

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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Plasma Selenium Concentration and Glutathione Peroxidase Activity in Red Blood Cells of Laying Hens Fed Sodium Selenite or Zinc-L-Selenomethionine

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Abstract: The objective of this study was to determine the effect of sodium selenite and zinc-L-selenomethionine on plasma selenium (Se) concentration and glutathione peroxidase (GSH-Px) activity in the red blood cells (RBC) of laying hens. Two hundred twenty four CP Browns aged 71 weeks were divided according to a 2x3 factorial in completely randomized design. One more group without additional Se supplementation was used as negative control. Each treatment consisted of four replicates and each replicate contained eight hens. The dietary treatments were T1: basal diet, T2, T3 and T4: basal diets added 0.3, 1.0 and 3.0 mg Se from sodium selenite/kg, respectively, T5, T6 and T7: basal diets added 0.3, 1.0 and 3.0 mg Se from zinc-L-selenomethionine/kg, respectively. The findings revealed that the plasma Se concentration of hens received supplemented zinc-L-selenomethionine diets was higher ($p < 0.05$) than that of hens received supplemented sodium selenite diets. Plasma Se concentration statistically increased ($p < 0.01$) with increasing Se levels. The Se sources did not dramatically alter GSH-Px activity in RBC. However, GSH-Px activity significantly increased ($p < 0.01$) with increasing dietary selenium levels. The results in this experiment indicate that zinc-L-selenomethionine increases higher plasma Se concentration than sodium selenite, however GSH-Px activity in RBC of laying hens is not affected by Se sources.

Key words: Sodium selenite, zinc-L-selenomethionine, plasma selenium concentration, glutathione peroxidase activity, laying hens.

Introduction

Selenium is an essential nutrient for poultry. N.R.C. (1994) recommended 0.05 mg Se/kg in the diet of laying hens for the maintenance and egg production. Moreover, Se allowance higher than 0.1 mg/kg can improve immunity (Song *et al.*, 2006). Selenium is an important component of the antioxidant defense mechanism and functions by controlling the body's glutathione pool and its major Se-containing antioxidant enzyme, GSH-Px (Mahmoud and Edens, 2003). The main physiological function of GSH-Px is to maintain low levels of H_2O_2 and others hydroperoxides in the cell to prevent tissues from peroxidation damages (Kim and Mahan, 2003). Recent studies found that activity of blood GSH-Px in chicks fed organic Se in the form of Se-enriched yeast and sodium selenite was not different (Kuricova *et al.*, 2003; Payne and Southern, 2005). On the other hand, Mahmoud and Edens (2003) reported that Se-enriched yeast had elevated higher GSH-Px activity in blood of chickens in a thermoneutral environment and after heat distress than inorganic Se. Nevertheless, GSH-Px activity increased significantly ($P < 0.05$) with increasing Se supplementation in the diet of broilers (Omaye and Tappel, 1974; Kuricova *et al.*, 2003; Yoon *et al.*, 2007) and laying hens (Payne and Southern, 2005). Furthermore, plasma Se concentration of chickens fed

Se-enriched yeast increased significantly ($P < 0.05$) when compared that of chickens fed inorganic Se (Kuricova *et al.*, 2003; Payne and Southern, 2005). The above results indicated that Se-enriched yeast was more beneficial than inorganic Se. However, there is lack information of the use of zinc-L-selenomethionine in laying hens. Zinc-L-selenomethionine is designed to be highly soluble and increase bioavailability of selenium (Ward, 2003). The previous studies showed that zinc-L-selenomethionine higher improved Se status in horses (Richardson *et al.*, 2006) and increased muscle (George *et al.*, 2004) and plasma Se concentrations in broilers (Spears *et al.*, 2003) than sodium selenite. Thus, the aim of this study was to determine the effect of zinc-L-selenomethionine and sodium selenite on plasma Se concentration and GSH-Px activity in RBC of laying hens.

Materials and Methods

Two hundred and twenty four CP Brown laying hens, 71 weeks old, were housed in evaporative cooling system housing. Internal temperature was set at 24°C. Lights were on continuously. The hens were randomly divided into 7 groups. Each group consisted of 4 replicates with 8 hens per replicate. The basal diet (Table 1) was formulated to meet or exceed nutrient requirement according to N.R.C. (1994) and without Se

Table 1: Feed ingredient and chemical composition of basal diet¹

Ingredients	%
Corn	59.00
Rice bran	4.25
Soybean meal (44%CP)	16.00
Fish meal	6.36
Soybean oil	2.78
Dicalcium phosphate	1.65
Oyster shell meal	8.44
DL-Methionine	0.15
Salt	1.12
Vitamin-mineral premix ²	0.25
Analyzed chemical composition (%DM)	
Dry matter	91.37
Crude protein	15.86
Ether extract	3.52
Crude fiber	2.87
Ash	12.85
ME ³ , kcal/kg	2,950.12

¹Sodium selenite and zinc-L-selenomethionine were mixed in corn and added to the diet to achieve the treatment levels.

²Vitamin-mineral premix provide (per kg diet): 10,000 IU vitamin A, 2,000 IU vitamin D₃, 11mg vitamin E, 1.5 mg vitamin K, 1.5 mg thiamin, 4 mg riboflavin, 10 mg pantothenic acid, 0.4, folic acid, 4 mg pyridoxine, 22 mg niacin, 0.4 mg colabamin, 0.1 mg biotin, 60 mg Fe, 70 mg, Mn, 50 mg Zn, 8 mg Cu, 0.5 mg Co, 0.7 mg I. ³Calculated value.

supplementation. The 0.3, 1.0, and 3.0 mg Se/kg from sodium selenite or zinc-L-selenomethionine (Availa[®]Se, Zinpro Corporation) were supplemented to the basal diet. Total Se concentration of zinc-L-selenomethionine was 1000 mg Se/kg. The hens received the basal diet or Se supplemented diets and water *ad libitum* throughout the 6 weeks experimental period. The experimental diets were randomly collected at the end of each week and determined for chemical composition (AOAC, 1999) and Se content. The same 2 hens in each replicate of the treatments were bled at the beginning of the experiment and the end of each tested week. Blood samples were collected via the main wing vein and placed into 10-ml tubes containing EDTA. After collection, the blood samples were centrifuged at 3,000 x g for 10 min. The plasma was harvested and stored at -20°C prior to determination of Se concentration. The dietary and plasma samples were digested with nitric acid until the solution was cleared. Selenium was analyzed by inductively coupled plasma mass spectrometer (ICP-MS model Elan-e, Perkin-Elmer SCIEX, USA) following the procedure of Sieniawska *et al.* (1999). The activity of GSH-Px in RBC was investigated on weeks 0, 1, 3 and 5. After separating plasma, RBC were washed three times with 0.9% saline solution. The distilled water was added to RBC to make a ratio of 4:1 and frozen for 24h to hemolyze the RBC (Patricia *et al.*, 1982). The activity of GSH-Px in RBC was determined using the proposed procedure of Koller *et al.* (1984).

Statistical analysis: The data of plasma Se concentration and activity of GSH-Px in RBC were analyzed using GLM procedure appropriate for Factorial in Completely Randomized Design (SAS, 1996). Treatment differences were determined by orthogonal contrasts

- (1) basal diet vs. Se supplemented diets,
- (2) sodium selenite vs. zinc-L-selenomethionine,
- (3) levels of Se supplementation. Values of $P < 0.05$ were considered significant.

Results and Discussion

The Se determination in experimental diets indicated that the basal diet provided 0.30 mg Se/kg. Consequently, the basal diets added 0.3, 1.0 and 3.0 mg Se/kg from sodium selenite or zinc-L-selenomethionine contained 0.68, 1.14 and 3.37, or 0.77, 1.43 and 3.47 mgSe/kg, respectively. Plasma Se concentration of laying hens fed control diet was lower ($P < 0.05$) than that of hens fed Se supplemented diets (Table 2). Zinc-L-selenomethionine increased higher ($P < 0.05$) plasma Se concentration than sodium selenite since the first week of the trial. The results in this experiment are in agreement with the previous reports (Kuricova *et al.*, 2003; Spears *et al.*, 2003; Payne and Southern, 2005; Yoon *et al.*, 2007). Those reports found blood Se concentration of birds receiving organic Se in the forms of zinc-L-selenomethionine or Se-enriched yeast was significantly higher ($P < 0.05$) than that of birds kept on basic diet or diet supplemented with sodium selenite. Normally, organic Se in the form of selenomethionine increased blood Se levels to a much greater extent than did selenite (Thomson, 1998) or selenate (Beilstein and Whanger, 1986). On the other hand, Pan *et al.* (2007) observed whole-blood Se concentrations of hens fed sodium selenite were higher ($P < 0.05$) than those of hens fed Se-enriched yeast. That study explained that organic Se is mainly deposited in egg and body tissue, whereas inorganic Se remains in the blood. Plasma Se concentration increased significantly ($P < 0.05$) with the increment of dietary Se, regardless of Se source (Table 2). Similarly, Jiakui and Xiaolong (2004) and Zuberbuehler *et al.* (2006) observed plasma Se of laying hens increased dramatically ($P < 0.05$) with levels of Se supplementation. The significant differences between the effects of inorganic and organic selenium on plasma Se showed since the first week of the present experiment. However, Kuricova *et al.* (2003) found those effects after six weeks. This discrepancy in results may be due to the differences of Se content in the basal diet and concentration of Se used. The results demonstrated that GSH-Px activity in RBC of laying hens fed control diet was lower ($P < 0.05$) than that of hens fed Se supplemented diets. Sources of Se did not markedly alter ($P > 0.05$) GSH-Px activity in RBC of laying hens (Table 3). The outcome was in agreement with the results of Omaye and Tappel (1974), Cantor *et al.* (1975), Kuricova *et al.* (2003); Payne and Southern

Table 2: Plasma selenium concentrations ($\mu\text{g/ml}$) of laying hens fed sodium selenite or zinc-L-selenomethionine

Experimental week	Basal Diet	Sodium selenite (mg/kg)			Zinc-L-selenomethionine (mg/kg)			SEM	P-value ¹			
		0.3	1.0	3.0	0.3	1.0	3.0		B	S	L	SxL
0	0.13	0.14	0.13	0.14	0.13	0.13	0.14	0.00	NS	NS	NS	NS
1	0.10	0.14	0.18	0.25	0.15	0.23	0.41	0.02	*	*	*	*
2	0.10	0.16	0.19	0.25	0.21	0.27	0.53	0.02	*	*	*	*
3	0.10	0.17	0.18	0.31	0.20	0.35	0.67	0.03	*	*	*	*
4	0.11	0.16	0.18	0.23	0.18	0.28	0.52	0.02	*	*	*	*
5	0.14	0.19	0.22	0.31	0.22	0.34	0.71	0.03	*	*	*	*
6	0.12	0.12	0.23	0.32	0.17	0.27	0.48	0.02	*	*	*	*
Average	0.11	0.15	0.18	0.26	0.18	0.27	0.49	0.00	*	*	*	*

¹B = basal diet vs. others, S = sodium selenite vs. zinc-L-selenomethionine, L = levels of Se supplementation, SxL = Se sources x levels.

* = significantly difference at $P < 0.05$. NS = not significantly difference at $P > 0.05$.

Table 3: Glutathione peroxidase activity in red blood cell (mUnit/Hb) of laying hens fed sodium selenite or zinc-L-selenomethionine

Experimental week	Basal diet	Sodium selenite (mg/kg)			Zinc-L-selenomethionine (mg/kg)			SEM	P-value ¹			
		0.3	1.0	3.0	0.3	1.0	3.0		B	S	L	SxL
0	2.68	5.76	3.33	6.99	4.13	4.04	4.46	0.39	NS	NS	NS	NS
1	4.76	8.75	14.70	15.49	11.60	14.03	16.05	0.94	*	NS	*	*
3	5.10	9.11	10.60	11.97	7.90	10.74	13.24	0.79	*	NS	*	NS
5	3.27	7.58	10.27	11.90	4.04	11.51	12.13	0.72	*	NS	*	NS
Average	4.00	7.78	9.65	11.58	8.03	10.02	11.52	0.46	*	NS	*	NS

¹B = basal diet vs. others, S = sodium selenite vs. zinc-L-selenomethionine, L = levels of Se supplementation, SxL = Se sources x levels.

* = significantly difference at $P < 0.05$. NS = not significantly difference at $P > 0.05$.

(2005) and Yoon *et al.* (2007). The current results reflected that bioavailability of zinc-L-selenomethionine and sodium selenite with respect to GSH-Px activity in RBC was similar. This might be explained by their metabolic route. Selenomethionine is converted into selenocysteine that can be degraded further in liver to serine and selenide. Sodium selenite is converted initially to selenogluthathione trisulfide and then degraded in liver to form selenide. The selenide is finally used for selenoprotein synthesis, such as GSH-Px (Schrauzer, 2000). The activity of GSH-Px was normally dependent on the amount of Se supplied to the diet (Omaye and Tappel, 1974; Kuricova *et al.*, 2003). Subsequently, the present result found that GSH-Px activity in RBC increased ($P < 0.05$) accordingly with levels of Se supplementation (Table 3). From the obtained results, it could be concluded that zinc-L-selenomethionine increased significantly ($P < 0.05$) plasma Se concentration. However, zinc-L-selenomethionine and sodium selenite were equally effective in stimulating GSH-Px activity in RBC of laying hens.

Acknowledgments

This work was supported by a grant from Masarakham University. Zinc-L-selenomethionine (Availa®Se) was provided by Zinpro Animal Nutrition (Thailand), Inc. The authors wish to thank Mr. Witpol Thosaikham, Miss. Galyanee Kunchree and Mr. Jakrit Charoensin for data collection and laboratory assistance.

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