

Effects of Organic Selenium on Laying Performance, Selenium Absorption and Utilization, Antioxidant Activity and Immunity in Laying Hens

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Research

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ABSTRACT

To study on influences of organic selenium on laying performance, Se absorption and utilization, immunity and antioxidant activity, 400 (laying rates 85%) hens during the period of 43-week were randomly allocated to 1 of 4 homogeneous treatments: (control treatment, no added Se feed; Na selenite, 0.3 mg Se/kg; yeast selenium, 0.3 mg Se/kg and DL-methionine selenium 0.3 mg Se/kg). Every treatment had 10 replicates, every replicate had 5 hencoops and every hencoop had 2 hens. We hypothesized that organic selenium was better than inorganic selenium, and DL-selenium had advantages over them of yeast selenium in some ways for laying hens. The experiment lasted for 77 d, with the first 7 d for adaptation. Egg production, laying rates and dry matter intake were recorded every day. And Se contents of serum, whole eggs, heart and liver, blood antioxidant and immunity index were analyzed in the 78th d of the study. The results showed Se sources had no significant effects on laying performance and eggs qualities ($P > 0.05$) except for the groups of organic selenium increased egg mass or had trends of increasing laying rates compared with the groups of control and inorganic selenium in the tenth week significantly respectively ($P < 0.05$ or $P = 0.07$, $P = 0.06$ and $P = 0.08$). The groups of organic selenium had increased the contents of Se in serum, whole eggs, heart and liver very significantly ($P < 0.01$); and increased glutathione peroxidase activity and antioxidant capacity significantly ($P < 0.05$). At same time, the groups of organic selenium had increased significantly or had trends to increase the immunoglobulin G in serum ($P < 0.05$ or $P = 0.05$). In a word, adding organic selenium in diets could improve laying performance, strengthen Se absorption and utilizations, antioxidant capacity and immunity of laying hens to the extent in a longer experiment. And the effects of DL-selenium had advantages over them of yeast selenium in some ways. It implicated that DL-selenium was benefited to production of laying hens and selenium-enriched eggs.

Key words: Selenium; Laying performance; Absorption and utilization; Antioxidant activity; Immunity.

Summary text: Selenium is an essential mineral not only for animal nutrition, but also for humans' nutrition. Organic selenium had more advantages in improvement of laying performance, Se absorption and utilizations, antioxidant capacity and immunity of laying hens. Especially, DL-selenium was a valuable product, which was benefits to improve production of laying hens and develop functional food, selenium-enriched eggs.

1. INTRODUCTION

Selenium (Se) is an essential mineral for animal nutrition as well as for humans, which played important role in antioxidant defense, redox regulation of gene expression, thyroid hormone metabolism, fertility and reproduction and immunocompetence development (Levkut et al., 2009; Rayman., 2000). As a constituent of selenoproteins, Se had structural and enzymic roles, which was well known as an antioxidant, as the Se-dependent glutathione peroxidase could prevent the body from oxidative stress and was a catalyst to produce activating thyroid hormone (Surai and Fisinin., 2014). Hence, it is essential to add adequate Se in animals' diets. In practices of production, Se often is added as form of inorganic sources, for example sodium selenite, in poultry diets. However, over the last few years, some researchers gave wide interest to organic sources of Se, such as the DL-methionine selenium and yeast selenium, which had a high bioavailability and low toxicity compared to inorganic Se (Yoon, et al., 2007; Reis, et al., 2009). However, the Se compound presented in yeast selenium was main L-selenomethionine according to previous research (Mendez, et al., 2000; Huang, et al., 2005). And L-selenomethionine was regarded as an exclusive form in natural selenomethionine compounds, and D-selenomethionine was another form of stereoisomer (Cukierski, et al., 1989). As a synthetic product, DL-selenomethionine was an equimolar mixture of D-selenomethionine and L-selenomethionine (Jing et al., 2015). Hence, yeast selenium and DL-methionine selenium had certain differences in a way. However, there was not sufficient research to compare DL-methionine selenium with yeast selenium and sodium selenite, or to compare their effects of in improving the antioxidant activity and selenium status of laying hens. On the other hand, functional or designer foods and their roles in human diet are becoming more and more popular. They represent one of the fastest growing parts in the world food industry. Eggs are a good source of nutrients and may potentially play an important role as a functional food in human nutrition. And selenium-enriched eggs have been shown to be a valuable source of Se for humans as well as for livestock animals. Studies reported that production of en-rich Se

eggs could provide several important nutrients including omega-3 polyunsaturated fatty acids, various vitamins or provitamins and minerals (Sparks, 2006; Bourre and Galea, 2006). Especially, with improvement of people's living levels and health consciousness, more and more people will need more and more en-rich products of animals. Therefore, using organic Se to produce en-rich products of animals will have wide prospect of development and make great progress in future. It is an interesting and meaningful to human. Hence, the objective of experiment was to compare the effects of DL-methionine selenium on laying performance, antioxidant and immunity capacities, Se contents of blood and tissues to them of yeast selenium and sodium selenite by adding them in diets to provide theory evidence and reference value for developing and utilizing organic Se to producing the en-rich Se food furthermore.

2. MATERIALS AND METHODS

2.1. Animal, Diets, and Experimental Design

The experiment was conducted at Henan Weishi Laying Experiment Farm (Kaifeng, China). The 400 (laying rates 85%) hens during the period of 43-week were randomly allocated to 1 of 4 homogeneous treatments (A, control treatment, no added Se; B, Na selenite, 0.3 mg Se/kg feed; C, yeast selenium, 0.3 mg Se/kg; D, DL-methionine selenium 0.3 mg Se/kg). Sodium selenite, yeast selenium and DL-methionine selenium was added to the basal diets at the expense of premix. Every treatment had 10 replicates, every replicate had 5 hencoops, and every hencoop had 2 hens. The experiment lasted for 77 d, with the first 7 d for adaptation. The sodium selenite was bought from Guangxi Nanning Junwei: feed co., LTD, and its content was 1000 mg/kg in product. The yeast selenium and DL-methionine selenium were bought respectively from Le Fu yeast companies in the Unites States and Puno (Chengdu) biological technology co., LTD, and their contents were 2000 mg/kg in product. Every 2 hens were housed in one hencoop with access to water and feed ad libitum. Furthermore, the insects were expelled in bodies of all the experimental hens, and all the experiment houses were swept and fumigated before the experiment began. During experiment, all the experiment houses

were well ventilated. All experimental protocols used in this experiment were in accordance with those approved by the Henan University of Animal Husbandry and Economy Institutional Animal Care and Use Committee (protocol number HNUAHE20200037) and the institutional safety procedures were followed. As showed for the formula and nutrient levels of the experiment diet in Table 1. The basal diets and premix were all prepared according to the People's Republic of China feed standard in laying hens (2004) by Henan Hyrum feed co., LED. And the feeds were offered 2 times per day, respectively 5:30 am and 16:30 pm. All ingredients were same except for Se sources in four diets.

Table1. Ingredients and nutrient content of the basal diet (based on dry matter)

Ingredients	%
Corn	66.2
Soybean meal,43%	23.7
CaHPO ₄	1.1
Limestone	7.7
DL-Methionine	0.1
NaCl	0.3
premix ^a	0.9
In total	100
Nutrient levels	
Metabolizable energy (ME/kg,DM)	11.16
Crude protein (%)	16.10
Calcium (%)	3.39
Available phosphorus (%)	0.33
Lysine (%)	0.80
L-Methionine (%)	0.38
L-Methionine+ Cystine (%)	0.66
Threonine (%)	0.60

Note: ^a provided per kilogram of complete diet: vitamin A, 7700IU; cholecalciferol, 3300 IU; vitamin E, 12 IU; vitamin B₁₂, 0.009 mg; riboflavin, 4.4 mg; niacin, 22 mg; calcium pantothenate, 5.5 mg; menadione, 0.75 mg; folic acid, 0.2 mg; thiamine, 3 mg; pyridoxine, 5.5 mg; biotin, 0.04 mg; choline, 275 mg; Mn, 66 mg; Zn, 60 mg; Fe, 30 mg; Cu, 8.8 mg; I, 0.9 mg. ^b Crude protein, calcium, available phosphorus were measured. The Se content of basal diet was 0.04 mg/kg DM. Other values were calculated based on data from the diet supplier.

2.2. Sampling, Measurement, and Analyses

The quantities of laying eggs, soft and cracking eggs were recorded and weighed respectively every day. At same time, the intake feed, egg production, laying egg rates, soft and cracking egg rates, average egg weight, feed and egg ratio were calculated respectively. The formula was as following.

Laying egg rates (%) = total egg quantities / (hen quantities×d) ×100; Daily egg mass (g / every hen·d) = total egg production / (hens quantities×d); Average egg weight (g / every egg) = total egg production / total egg quantities; Feed and egg ratio = total feed consumption quantities / total egg weight; Daily intake (g / every hen·d) = total intake / (hen quantities×d).

In the 78th d of experiment, the egg samples were collected from 4 treatments (one egg / treatment·replicate) to determinate the contents of Se by atomic absorption spectrometry, the egg weight, yolk height, yolk colors, Hough units by Egg Multitester (Japan ENT7300), the eggshell strength and response time by eggshell strength tester (Nanjing yao en ESTG-1). However, the determination of egg shape index needed to determinate the length of the egg's longitudinal and transverse diameter firstly by Vernier caliper, then calculated the ratio of their longitudinal and transverse diameter. And the determination of eggshell thickness needed to determinate the thickness of eggshell's middle, big and small parts firstly by microcalliper, and calculated their average thickness.

In the 78th d of experiment, the hens' samples of livers and lungs were collected respectively from 4 treatments (one hen / treatment·replicate) by slaughter, then kept under -30°C to prepare for determination.

Before the hens were slaughtered, the 5ml blood samples (2ml anti-freezing and 3ml no anti-freezing) were collected by vein of chicken wings from 4 treatments (one hen / treatment·replicate) in the 78th d of experiment and kept under -20°C, then prepared to determinate the blood cell parameters by automatic

hematology analyzer, the biochemistry parameters and immunological indexes by blood biochemical analyzer, the blood enzymatic activities by reagent kits and the Se contents by atomic absorption spectrometry.

Among the determinations, egg qualities and Se contents were determined in the experiment center and testing center, Henan University of Animal Husbandry and Economy. The glutathione peroxidase (GSH-PX) activities were determined in Nanjing Jiancheng bioengineering institute, and the other blood cell and biochemistry parameters, blood enzymatic activities and immunological indexes were determined by Zhengzhou Yihe hospital.

2.3. Calculations and Statistical Analysis

Analysis of all the data was used to MIXED procedure based on SAS 9.1.3 software, and Hens in treatments were subjected as random to test for main effects and interactions using the covariance type AR (1), and the residual error was used to test for week and week \times treatment interaction. Mean comparisons across treatments were made when the interaction terms of the model were significant ($P < 0.05$) using LSMEANS and

PDIF separation of all the treatments. Significant differences were declared at $P < 0.05$ and trends at $0.05 \leq P \leq 0.10$.

3. RESULTS

3.1. Laying Performance and Egg Qualities

As shown in Table 2, there was no significant effect of supplementation of Na selenite, yeast selenium and DL-methionine selenium on daily intake, feed and egg ratios, average egg weight, soft and egg cracking rates, yolk height, yolk colors and Hough units respectively. And there was no significant difference in laying egg rates either. However, the daily egg mass of the treatments of yeast selenium and DL-selenium methionine were higher than them of control and inorganic treatment in the tenth week ($P < 0.05$) and had higher trends to them of control and inorganic treatment in the ninth week and in the whole period respectively ($P=0.06$ and $P=0.08$). At same time, the laying egg rates of the treatments of yeast selenium and DL-selenium methionine had higher trends to them of control and inorganic treatment ($P=0.07$).

Table 2. Effects of organic selenium on production performance in laying hens (n=10)

Controls	A	B	C	D
supplemented	No added selenite	Na selenite (0.3 mg Se/kg)	yeast selenium (0.3 mg Se/kg)	DL-methionine selenium (0.3 mg Se/kg)
Daily Intake (g/d)	102.8 \pm 2.4	102.9 \pm 2.2	103.3 \pm 3.7	103.7 \pm 1.2
Egg mass(the first week,g/d)	50.86 \pm 3.22	50.39 \pm 0.79	51.76 \pm 1.52	51.58 \pm 2.45
Egg mass(the second week,g/d)	51.81 \pm 3.81	51.54 \pm 0.61	51.34 \pm 2.21	50.50 \pm 2.10
Egg mass(the third week,g/d)	50.60 \pm 1.67	51.70 \pm 1.08	51.14 \pm 3.00	51.51 \pm 0.92
Egg mass(the fourth week,g/d)	51.88 \pm 4.17	50.71 \pm 1.38	51.93 \pm 1.47	51.88 \pm 1.05
Egg mass(the fifth week,g/d)	50.47 \pm 2.04	51.35 \pm 0.46	51.71 \pm 1.60	50.65 \pm 1.82
Egg mass(the sixth week,g/d)	51.08 \pm 0.67	50.82 \pm 0.51	51.49 \pm 1.13	51.51 \pm 1.74
Egg mass(the seventh week,g/d)	51.28 \pm 0.57	50.83 \pm 0.67	51.92 \pm 1.88	51.62 \pm 2.43
Egg mass(the eighth week,g/d)	51.08 \pm 1.43	50.97 \pm 0.77	51.44 \pm 1.55	51.60 \pm 1.98
Egg mass(the ninth week,g/d)	50.58 \pm 0.53	51.36 \pm 0.47	51.92 \pm 0.92	51.88 \pm 1.60
Egg mass(the tenth week,g/d)	50.77 ^b \pm 1.35	51.18 ^b \pm 0.68	52.36 ^a \pm 1.43	52.42 ^a \pm 1.03
Egg mass(the whole period,g/d)	51.02 \pm 2.22	51.09 \pm 0.82	51.76 \pm 1.66	51.51 \pm 1.75
Feed and egg ratios (g/g)	2.02 \pm 0.09	2.01 \pm 0.05	2.00 \pm 0.09	2.01 \pm 0.07
Average egg weight (g)	60.42 \pm 1.29	60.51 \pm 1.21	60.65 \pm 1.29	60.31 \pm 0.96
Soft and cracking egg rates (%)	1.50 \pm 0.10	1.59 \pm 0.57	1.52 \pm 0.07	1.45 \pm 0.05
laying rates (%)	84.47 \pm 3.82	84.45 \pm 1.83	85.47 \pm 3.39	85.42 \pm 2.73
Yolk height (mm)	5.82 \pm 1.17	5.44 \pm 0.93	6.20 \pm 1.83	5.60 \pm 1.37
yolk colors	5.56 \pm 0.64	5.74 \pm 0.93	5.44 \pm 1.20	5.14 \pm 1.27
Hough units	74.92 \pm 8.00	72.10 \pm 8.11	74.62 \pm 14.35	71.48 \pm 9.55
Egg shape indexes	1.29 \pm 0.07	1.27 \pm 0.06	1.28 \pm 0.03	1.28 \pm 0.05
Eggshell strength (Kilogram force)	4.60 \pm 0.87	4.61 \pm 0.44	4.33 \pm 0.16	4.72 \pm 0.84
Response time (s)	0.77 \pm 0.07	0.79 \pm 0.04	0.74 \pm 0.02	0.79 \pm 0.08
Eggshell thickness (mm)	0.39 \pm 0.02	0.39 \pm 0.01	0.39 \pm 0.01	0.37 \pm 0.02

Note: In the same column, values with different capital and small letters mean very significant difference at $P < 0.01$ and significant difference at $P < 0.05$.

Table 3. Effects of organic selenium on Se content in tissue, blood cell and biochemistry parameters and immunity indexes in laying hens (n=10)

Controls	A	B	C	D
supplemented	No added selenium	Na selenite (0.3 mg Se/kg)	yeast selenium (0.3 mg Se/kg)	DL-methionine seleni- um (0.3 mg Se/kg)
Se in serum (mg/kg)	0.09 ^C ±0.05	0.14 ^{BC} ±0.04	0.17 ^{AB} ±0.02	0.19 ^A ±0.10
Se in the whole eggs (mg/kg)	0.05 ^B ±0.01	0.20 ^{AB} ±0.08	0.24 ^{AB} ±0.05	0.39 ^A ±0.02
Se in hearts (mg/kg)	0.17 ^C ±0.04	0.20 ^{BC} ±0.05	0.25 ^{AB} ±0.04	0.28 ^A ±0.29
Se in livers (mg/kg)	0.21 ^B ±0.05	0.22 ^B ±0.03	0.42 ^A ±0.04	0.50 ^A ±0.05
Red blood cells, ×10 ¹² (quantities /L)	2.14±0.12	2.16±0.17	2.01±0.12	2.13±0.10
White blood cells, ×10 ¹¹ (quantities /L)	3.65±0.10	3.73±0.21	3.44±0.14	3.87±0.85
Platelet, ×10 ¹¹ (quantities /L)	2.09±0.46	2.60±0.82	2.04±0.95	2.48±0.50
Hemoglobin (g/L)	70.1±5.2	73.6±5.2	70.4±5.8	71.0±6.2
Hematocrit (%)	26.8±1.8	27.8±2.6	26.1±1.6	27.6±2.0
Total protein (g/L)	52.7±4.7	56.5±6.4	53.3±6.8	60.3±9.4
Albumin (g/L)	19.0±1.7	18.5±1.6	18.5±1.8	17.2±2.9
Urea (m mol/L)	1.82±0.16	1.76±0.16	1.67±0.11	1.69±0.06
Creatinine (u mol/L)	15.0±11.2	14.4±6.2	9.20±3.43	9.60±2.46
Glucose (m mol/L)	10.5±2.0	11.3±1.3	11.2±1.2	10.0±1.6
Cholesterol (m mol/L)	3.38±0.95	3.10±1.00	3.31±1.20	2.95±0.65
Total bilirubin (u mol/L)	4.14±2.08	3.76±2.84	2.30±1.34	2.90±1.78
glutathione peroxidase activity, ×10 ³ (U/ml)	2.30 ^b ±1.19	2.21 ^b ±0.27	3.38 ^a ±0.73	3.85 ^a ±0.86
Alanine aminotransferase (U/L)	3.75±0.46	3.40±0.52	3.00±0.67	3.40±0.52
Aspartate aminotransferase (U/L)	191±38	222±62	169±11	193±39
Gamma-glutamyltransferase (U/L)	39.6±32.1	23.0±6.6	23.2±4.0	23.8±6.6
Alkaline phosphatase (U/L)	396±77	407±258	398±202	413±202
Immunoglobulin A (g/L)	2.85±0.98	2.66±1.10	2.94±0.76	3.45±0.57
Immunoglobulin G (g/L)	1.44 ^b ±0.99	2.33 ^b ±1.28	2.85 ^{ab} ±0.71	3.15 ^a ±1.56
Immunoglobulin M (g/L)	3.35±2.54	4.22±1.99	6.64±3.39	5.04±2.00

Note: In the same column, values with different capital and small letters mean very significant difference at $P < 0.01$ and significant difference at $P < 0.05$.

3.2. Se Contents in Tissue and Whole Eggs, blood cell and biochemistry parameters, enzymatic activities and immunological indexes

The effects of organic selenium on Se contents in tissue and whole eggs, blood cell and biochemistry parameters, enzymatic activities and immunological indexes were shown in table 3. The Se contents of treatment D in serum and hearts were very significantly higher than them of treatment A and B, and the Se contents of treatment C were very significantly higher than them of treatment A ($P < 0.01$). The Se contents of treatment D in whole eggs were very significantly higher than them of treatment A ($P < 0.01$), and the Se contents of treatment C and B had higher trends than them of treatment A. The Se con-

tents of treatment D and C in livers were very significantly higher than them of treatment A and B ($P < 0.01$).

At same time, we could know that the GSH-PX activity of treatment C and D were significantly higher than them of A and B ($P < 0.05$). The Immunoglobulin G (IgG) of treatment D were significantly higher than them of A and B ($P < 0.05$), and the IgG of treatment C had higher trends to them of A and B ($P = 0.05$). But the other blood cell and biochemistry parameters, enzymatic activities had no significant effects among groups ($P > 0.05$).

4. DISCUSSION

Many studies proved that selenium not only was an indispensable ingredient of glutathione peroxidase, but also was a key synthetase of triiodothyronine (T_0) - important cofactors and activating agent of 5'-iodinase in the bodies of animals. Selenium might eliminate the peroxide and hydroxyl free radicals which animals' cells produced during the period of their respiratory and metabolism, maintained the integrity of biomembrane, protected cells from damages of oxidized low-density lipoprotein and so on. Furthermore, T_0 was an important component of animal growth, especially poultry. It could regulate and control the growth of animals by regulating and controlling the assimilation of energies and proteins in the bodies of animals (Neve J., 2000). At same time, the studies proved that the selenium partook and constituted the selenoprotein in the bodies of animals and worked by the form of organic matters, for example, amino acids selenium. Among them, the selenium methionine and cysteine selenium were the final active forms. Compared to inorganic selenium, they had higher bioavailabilities and were nontoxic to people and environments (Zou, et al., 2005). In this experiment, adding 0.3 mg/kg organic selenium in diets of laying hens had no significant effect on the other laying performance, but increased egg mass from 63d to 77d. The results were similar to the results obtained in previous reports. They found no effects when sodium selenite (SS) or se-enriched yeast supplemented in poultry diet on the first 8 weeks, whereas exhibited higher laying egg rates than SS from the ninth weeks when added yeast selenium in diets. Laying egg rates for the entire flock reached a peak averaged 96.1% on days 61 to 75 in the 0.3 mg/kg yeast selenium group (Pavlovic et al., 2009). Same, although the laying egg rates of groups of organic selenium were not increased, their egg mass reached a peak averaged 52.36 and 52.42 g per day in the tenth week in this experiment. These results showed the organic selenium were benefits for increasing eggs production in a longer experiment period. Maybe as time goes on, the effects would be more obvious.

In current experiment, dietary treatment did not negative affect any parameters correlated to egg or shell quality. The overall values obtained in current study were very acceptable for optimal egg quality at this age of hens in production. Furthermore, there were similar Hough unit, shell thickness and strength among dietary treatments. The standard commercial egg production guides and other available literature reported average values obtained egg quality parameters conformed adding organic selenium in diets of laying hens had not adverse effects on egg qualities (Mohitiasli et al., 2008; Tufarelli et al., 2016; Pavlović et al., 2010). Hence, the results were consistent with previous report.

Egg Se concentration was elevated by SS as well as SY supplementation as dietary levels increase, but selenium yeast is more effective in increasing total egg Se than selenite (Payne et al., 2005; Utterback et al., 2005). It was demonstrated that organic Se sources such as SY and Se-chlorella were equally effective for Se transfer from the feed into the eggs, while SS was much less potent likewise (Skřivan et al., 2006). It was reported that adding the selenium enriched yeast in diets could improve the Se content in the tissues of chicken significantly (Pane et al., 2005). Leeson et al. (2008) reported that compared with chicken of no intake Se, adding Se could improve the Se contents in livers and muscle tissue of chicken significantly, also. In this experiment, adding 0.3mg/kg organic selenium in diets improved the Se contents in the serum, whole eggs, hearts and livers to some extent. Especially, all the Se contents in organic treatments were higher or very higher than them of control and sodium selenite treatment significantly ($P<0.01$). They were consistent basically with their results. On the other hand, they were consistent with our results in dairy cow's experiment. They further showed the organic selenium had higher bioavailability than inorganic selenium. It was very benefits to produce the selenium enriched products. Relatively speaking, DL-selenium methionine had higher bioavailability and deposition rates compared to yeast selenium in serum and tissue.

It was reported that some proportion of ingested Se methionine (SeMet) escaped the Se metabolism and was nonspecifically incorporated into the general body proteins. This metabolic ability of SeMet was based on the truth that tRNA methionine (tRNAMet) in plants, bacteria, birds and mammals had not differences between the common amino acid methionine (Met) and SeMet. Hence, both Met and SeMet could substitute each other during course of incorporating into general proteins (Schrauzer, 2000). These were reasoning that animal products such as meat, milk proteins and egg albumen might contain significantly more Se when animal feed was supplemented with SY containing SeMet as the predominant Se-compound. The possible reasons, which DL-methionine selenium had more absorption and utilization rates compared to yeast selenium were DL-methionine selenium included D-methionine and L-methionine, and yeast selenium only had L-methionine.

The selenoenzyme GSH-Px could reflect well the antioxidant status of cell bodies and functions to remove hydrogen and organic peroxides (Kyriakopoulos and Behne, 2002). At present experiment, the GSH-PX activity in organic treatments were higher than them of control and sodium selenite treatment significantly ($P < 0.05$), and DL-selenium methionine treatment were higher than them of yeast selenium treatment. These results concerning the relationship between dietary Se supplement and the activity of GSH-Px were consistent with previous reports (Spears, et al., 2003; Surai and Fisinin., 2014). Cantor, et al. (1982) reported that different Se sources affected the GSH-Px activity and the GSH-Px mRNA levels in the tissue of pigs fed organic sources were higher than those fed SS (Gan, et al., 2013). In this experiment, the GSH-PX activity of organic selenium were higher than them of control and sodium selenite treatment significantly, and the reasons might be attributed to better bioavailability of organic selenium because of functional selenoprotein activity to express GSH-Px mRNA and the formation of superoxide anions. Moreover, as an analog to the selenomethionine compounds, methionine played important roles to support cysteine for GSH

synthesis. Hence, these results might be due to the supplementation of organic selenium led to increase the activity of GSH-Px in the plasma (Jing et al., 2015).

Increasing of red blood cells (RBC) could promote the phagocytic function of white blood cells (WBC), improve the conveying gas capacity of blood, and strengthen the metabolic and immunity of bodies. The WBC, neutrophils and lymphocyte were the important indexes which indicated immunity of bodies in blood. Their changes of quantities expressed the stronger or weaker function of cells. Moreover, the RBC had immune adherence functions, which could promote the phagocytic function of WBC, and were the host's parts of defense mechanism. Now it has been proved that the RBC caught antigen by immune adherence functions, then eliminated the oxidation metabolite produced during the course of phagocytosis by stronger oxidation of higher contents catalase and superoxide dismutase, promoted their phagocytic function finally. At same time, the RBC took part in the production of γ -interferon, IL-1, IL-2 and Ig. They were a subsystem of immune system in the whole bodies (Zhao, et al., 2012). The previous experiment showed that selenium could strengthen the immunity functions of animals (Zhou, 2005). In this experiment, all the blood cell parameters had no significant differences among groups ($P > 0.05$) and did not appear obvious fluctuation. It showed as far as blood cell parameters, they did not effect on the immunologic functions of laying hens.

IgG、Immunoglobulin A (IgA)、Immunoglobulin M (IgM) were the immunoglobulin of human and animals. Among them, the IgG was the most important one. Its main function was playing a role of protection in the immunization of bodies. Huang et al. (2004) reported Se enriched Lactobacillus enhanced serum IgG and IgA in broiler chickens. It was reported by Wang et al. (2007) that despite being an essential trace element, selenium in fact was toxic at a level much higher than the requirement. This suggested that feeding of diet containing 0.30 mg/kg of nano-Se could produce the greatest improvement in chickens

for the humoral immunity (Cai et al., 2012). In this experiment, when fed diets contained 0.30 mg/kg of DL-selenium and yeast selenium methionine, they had improved the immunity of laying hens to the extent. This was basically consistent with the former results. They showed adding organic selenium in diets could enhance immunity of laying hens to the extent.

CONCLUSION

In this experiment, adding organic Se in diets not only could improve the laying performance in some ways, but also had no adverse effects on eggs qualities. Moreover, it could increase the Se contents in serum, whole eggs, hearts and livers obviously, and improve the immunity and antioxidant capacity to the extent. In a word, organic Se was very benefit to production of laying hens and en-rich products of animals in a longer experiment.

The effects of DL-selenium methionine were like yeast selenium, but they had advantages over them of yeast selenium in the ways of Se absorption and utilization, improving antioxidant capacity and immunity of laying hens overall. Hence, DL-selenomethionine was a more efficient Se source in laying production.

CONFLICTS of INTEREST

The authors declared that no conflicts of interest exist.

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ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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