, 5H2O): 6 mg; I (KI): 0.86 mg; and Se (Na2SeO3): 0.2 mg.

Cell-mediated immune response was measured as the reaction to 2,4-dinitrochlorobenzene (DNCB). At the end of the experiment, two birds from each replicate were randomly selected and 0.1 ml DNCB was applied to an approximately 10 cm^2 area of skin without feathers on the left lateral abdomen. The corresponding area on the right side was treated solely with the solvent (a mixture of acetone and olive oil). Skin thickness on both sides was measured with a digital calliper before and 24 hours after the challenge. The increase in skin thickness was calculated as the difference before and after the challenge (Verma *et al.*, 2004).

Data related to immune response and yolk antioxidant properties were analysed by a general linear model (GLM) procedure for a completely randomized experimental design using SAS software (Version 9.1; SAS Institute, Inc.). The Se concentration of the yolk and albumen were analysed as repeated measures using the MIXED procedure of SAS. Means were compared using Tukey's Test. Effects were considered significant at $P \leq 0.05$.

Results

The Se concentrations of egg yolks and albumen are summarized in Table 3. Because no interactions were found between Se source and flaxseed, only the main effects are presented. In addition, these traits were not affected by flaxseed level in the diets. However, the Se dietary treatments resulted in significantly different Se concentration in egg. The greatest concentration of Se in the yolk was observed with diets containing SOH and SY, which were different from SS (P <0.01). The albumen Se concentration in the

albumen was greatest in response to the diet supplemented with SOH, followed by SY and then SS (*P* <0.01). Thus, feeding the hens SS, SY and SOH caused about 133%, 215% and 231% relative increases in the Se content in yolks, respectively. Similarly, the relative increases in Se concentrations in the albumen were 57%, 229%, and 265%, respectively.

Table 3 Means (± SE) of selenium concentrations observed in egg yolk and albumin (dry matter basis) for selenium source and flaxseed supplementation

Main effects	Selenium concentration (µg/g)	
	Egg yolk	Egg albumen
Flaxseed		
0	1.54 ± 0.03	2.57 ± 0.06
5	1.55 ± 0.03	2.61 ± 0.06
10	1.55 ± 0.03	2.68 ± 0.06
Selenium (Se) source		
SN	0.63^{c} ± 0.03	1.10^{d} ± 0.07
SS	1.47^{b} ± 0.03	1.73^{c} ± 0.07
SY	1.99^{a} ± 0.03	3.62^{b} ± 0.07
SOH	2.09^{a} ± 0.03	4.02^{a} ± 0.07
	P–values	
Flaxseed	0.93	0.23
Se source	<0.0001	<0.0001
Flaxseed × Se source	0.49	0.54
Time	0.07	<0.0001
Flaxseed × time	0.72	0.56
Se sources × time	0.62	0.0001
Flaxseed× Se sources × time	0.15	0.37

$^{a-d}$ Column means with different superscripts differ significantly (P<0.01)
SN: without selenium, SS: selenium selenite, SY: Se-enriched yeast, SOH: hydroxy selenomethionine

Antioxidant indices at the end of the trial are shown Table 4. Glutathione peroxidase activity was increased by supplementation of Se. Birds fed SOH and SY had increased TAC compared with birds that had not received supplemental Se (P<0.01). SOD activity and MDA level were not affected by Se source. Nor was any effect of flaxseed observed. Neither were interactions found between flaxseed and Se source.

Indicators of the immune response of the hens at the end of the trial are shown in Table 5. The interactions between the level of flaxseed in the diets and sources of Se and the main effect of flaxseed were not significant. Antibody response against SRBC was not affected, except IgM was greater in birds fed diets containing SS and SY than those fed SN (P<0.05). Based on DNCB, birds that were provided with supplemental SS had increased cell-mediated response when compared with birds that received no supplemental Se (P<0.01). Interactions between flaxseed and Se source were not observed.

Discussion

The results support the general conclusion that Se concentration in eggs is a consequence of both dietary level and form of Se. Some reports suggested that Se sources such as SY produce greater deposition of Se in the whole egg compared with SS (Chinrasri *et al*., 2009; Briens *et al*., 2013; Fasiangova *et al*., 2017). It is believed that organic sources have a higher absorption efficiency compared with SS, with 50–75% of it being excreted (Hu *et al*., 2012). Chinrasri *et al*. (2009) reported that organic Se-Met is actively absorbed from the gut, and can be incorporated directly into body tissues and in products such as eggs as effectively as methionine, whereas inorganic forms are absorbed passively and are probably required for Se-Cys synthesis. Inorganic Se compounds are used primarily in the Se metabolism pathway to synthesize seleno-protein and not to replenish Se deposits in existing tissues.

Table 4 Means (± SE) of indicators of antioxidant content of egg yolk for selenium source and flaxseed supplementation

Main effects	MDA[1] (µg/g)	GSH-Px[1] (U/g protein)	SOD[1] (U/g protein)	TAC[1] (% IC)
Flaxseed				
0	0.56 ± 0.02	23.42 ± 1.06	89.78 ± 4.36	21.46 ± 1.11
5	0.55 ± 0.02	23.08 ± 1.06	85.52 ± 4.36	20.55 ± 1.11
10	0.59 ± 0.02	22.66 ± 1.06	83.30 ± 4.36	20.53 ± 1.11
Selenium (Se) source[2]				
SN	0.55 ± 0.02	18.21^{b} ± 1.23	87.20 ± 5.03	16.77^{c} ± 1.28
SS	0.59 ± 0.02	23.21^{a} ± 1.23	84.86 ± 5.03	19.73^{bc} ± 1.28
SY	0.54 ± 0.02	25.13^{a} ± 1.23	90.44 ± 50.3	22.14^{ab} ± 1.28
SOH	0.60 ± 0.02	25.42^{a} ± 1.23	82.29 ± 5.03	24.76^{a} ± 1.28
		P-values		
Flaxseed	0.32	0.88	0.57	0.79
Se source	0.09	0.0006	0.70	0.0007
Flaxseed × Se source	0.78	0.95	0.94	0.93

[a-d] Column means with different superscripts differ significantly (P<0.01)
[1] MDA: malondialdehyde, GSH-Px: glutathione peroxidase activity, SOD: superoxide dismutase, TAC: total antioxidant capacity (% Inhibition concentration)
[2] SN: without selenium, SS: selenium selenite, SY: Se-enriched yeast, SOH: hydroxy selenomethionine

Table 5 Means (± SE) of indicators of immune response by laying hens for selenium source and flaxseed supplementation

Main effects	SRBC[2]	IgG	IgM	DNCB[2]
Flaxseed				
0	6.56 ± 0.25	5.23 ± 0.23	1.31 ± 0.11	0.453 ± 0.017
5	6.50 ± 0.25	5.25 ± 0.23	1.25 ± 0.11	0.426 ± 0.017
10	6.75 ± 0.25	5.50 ± 0.23	1.25 ± 0.11	0.404 ± 0.017
Selenium (Se) source[1]				
SN	6.33 ± 0.29	5.42 ± 0.27	0.91^{b} ± 0.13	0.366^{b} ± 0.020
SS	6.66 ± 0.29	5.25 ± 0.27	1.41^{a} ± 0.13	0.490^{a} ± 0.020
SY	6.75 ± 0.29	5.44 ± 0.27	1.31^{a} ± 0.13	0.440^{ab} ± 0.020
SOH	6.66 ± 0.29	5.33 ± 0.27	1.33^{ab} ± 0.13	0.414^{ab} ± 0.020
		P-values		
Flaxseed	0.765	0.613	0.902	0.160
Se source	0.750	0.924	0.027	0.001
Flaxseed× Se source	0.741	0.711	0.137	0.768

[a-b] Column means with different superscripts differ significantly (P<0.05)
[1] SN: without selenium, SS: Selenium selenite, SY Selenium -enriched yeast, SOH: hydroxy selenomethionine
[2] SRBC: sheep red blood cell, DNCB: 2, 4-dinitro 1-chlorobenzene

In this study, organic Se sources improved the Se deposition in the egg yolk and albumen. The relative increase of Se in the eggs produced by hens that consumed SOH was greater than that of those that consumed SY. In fact, SOH is a relatively new organic Se source, with increased bioavailability compared with SY and SS, which has been introduced to the animal feed industry. Also, the increased efficacy of SOH

may arise from its greater purity (99%) as a precursor of Se-Met, compared with SY, which contains about 60–65% Se in the form of Se-Met, and the remaining 37% is bound in other forms or as inorganic Se (Jlali *et al*., 2014). In fact, organic Se is mostly retained in the body, but there is a high excretion of inorganic Se through the gut and from the tissues (Rajasekaran & Kalaivani, 2015).

It has been shown that SOH influences the egg yolk quality positively through providing a greater Se supply to the egg (Tufarelli *et al*., 2015). Previous researchers demonstrated that the organic and inorganic forms of Se were deposited primarily in egg albumen and yolks, respectively (Fasiangova *et al*., 2017; Pappas *et al*., 2006). Together with the results of the present study, the previous research suggested that SOH had a remarkable tendency towards egg Se deposition, especially in the albumen. However, relatively greater Se enrichment of the yolk relative to albumen has been reported from SY (Pappas *et al*., 2006).

Gurbuz *et al*. (2012) provided SY and SS to hens at 0.3 mg/kg diet, and observed that Se content of the eggs produced by hens that were given SY was greater on the 60th and 90th days of the experiment, with no difference being observed the 30th day. Kralik *et al*. (2016) showed that albumen Se content was affected by supplementation with a low level of Se-Met by the 26th day of the experiment, but the yolk was not affected at that time. It has also been shown that both the concentration and form of dietary Se can influence Se content of the yolk, so that organic sources supplemented at a higher level (0.6 ppm compared with 0.3 ppm) resulted in greater deposition (Asadi *et al*., 2017). In confirmation of results from Jlali *et al*., 2014, the present results show that Se deposition in the yolk and albumen increases 3- to 3.5-fold as a consequence of supplementation with organic sources, while SS increased Se deposition in the yolk more than in albumen (approximately 2.5-fold versus1.5-fold increase rate).

Dalle-Zotte *et al*. (2015) reported that malondialdehyde (MDA) index increased in birds that were fed omega-3 PUFA sources such as flaxseed. However, it has been reported that omega-3 PUFA enhances the antioxidative status, such as GSH-Px activity and TAC, and decreases lipid peroxidation in the serum of laying hens (Ebeid, 2011). Further, flaxseed-augmented diets that were fed to geese improved liver SOD and GSH-Px in their progeny (Chen *et al*., 2015). Increased dietary omega-3/omega-6 PUFA may increase antioxidant capacity and alleviate inflammation by inhibiting synthesis of omega-6 PUFA (Chen *et al*., 2015). However, no effect was observed in the current study of dietary n-3 PUFA from flaxseed on the antioxidant indices in the yolk and plasma of the birds. This study was conducted with fresh eggs, so possibly changes might become apparent only after a period of storage, during which more peroxidation occurs in the yolk.

Contrary to other studies (Mohiti-Asli *et al*., 2008; Wang *et al*., 2010, Fasiangova & Borilova, 2017) that indicated effects of Se supplementation on the antioxidant content of serum or yolk, in this study neither MDA nor SOD responded differently to the Se sources. However, the present results are consistent with the findings of other researchers (Petrovic *et al*., 2006; Lesson *et al*., 2008). In contrast, TAC and GSH-Px increased when Se was supplemented in this study. Among indices of antioxidant status in biological systems, TAC offers an integrated measure of enzymatic and non-enzymatic antioxidants, and it is assumed to be a better index of biological function than a single antioxidant (Mahmoud & Hijazi, 2007). The results from the current study for TAC are consistent with the effect of Se supplementation, especially from organic sources, enhancing antioxidant response. According to Boostani *et al*. (2015), antioxidant enzyme activities such as GSH-Px act as a protective response to oxidative stress in broilers and increase when the diet is supplemented with Se. In addition, Se supplementation can lower the level of MDA. However, Buckiuniene *et al*. (2018) reported that MDA content in yolks is greater when the diet is supplemented with Se-Met. A plausible explanation for these differences among studies may be that when birds are not exposed to sufficient stress, there is less opportunity for Se supplementation to be effective.

The lack of differences in GSH-Px that were observed in this study may result from both the organic and inorganic sources being converted to Se-Cys, which, in turn, leads to synthesizing additional seleno-protein enzymes (Papazyan *et al*., 2006). Rajashree *et al*. (2014) found the activity of GSH-Px in the albumen and yolk to be greater with dietary organic Se compared with Se from an inorganic source. However, some researchers reported that GSH-Px activity in laying hens was not affected by the source and dose of Se (Lesson *et al*., 2008; Delezie, 2014). It has also been observed that SS may be regarded as a pro-oxidant, which can stimulate lipid peroxidation, and thus decreases the absorption of various nutrients such as antioxidants (Papazyan *et al*., 2006; Skrivan *et al*., 2010).

In avian species, SOD and GSH-Px activities that function in cell adaptation to stress conditions such as oxidative stress and metabolic pressure can be influenced by dietary Se levels and forms (Chen & Wu, 2014; Surai, 2016). Greater SOD and GSH-Px activities and TAC and reduced MDA content were found in plasma of laying hens fed SY and Se-Met compared with hens fed SS (Chen & Wu, 2014; Jing *et al*., 2015; Surai, 2016). Furthermore, Se supplementation increases SOD activity and TAC in the serum of laying hens, probably owing to the synergy of Se with vitamins E and A (Zdunczyk *et al*., 2013). In agreement with the present results, Jing et al. (2015) observed that organic Se sources have greater ability to increase GSH-Px activity in the albumen and yolk, compared with SS. However, Jing *et al*. (2015) observed no difference in

SOD activity between organic Se and SS. The current results confirm the findings of Petrovic *et al*. (2006), who demonstrated that GSH-Px activity in various tissues depended on there being Se in the diet rather than on the source or amount of supplementation.

It has been suggested that Se sources, especially organic ones, and PUFA may interact. This interaction may occur through Se-dependent GSH-Px, which plays an important role in regulating the biosynthesis of prostaglandins from their precursor eicosanoids. However, the precise nature of this role is not fully understood (Mohiti-Asli *et al*., 2008). No interactions between flaxseed and Se source were observed in this study that affect GSH-Px.

Diets that are high in omega-3 PUFA reportedly increase serum IgG of hens and egg IgY, with the dietary ratio of omega-6/omega3 PUFA being the main factor in modulating IgG formation (Ebeid, 2011; Cherian & Quezada, 2016). The yolks of eggs from hens that were fed flaxseed oil or ground flaxseed have also been shown to be enriched in omega-3 fatty acids. (Ehr *et al*., 2017). Enriching eggs with omega-3 PUFA may enhance their antioxidant status and reduce lipid peroxidation, which may improve the immune response in laying hens. The protective effect of dietary omega-3 fatty acids from sources such as fish oil may be associated with EPA as a metabolite of arachidonic acid (Huang, 2016). The ratio of omega-6/omega3 PUFA can also influence cell-mediated immunity, but not the antibody response in broiler breeders (Khatibjoo et al., 2011). Fu *et al*. (2017) indicated that greater levels of linoleic acid, as the main omega-6 fatty acid in the diet, can undermine the immune response, while higher levels of omega-3 sources such as fish oil enhance the antibody titre, especially IgM in broilers. Although these references provide a good body of evidence that exhibits the effect of flaxseed on the functionality of immunity in poultry, no such response was recorded in this study.

Selenium is known to be vital for stimulation of neutrophil function, as well as the functioning of T and B lymphocytes in humans and animals. In fact, GSH-Px acts in cellular redox and other physiological reactions such as signal transduction and regulation of pro-inflammatory cytokine production (Pappas et al., 2005; Fasiangova & Borilova, 2017). It was demonstrated that GSH-Px activity can improve immune response through the production of antibody titre in aged laying hens, as a result of its antioxidant properties. In addition, GSH-Px tends to protect neutrophils from oxygen-derived radicals (Ebeid, 2011). It was also indicated that the interaction of Se and vitamin E affects antibody production against SRBC, including the total IgG and IgM in laying hens (Ziaei *et al*., 2013). Although the main cause of this influence is unknown, it may be due to their antioxidant properties.

It has been suggested that the insufficiency of Se affects humoral immune response, which is manifest as reduced levels of IgG and IgM antibodies, and that organic forms of supplemental Se are more effective than inorganic ones in improving immune functions in broilers (Suchy *et al*., 2014). Likewise, dietary supplementation of nano-Se increased humoral and cellular immune responses compared with SS and control groups in growing layers (Mohapatra & Swain, 2014). According to Boostani *et al*. (2015), oxidative conditions can decrease the weights of lymphoid organs and the negative effect may be attenuated by dietary supplementation of Se. They observed increased levels of IgG and IgM from supplementation with either organic Se or nano-Se. In contrast, Mohiti-Asli *et al*. (2010) observed that neither SY nor SS could alleviate the low level of SRBC caused by heat stress in laying hens.

The results of this study, for the most part, agree with the existence of an effect of Se supplementation on cellular immune function. Humoral immunity, contrary to these studies, total SRBC and IgG remained unchanged in the present study and only IgM was positively affected. Thus for humoral immunity, organic and inorganic Se forms were equally effective. This result confirms the findings by Boostani *et al*. (2015) that organic Se can result in higher serum IgM concentration in broilers. The presence of Se, irrespective of source, is an important factor in immune function and organic and inorganic forms have the same bioavailability.

Conclusion

No interactions were observed between dietary selenium sources and flaxseed levels that affected the selenium content in eggs, antioxidant status of egg yolk, and immune response of the laying hens. Nor were any effects of adding up to 100 g/kg flaxseed to the diet observed for these traits. However, dietary inclusion of Se at a greater level (1 mg/kg diet) than is normally used could improve Se deposition in the egg without considerable adverse effects. Organic sources, especially hydroxyl Se-Met, produce greater reserves in eggs, particularly in albumen, so it may be a better choice for enrichment of eggs. Selenium supplementation may also improve antioxidant status and some components of immune response.

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Authors' Contributions

All of the authors cooperated in implementing the trial and statistical analysis as well as interpreting and reviewing the results of the study.

Conflict of Interest Declaration

The authors declare that they have no conflict of interests.

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