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Effect of Glycine Nano-Selenium Supplementation on Production Performance, Egg Quality, Serum Biochemistry, Oxidative Status, and the Intestinal Morphology and Absorption of Laying Hens

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Abstract

The objective of this study was to investigate the feasibility of using glycine nano-selenium (NS-Gly) as a feed supplement and to evaluate its influence on production performance, egg quality, serum biochemistry, oxidative status, and the intestinal morphology and absorption of laying hens. A total of 864 hens at 40 weeks were randomly assigned into six groups including the basal diet (control, 0.13 mg Se/kg); basal diet + 0.30 mg Se/kg (Na_2SeO_3) diet; and basal diet + 0.15, 0.30, 0.45, and 0.60 mg Se/kg (NS-Gly) diet. After 8 weeks of Se supplementation, no difference was observed among the treatments on production performance and egg quality ($P > 0.05$). The levels of albumin (ALB) and alanine aminotransferase (GPT) were significantly influenced by dietary Se supplementation ($P < 0.05$). In the serum, the level of glutathione peroxide (GSH-Px) was significantly increased in the groups with the dietary NS-Gly supplementation ($P < 0.05$). The superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) levels in all groups of NS-Gly supplementation had a remarkable increase ($P < 0.05$). In the liver, GSH-Px was significantly increased in 0.45 and 0.60 mg/kg NS-Gly groups ($P < 0.05$). The activities of SOD and catalase (CAT) were significantly increased in the groups of 0.30 mg/kg NS-Gly diet ($P < 0.05$). The results of intestinal morphology showed that the crypt depth was affected by higher dose groups of NS-Gly diets in the duodenum, and the differences ($P < 0.05$) were obtained in villus height, the crypt depth, and the V/C in the jejunum. In the ileum, a significant increase ($P < 0.05$) of villus height was observed in 0.15 and 0.3 mg/kg Se-added groups. The V/C was the highest in the SS groups ($P < 0.05$). The mRNA levels of solute carrier family 3 member 1 (rBAT), solute carrier family 6 member 19 (B^0AT1), and solute carrier family 15 member 1 (PepT1) increased at different degrees in the duodenum, especially in 0.15 and 0.60 mg/kg NS-Gly groups ($P < 0.05$). In the jejunum, the expression of B^0AT1 was similar to that in the duodenum, and the expression of rBAT increased significantly in the 0.30 and 0.45 mg/kg NS-Gly groups ($P < 0.05$). The mRNA level of PepT1 increased significantly in the 0.30 mg/kg SS group. Conclusively, dietary NS-Gly supplementation could improve the antioxidant capacity, as well as the structure of small intestine in laying hens, although have no significant effects on the production performance and egg quality.

Keywords Glycine nano-selenium · Antioxidant · Selenium transport · Laying hens

Introduction

Selenium (Se), as an essential trace mineral, is vital for the healthy growth and development of animals and human beings [1]. For birds, an adequate intake of Se is required to overcome the risks of immunodeficiency, exudative diathesis, nutritional muscular dystrophy, and other diseases related to Se deficiency [2, 3]. Numerous studies reported that Se might

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improve the productive and reproductive indices of laying hens when supplied inadequate levels [4, 5]. It also affects the antioxidative status, immune response, and tolerance against different stressors [6, 7]. As some natural feeds are insufficient of Se, this element is commonly extra added. Normally, Se occurs in two chemical forms: organic and inorganic, both of them have been used in poultry diets [8]. Studies have shown that the organic form of Se is more conducive to the deposition of Se in human tissues and eggs, and reduces the transfer of Se to the environment through feces [9, 10]. However, the excessive Se addition can also cause toxic effects in animals.

Recently, Se in nanoparticle form (nano-selenium, NS) has been applied in animal feed due to its high bioavailability and low harmfulness [11–13]. Nanoparticles are easier to pass through cell membranes in organisms and interact rapidly with biological systems [14]. It suggested that the superiority of nanoparticles is on account of smaller particle size and larger surface area, which may increase mucosal permeability, and improve intestinal absorption due to the formation of nanoemulsion droplets [15]. It has been reported that the range between optimal and toxic dietary levels of NS was greater than that of SS [16]. It has been demonstrated that metal chelated with amino acid or protein has a better bioavailability to poultry [17, 18]. Most of the NS reported are single Se particles with protein as its nucleus, red selenium as its membrane and protein as its dispersant. NS-Gly is a new type of Se source that uses glycine as an adsorbent and a stabilizing agent of NS. There are 0.9 weight portion of Se and 85 to 95 weight portions of glycine form granular nano-selenium, and the average particle size is 60 nm [19]. It has stable physical and chemical properties and good dispersion and does not reunite. Compared with regular sodium selenite, NS can improve antioxidant status and Se absorption more efficiently on broiler chickens [20]. NS appears to be more effective than other forms of Se at upregulating selenoenzymes, scavenging free radicals, and increasing the antioxidant capacity [21, 22].

Accordingly, the objective of this study was to investigate the feasibility of using NS-Gly as a feed supplement for laying hens and to evaluate its influence on production performance, egg quality, serum biochemistry, oxidative status, and the intestinal morphology and absorption.

Materials and Methods

Birds Management

A total of 864 laying hens (Huafeng layers, 40 weeks) were obtained from a commercial poultry layer farm (Jiande, China). Hens were randomly assigned into 6 treatments, each of which included 6 replicates ($n = 24$ laying hens). All hens were raised in the naturally ventilated windowed poultry house with temperature between 23 and 26 °C, the relative humidity between 65 and 75%, and illumination at 16 h/day (20 lx). Feed and water were offered ad libitum. The environmental conditions were the same for all groups. The experiment lasted for 9 weeks, including 1 week of acclimation period and 8 weeks of test period.

Experimental Design and Diets

The protocol of treatments was as follows: (1) basal diet (control, 0.13 mg Se/kg); (2) basal diet + 0.30 mg Se/kg diet (Na_2SeO_3, SS); (3) basal diet + 0.15 mg Se/kg diet (NS-Gly); (4) basal diet + 0.30 mg Se/kg diet (NS-Gly); (5) basal diet + 0.45 mg Se/kg diet (NS-Gly); and (6) basal diet + 0.60 mg Se/kg diet (NS-Gly). NS-Gly, feed grade, with an average particle size of 60 nm and 1% content of Se, was provided by Weifeng Feed Co., Ltd. (Jiande, China). The composition and nutritional level of the basal diet are presented in Table 1. The different contents of glycine caused by the addition of graded NS-Gly were balanced by adding extra glycine in the premixes to make all nutrients in diets kept at the same levels except for the Se content. The analysis concentrations of dietary Se in the six experimental groups were 0.13, 0.44, 0.30, 0.42, 0.58, and 0.75 mg Se/kg diet, respectively.

Table 1 Ingredient compositions and nutrient levels of basal diet for laying hens

Items Ingredients	Composition Content (%)
Corn	62.00
Soybean meal	24.50
Soybean oil	0.50
Limestone	8.00
Premix[1,2]	5.00
Total	100.00
Nutrient[3]	
Metabolism energy, MJ/kg	10.99
Crude protein, %	15.67
Lysine, %	0.80
Methionine, %	0.34
Calcium, %	3.69
Total phosphorus, %	0.54

[1] The premix provided the following per kilogram of diet: vitamin A, 7500 IU; vitamin D_3, 2500 IU; vitamin E, 49.5 mg; vitamin K_3, 2.5 mg; vitamin B_1, 1.5 mg; vitamin B_2, 4 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.02 mg; niacin, 30 mg; folic acid, 1.1 mg; pantothenic acid, 10 mg; biotin, 0.16 mg; chloride choline, 400 mg; sodium chloride, 2500 mg; Fe, 80 mg; Cu, 20 mg; Mn, 60 mg; Zn, 80 mg; I, 0.8 mg

[2] The premix in 6 treatments provided per kilogram of diet: Na_2SeO_3, 0.30 mg, NS-Gly, 0.15, 0.30, 0.45, and 0.60 mg, respectively, and in the control without additional Se

[3] Values were calculated from Chinese feed database provided with tables of feed composition and nutritive values in China (21th edition)

Sample Collections

During the test period, the number of eggs and egg weight was recorded everyday (at 14:00) on a replication basis and the laying rate was calculated. Feed consumption was measured once a week on a replication basis and the average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. At the end of the experimental period, twelve birds per treatment (2 birds each replicate) were randomly selected. After the hens fasted 12 h (water was offered ad libitum), the blood samples were collected in 1.5 ml of Eppendorf tubes by puncture of the wing vein. These tubes were centrifuged at 3000×*g* for 15 min to separate serum and stored at − 80 °C for biochemical and serum antioxidants analysis. Then, birds were euthanized by cervical dislocation. Immediately, samples of liver were snap frozen in liquid nitrogen and stored at − 80 °C for antioxidant and molecular analysis. The intestinal mucosa was collected and stored at − 80 °C from the duodenum, jejunum, and ileum for molecular analysis. The small intestine was kept in 4% paraformaldehyde for histological evaluations.

Egg Quality Determination

A total of 18 eggs (3 eggs per replication, 6 replications per treatment) from each treatment group were randomly collected at the end of the experiment to determine the egg quality. Egg weight, eggshell strength, albumen height, Haugh unit, and yolk color were measured by a digital egg tester (DET-6000, Nabel Co., Ltd., Kyoto, Japan). Eggshell thickness was measured (without shell membrane) with an egg shell thickness gauge (ESTG-1, Orka Food Technology Ltd., Ramat Hasharon, Israel).

Serum Biochemical Analysis

Serum contents of total protein (TP), albumin (ALB), glucose (GLU), total cholesterol (T-CHO), and triglyceride (TG), along with the activities of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT), were determined and calculated following the instructions of commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Serum and Liver Antioxidant Enzyme Assays

Samples of liver were cut into small pieces, added with ice-cold physiological saline at a ratio of 1:9 (weight:volume) to prepare 10% tissue homogenate mechanically, and then centrifuged at 3000×*g* for 10 min to separate supernatant at 4 °C. The supernatant was collected and stored at − 80 °C for the following analysis. The activities of glutathione peroxide (GSH-Px), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and catalase (CAT) and the content of malondialdehyde (MDA) in the serum and hepatic supernatants were assayed using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer.

Histopathological Analysis

About 1 cm segment of the duodenum, jejunum, and ileum fixed with 4% paraformaldehyde were trimmed and embedded in paraffin wax. The paraffin sections were cut into 5–6 μm thick using a microtome (Leica Microsystems, RM2016), then stained with hematoxylin and eosin (H&E) for histopathological observation. In the villus height, villus width, villus area, and crypt depth of 8 villi, each intestinal sample was calculated by optical microscopy (Nikon Eclipse 80i, Nikon, Tokyo, Japan).

Total RNA Extraction and Quantitative Real-time PCR

Total RNA was extracted from the liver and the intestinal mucosa from duodenum, jejunum, and ileum samples with TRIzol (Takara code: 9109, Shiga, Japan). Its concentration and purity were detected and assessed by nucleic acid concentration analyzer NanoDrop 2000 (Thermo Fisher, Waltham, MA, USA). Complementary DNA (cDNA) was generated by a HiScript II Q RT SuperMix Reverse Transcriptase (Vazyme Biotechnology, Nanjing, Jiangsu, China). Quantitative real-time PCR was employed by a SYBR Premix PCR kit (Vazyme Biotechnology, Nanjing, Jiangsu, China) and normalized to β-actin in the CFX96 Touch Real-Time PCR detection system (Bio-Rad, USA). There were 6 samples in each group, each sample was conducted in duplicate, and no template control was included. The primer sequences for qRT-PCR are presented in Table 2. The relative expression of each gene was calculated by the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The data were statistically analyzed by one-way ANOVA using SPSS 20.0 (SPSS Inc., Chicago, IL) and expressed as means and SEM. The Duncan test was used to compare the significant differences ($P < 0.05$) between means.

Results

Production Performance

The effects of glycine nano-selenium supplementation on production performance are shown in Table 3. There is no significant difference in laying rate, egg weight, ADFI, and FCR among the experimental groups.

Table 2 Primer used for quantitative real-time PCR

Target gene	Primer	Primer sequence (5′-3′)	Accession no.
β-Actin	Forward Reverse	TCCCTGGAGAAGAGCTATGAA CAGGACTCCATACCCAAGAAAG	NM_205518.1
PepT1	Forward Reverse	CTTGGCAGATCCCTCAGTATTT GTTGGGCTTCAACCTCATTTG	XM_034074354.1
B^0AT1	Forward Reverse	CATGATCGGACACAAGCCCA AGCATAGACCCAGCCAGGATA	XM_419056.6
rBAT	Forward Reverse	GGAGAGGCACGAAGTGAAAT CGAGGGTAGACCTGGTAGATAG	XM_010727793.3

β-Actin beta actin, *PepT1* solute carrier family 15 member 1, *B^0AT1* solute carrier family 6 member 19, *rBAT* solute carrier family 3 member 1

Egg Quality

As shown in Table 4, no significant difference of egg weight, albumen height, yolk color, Haugh unit, eggshell thickness, and strength was observed among all groups.

Serum Biochemistry

The current study revealed the effects of dietary Se on the serum biochemical changes. The results presented in Table 5 showed that the groups of SS, 0.15, 0.3, and 0.45 mg/kg NS-Gly were improved ($P < 0.05$) in the ALB compared with the control group, whereas there was no significant difference between the group of 0.6 mg/kg NS-Gly and the control group. On the contrary, the level of GPT decreased significantly in the groups of 0.3, 0.45, and 0.60 mg/kg NS-Gly diet compared with the control group ($P < 0.05$).

Serum Antioxidant Parameters

The effect of Se on the antioxidant parameters in the serum is summarized in Fig. 1. The activity of GSH-Px was significantly increased in the groups with the dietary NS-Gly supplementation ($P < 0.05$) compared with the control group. The activities of SOD and T-AOC in serum in groups of 0.15, 0.3, 0.45, and 0.60 mg/kg NS-Gly had remarkable increases ($P < 0.05$) when compared to the control, whereas no marked differences were observed between the control and the SS group. There are no significant differences among all groups in serum CAT activity and MDA level.

Hepatic Antioxidant Parameters

The effects of Se on the liver antioxidant indices of laying hens are shown in Fig. 2. The activity of GSH-Px was increased with the increase of dietary NS-Gly supplementation, and significantly in the groups of 0.45 and 0.60 mg/kg NS-Gly diet ($P < 0.05$). The activity of SOD was significantly increased in the group of 0.30 mg/kg NS-Gly diet ($P < 0.05$), then decreased in the groups of 0.45 and 0.60 NS-Gly diets. Similarly, the activity of CAT increased with the increase of NS-Gly treatments, and significantly in the group of 0.30 mg/kg NS-Gly diet ($P < 0.05$), then decreased in the group of 0.45 and 0.60 mg/kg NS-Gly diet, compared with the control group. Furthermore, no significant differences were shown between the SS and control groups in hepatic antioxidant parameters.

The Intestinal Morphology

Histological changes in the small intestine were evaluated by a light microscope. As shown in Table 6, in duodenum, the

Table 3 Effects of Se supplementation on production performance of laying hens

Items	Control	SS (mg/kg)	NS-Gly (mg/kg)				SEM	*P* value
		0.3	0.15	0.3	0.45	0.6		
Laying rate, %	73.84	72.97	72.97	72.76	73.40	74.91	1.45	0.694
Egg weight, g	49.63	49.47	49.23	49.93	49.54	50.36	0.38	0.077
ADFI, g	91.69	88.13	87.86	89.66	88.05	94.84	3.49	0.300
FCR	2.42	2.37	2.34	2.41	2.33	2.48	0.10	0.656

Values are represented as the mean and SEM ($n = 6$). *SEM* standard error of the means. *SS* Na_2SeO_3, *NS-Gly* glycine nano-selenium, *ADFI* average daily feed intake, *FCR* feed conversion ratio

Table 4 Effects of Se supplementation on egg quality of laying hens

Items	Control	SS (mg/kg)	NS-Gly (mg/kg)				SEM	*P* value
		0.3	0.15	0.3	0.45	0.6		
Egg weight, g	49.97	51.51	49.57	49.99	50.26	51.10	0.92	0.258
Albumen height, mm	4.97	5.24	5.02	5.06	5.07	5.16	0.29	0.956
Yolk color	6.33	6.47	6.47	6.43	6.57	6.73	0.18	0.346
Haugh unit	71.78	73.47	72.63	72.12	73.04	73.06	2.43	0.983
Eggshell thickness, mm	0.36	0.36	0.36	0.35	0.37	0.37	0.01	0.532
Eggshell strength, kg/m^2	4.34	4.19	4.06	3.97	4.43	4.32	0.21	0.224

Values are represented as the mean and SEM ($n = 18$). *SEM* standard error of the means. *SS* Na_2SeO_3, *NS-Gly* glycine nano-selenium

crypt depth was the lowest in the 0.45 mg/kg NS-Gly groups, and significantly reduced in the NS-Gly groups, except for the 0.15 mg/kg NS-Gly group, compared to the control ($P < 0.05$). The significant differences were not detected in villus height and V/C. In the jejunum, the differences ($P < 0.05$) were obtained between the 0.15 mg/kg NS-Gly and the control group in villus height. Crypt depth was reduced in all NS-Gly groups ($P < 0.05$), leading to the V/C reduced significantly in the 0.15 and 0.45 mg/kg NS-Gly groups, compared to the control group. In the ileum, a significant increase ($P < 0.05$) of villus height was observed in the groups with 0.15 or 0.3 mg/kg Se added. Treated with Se shown no obvious difference in crypt depth compared with the control group, and only the V/C of SS group was significantly different from that of the control group ($P < 0.05$).

Gene Expression of rBAT, B^0AT1, and PepT1

The gene expression of transporters in the small intestine is shown in Fig. 3. In the duodenum, the mRNA levels of rBAT, B^0AT1, and PepT1 were the highest in 0.15, 0.15, and 0.60 mg/kg NS-Gly groups ($P < 0.05$), respectively. In the jejunum, the expression of rBAT presented an increase trend, significant in the 0.30 and 0.45 mg/kg NS-Gly groups ($P < 0.05$), and then, it went down in 0.6 mg/kg NS-Gly group. The mRNA level of B^0AT1 increased significantly in the group of 0.15 mg/kg NS-Gly diet ($P < 0.05$), and the expression of PepT1 was the highest in both the 0.15 mg/kg NS-Gly and 0.30 mg/kg SS groups.

Discussion

Although Se is essential for animal nutrition, the excess level of Se can be toxic when provided above the biological requirement [23]. The addition of Se at 0.30 mg/kg in the diet is recommended for laying hens, while the study showed that 1.5 mg/kg of NS is safe for chicken and indicated that NS operates a similar way to organic selenium and could potentially be used in poultry feed as a trace element additive [24]. In this study, after 8 weeks of Se supplementation, there were no significant differences among treatments in production performance and egg quality. Similarly, the previous reports found that dietary Se supplementation at 0.3 mg/kg from SS,

Table 5 Effects of Se supplementation on serum biochemistry of laying hens

Items	Control	SS (mg/kg)	NS-Gly (mg/kg)				SEM	*P* value
		0.3	0.15	0.3	0.45	0.6		
TP, g/L	40.76	39.90	47.26	47.26	50.69	48.46	5.32	0.310
ALB, g/L	14.26^{c}	17.16^{a}	15.48^{abc}	17.13^{a}	16.43^{ab}	14.94^{bc}	0.94	0.018
GLU, mmol/L	16.47	16.48	16.24	17.47	16.39	16.81	1.87	0.989
T-CHO, mmol/L	4.62	4.28	5.13	5.85	5.84	4.77	0.81	0.315
TG, mmol/L	15.99	15.32	19.11	14.17	14.42	14.79	1.93	0.191
GOT, IU/L	28.29	27.67	26.92	23.68	24.76	24.40	3.12	0.592
GPT, IU/L	5.58^{a}	4.78^{ab}	4.82^{ab}	2.80^{c}	3.65^{bc}	3.25^{c}	0.64	0.030

$^{a–c}$ Values are represented as the mean and SEM ($n = 8$). Means within a column with different superscripts are significantly different ($P < 0.05$). *SEM* standard error of the means. *SS* Na_2SeO_3, *NS-Gly* glycine nano-selenium, *TP* total protein, *ALB* albumin, *GLU* glucose, *T-CHO* total cholesterol, *TG* triglyceride, *GOT* aspartate aminotransferase, *GPT* alanine aminotransferase

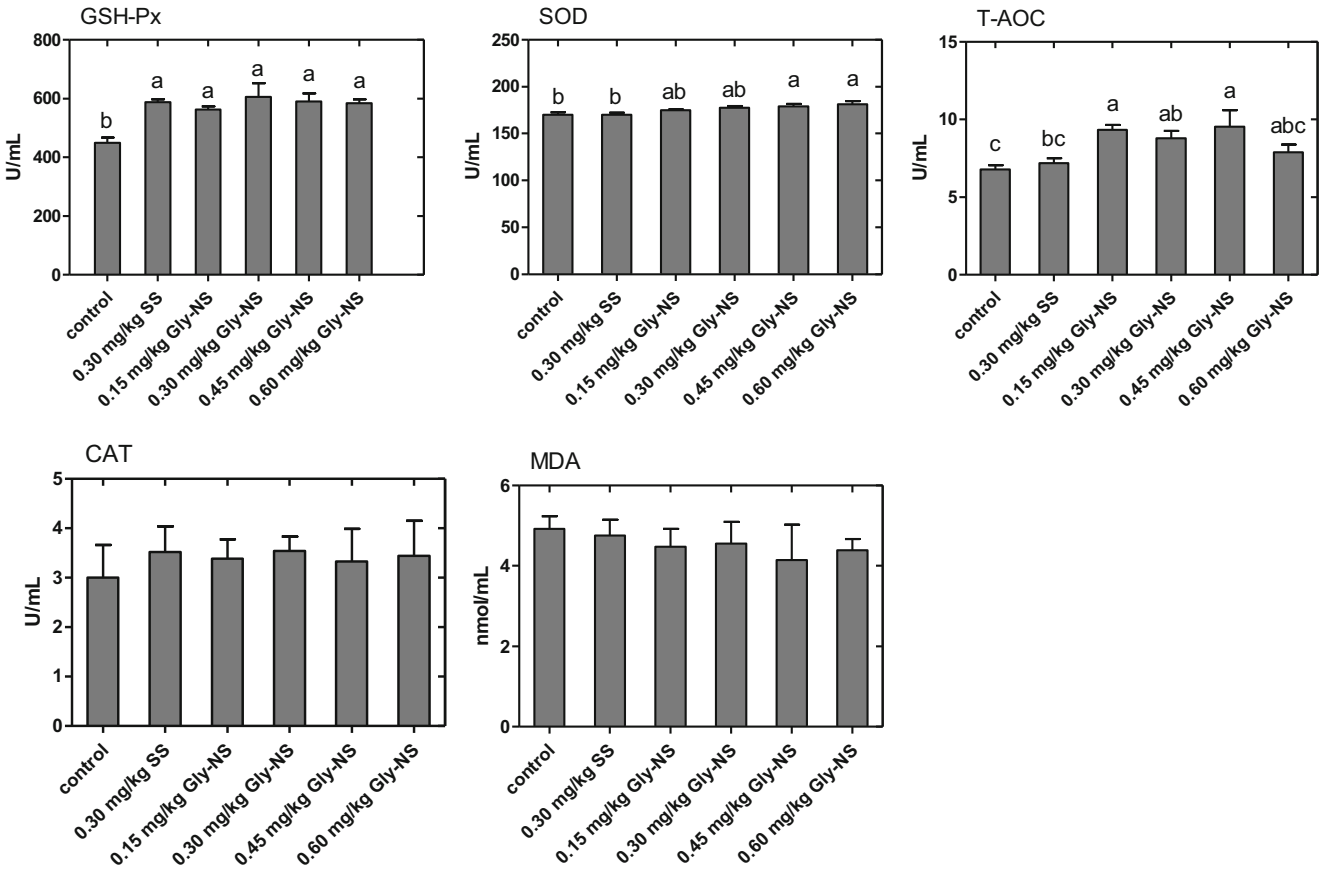

Fig. 1 Effects of Se supplementation on antioxidant parameters in the serum of laying hens. Values are represented as the mean ± SE ($n = 8$). (a–c) Bars with different superscript letters are significantly different ($P < 0.05$). SS, Na_2SeO_3; NS-Gly, glycine nano-selenium; GSH-Px, glutathione peroxide; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; CAT, catalase; MDA, malondialdehyde

selenium methionine, NS, Se-enriched yeast, and Se-enriched bean sprout did not affect productivity and egg quality [25, 26]. It is contrast to the results that supplementation with 0.3 mg Se/kg of SS, SY, or NS was beneficial for the performance of laying hens [5, 27]. These differences could be associated with the background Se in the feedstuffs, breeds, or the environment.

Serum biochemical indexes reflect the metabolism of the body, some of them could be used to measure liver function in clinical practice, which reflects body health [28]. In the current study, Se supplementation significantly enhanced the levels of serum ALB, except for the group of 0.6 mg/kg NS-Gly. Serum ALB is a major extracellular source of reduced sulfhydryl groups, which are potent scavengers of reactive oxygen and nitrogen species [29]. GOT and GPT are important amino acid transferases in animals, which are the indicators of protein metabolism in the body. They are released from the liver or cardiac cells into the plasma and the increase beyond the normal range indicates liver injury or damage [30]. Studies demonstrated that Se may prevent the decrease in GOT and GPT activities in the liver and kidneys caused by harmful substances and it was speculated that Se has the function of protecting animal liver [31]. Similarly, our results showed that, with the Se administration, the activities of GPT were significantly reduced in the NS-Gly addition groups compared with the control group. Therefore, these results indicated that NS-Gly is associated with the liver function of laying hens and may promote the utilization of amino acids by the body.

Oxidative stress is defined as the presence of metabolic and radical substances or the so-called reactive (oxygen, nitrogen, or chlorine) species [32, 33]. Se is a vital antioxidant, it forms selenocysteine which is a part of the active center of GSH-Px, and at least another seven selenoproteins may play a role in combating oxidative stress and removing toxins associated with reactive oxygen species [34, 35]. It has been previously reported that different forms of Se (organic, inorganic, or Nano-Se) affect antioxidant activity [36–39]. Our results showed that dietary SS or NS-Gly supplementation led to significant increases in serum GSH-Px activity compared to the control, consistent with the previous studies. Also, we found that, with the increasing supplementation of Gly-NS, the levels of hepatic GSH-Px increased. However, there was no significant effect on serum and liver SOD activity under 0.30 mg/kg Se supplementation observed. In terms of T-AOC of the liver or serum, high Se supplementation did not seem to be necessary to resist oxidation; similar reports were obtained

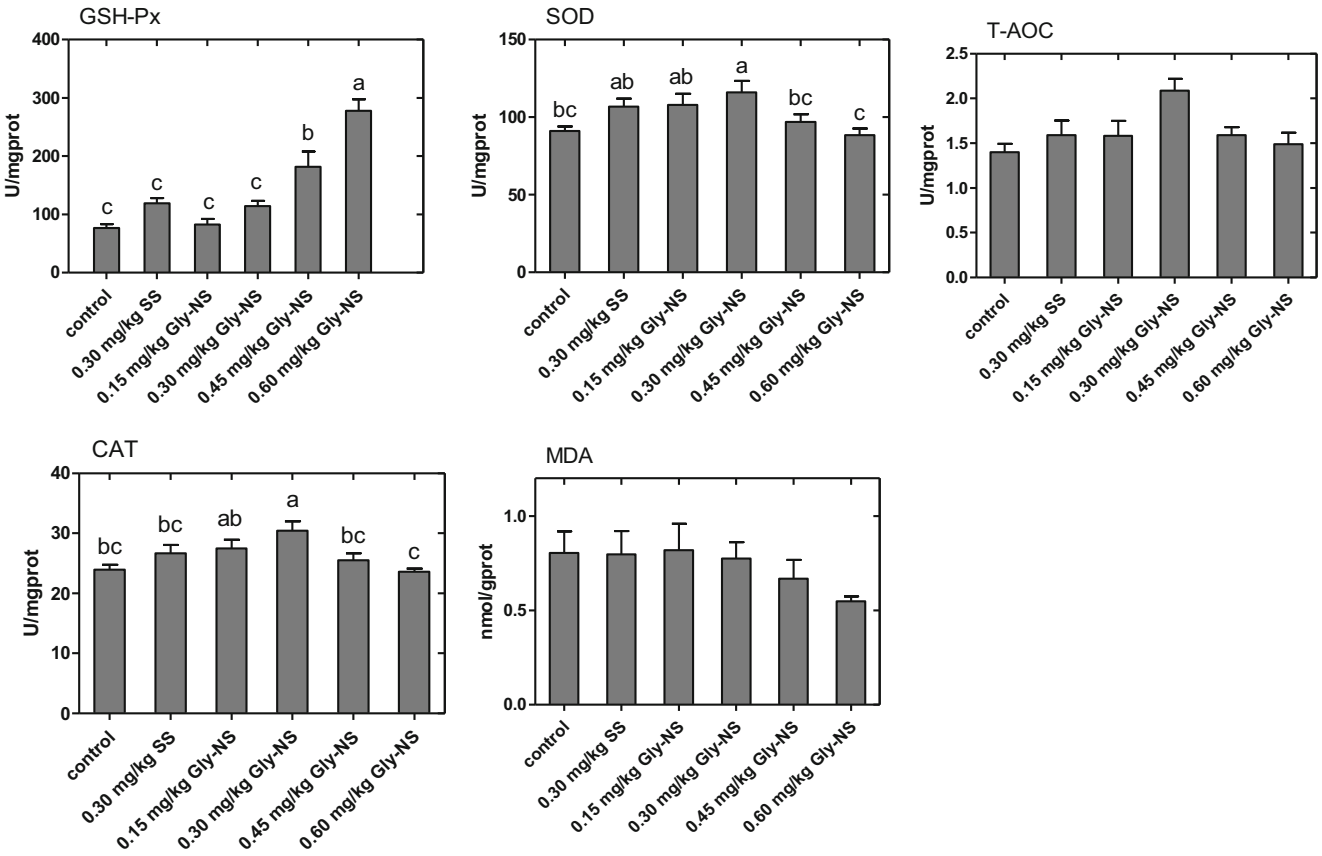

Fig. 2 Effects of Se on liver antioxidant indices of laying hens. Values are represented as the mean ± SE ($n = 8$). (a–c) Bars with different superscript letters are significantly different ($P < 0.05$). SS, Na_2SeO_3; NS-Gly, glycine nano-selenium; GSH-Px, glutathione peroxide; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; CAT, catalase; MDA, malondialdehyde

by Gan et al. [40]. Therefore, Se supplementation can improve the antioxidant capacity of laying hens to ensure the maintenance of health.

The small intestine is the main site for nutrient digestion and absorption [41]. The basic function of the small intestinal mucosa, which consists of a single layer of epithelial cells, is to digest and absorb nutrients and block pathogenic bacteria and toxic substances in the intestinal cavity [42]. The crypt is a tubular gland formed by the small intestinal epithelium descending into the lamina propria at the root of the villi [43]. A higher villus height to crypt depth ratio (V/C) indicates a higher rate of the digestion and absorption function; it is more representative when measure of individual [44]. On the current trial, dietary Se intake was conducive to the structure of duodenum, jejunum, and ileum, which were agreed with the previous researches of Lianping et al. [45] and Ahmed [46]. They found that dietary organic Se supplementation produced positive effects on some villi morphological characteristics in both duodenum and jejunum. The changes in crypt depth indicated that Gly-NS may improve the intestinal structure and function by promoting the proliferation of intestinal epithelial cells. Our results also suggested that Gly-NS performed better in the duodenum and jejunum than in the ileum. Meanwhile, the SS group just showed significant difference in villi height of ileum compared with the control group. Those may be related to the main site and mode of absorption of different forms of Se in the intestine.

The transportation of NS across the biological body is determined by its physicochemical properties, including size and shape [47]. Theoretically, nano-sized particles (NPs) can pass through the intestinal epithelium in two ways: paracellular or transcellular [48]. Gly-NS is too large to get into paracellularly, restricted by the narrow region of intercellular spaces and by the tightness of the junctions between the epithelial cells [49]. Transcellular transport of NPs takes place through a process called transcytosis, which starts with endocytosis in the apical membrane of the cells. Subsequently, the NPs are transported through the cells and released on the basolateral pole [50]. Gangadoo et al. [24] and Shi et al. [51] suggested an active transport mechanism of NS similar to organic Se. Perhaps it is possible for Gly-NS to enter cells through organic Se transporters. Selenomethionine is commonly used as organic Se source. The major route for the uptake of selenomethionine is the system b0 and + rBAT [52], and the B0 system may dominate overall transport of selenoamino acids [53, 54]. So, we evaluated the expression levels of rBAT, B^0AT1,

Table 6 Effects of Se supplementation on villi morphology of the small intestine of laying hens

Items	Control	SS (mg/kg)	NS-Gly (mg/kg)				SEM	*P* value
		0.3	0.15	0.3	0.45	0.6		
Duodenum								
Villus height, um	1342.17	1376.19	1394.49	1375.14	1285.98	1281.75	52.57	0.184
Crypt depth, um	165.61^{a}	144.70^{abc}	151.06^{ab}	127.50^{bc}	123.95^{c}	126.78^{bc}	11.30	0.004
V/C	8.56	10.12	9.73	10.04	9.72	9.70	0.93	0.602
Jejunum								
Villus height, um	1105.24^{bc}	1191.96^{ab}	1236.96^{a}	1114.68^{abc}	1120.21^{abc}	1060.96^{c}	56.25	0.042
Crypt depth, um	143.66^{a}	130.22^{ab}	114.92^{b}	113.56^{b}	103.15^{b}	104.59^{b}	12.06	0.018
V/C	7.68^{b}	9.67^{ab}	10.94^{a}	8.72^{b}	11.10^{a}	8.12^{b}	0.92	0.030
Ileum								
Villus height, um	737.27^{c}	918.66^{a}	938.59^{a}	903.37^{ab}	824.86^{bc}	800.14^{c}	41.33	0.000
Crypt depth, um	95.53	93.71	95.55	94.65	93.11	99.97	6.44	0.886
V/C	7.84^{b}	9.98^{a}	9.46^{ab}	9.61^{ab}	9.59^{ab}	8.50^{ab}	0.63	0.024

$^{a–c}$ Values are represented as the mean and SEM ($n = 8$). Means within a column with different superscripts are significantly different ($P < 0.05$). *SEM* standard error of the means. *SS* Na_2SeO_3, *NS-Gly* glycine nano-selenium, *V/C* Villus height/Crypt depth

and PepT1, which play a critical role in the epithelial amino acid and small peptide transportation, and found that the expression of rBAT, B^0AT1, and PepT1 increased at different degrees in the duodenum, but oddly, they did best in 0.15 and 0.60 mg/kg NS-Gly groups. In the jejunum, the expression of B^0AT1 was similar to the duodenum, and the rBAT presented increase trend, significant in the 0.30 and 0.45 mg/kg NS-Gly groups, but then it went down in the 0.6 mg/kg NS-Gly group. The mRNA expression of PepT1 caught our attention as it significantly increased in the 0.30 mg/kg SS group. We speculated that the absorption efficiency and mode of NS-Gly may be different in different intestinal segments. It is generally believed that the duodenum is the main site of Se absorption. Liu et al. [55] indicated that the jejunum was the main SS absorption site, and the Se absorption is a saturated carrier-mediated process in the duodenum, but a non-saturated diffusion process in the jejunum and ileum. It was shown that the addition of NS-Gly did change the expression of amino acid transport carrier, but whether it means that NS-Gly can be

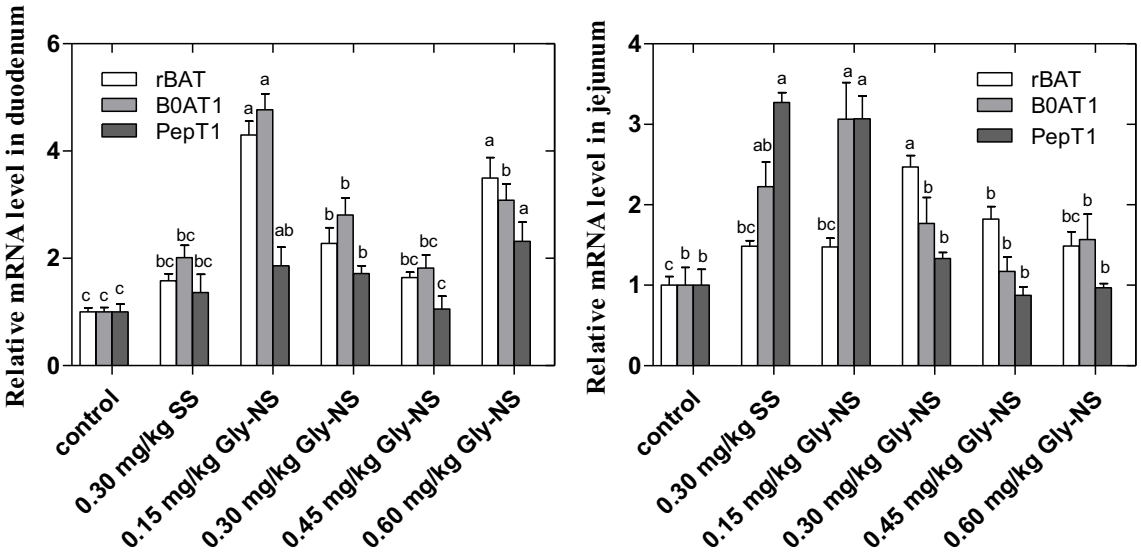

Fig. 3 Effects of Se on mRNA expression of rBAT, B^0AT1, and PepT1 of laying hens. Method of $2^{-\Delta\Delta Ct}$ was applied for the calculation of relative gene expression with β-actin as the endogenous control and the average ΔCt value of control group as the calibrator to normalize the signal. Values are represented as the mean ± SE ($n = 6$). (a–c) Bars with different superscript letters are significantly different ($P < 0.05$). SS, Na_2SeO_3; NS-Gly, glycine nano-selenium; rBAT, solute carrier family 3 member 1; B^0AT1, solute carrier family 6 member 19; PepT1, solute carrier family 15 member 1

absorbed and transported by amino acid transport carrier needs further study.

Conclusion

The present study indicated that laying hens with NS-Gly supplementation were more tolerant to oxidative stress, and NS-Gly was conducive to the structure of the small intestine but have negligible effects on their growth performance and egg quality. There was slight difference between the 0.15 mg/kg NS-Gly and 0.30 mg/kg SS group. It is practical to use lower concentration of NS-Gly as a feed Se supplement for laying hens.

Authors' Contributions Wenting Zhou, Xinyang Dong, and Xiaoting Zou conceived the idea; Wenting Zhou and Sasa Miao performed the experiment; Wenting Zhou, Sasa Miao, and Mingkun Zhu performed the data analyses and wrote the manuscript; all authors contributed to the revisions.

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Compliance with Ethical Standards

The current study was approved by the Animal Care and Welfare Committee of Animal Science College and the Scientific Ethical Committee of the Zhejiang University (No. ZJU2013105002) (Hangzhou, China).

Conflict of Interest The authors declare that they have no conflict of interest.

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