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Effects of different levels of nano-selenium on growth performance, antioxidant capacity, biochemical parameters, and selenium content in Landes geese

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ABSTRACT - The objective of this study was to evaluate the effects of dietary supplementation with different levels of nano-selenium (NS) on growth performance, antioxidant activity, biochemical parameters, and selenium content in Landes geese. A total of 120 80-week-old healthy Landes geese (4.44±0.03 kg) were randomly assigned to three groups, each with four replicates of 10 birds. The control birds were fed a basal diet without further dietary supplementation (0.0 mg/kg of NS) and the two experimental groups were fed the basal diet supplemented with dietary NS at 0.2 or 0.4 mg/kg of feed. The results demonstrated that NS dietary supplementation had no significant effect on growth performance. Increased total superoxide dismutase activity in serum, breast muscle and liver, glutathione peroxidase level in serum and liver, and catalase in breast muscle and liver were observed for both NS supplemented groups. Additionally, reduced malondialdehyde in serum, breast muscle, and liver was detected in both NS-supplemented groups. Compared with the control, the birds fed diets supplemented with NS had lower concentrations of alanine aminotransferase, triglyceride, aspartate aminotransferase, and low-density lipoprotein cholesterol in serum, while high-density lipoprotein cholesterol was increased. Furthermore, increased selenium, especially in the liver, was found in groups with dietary supplementation of NS. These findings suggest the supplementation of NS in diets can improve antioxidant status, biochemical parameters, and tissue selenium content, although it has no significant effect on growth performance of Landes geese.

Keywords: antioxidant status, average daily gain, diets, serum biochemical parameters, supplementation

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1. Introduction

Selenium (Se) is a basic trace element in animals and humans. The dietary shortage of Se might lead to white muscle disease (Bakhshalinejad et al., 2018; Semenenko et al., 2021), low glutathione peroxidase (GSH-Px) (Silva et al., 2021), and reproduction inefficiency (Hemingway et al., 2003). Selenium has many physiologically relevant regulatory functions such as maintaining normal immunity, reducing free radicals, and improving growth performance (Li et al., 2021; Nemati et al., 2021). The biological functions of Se are carried out utilizing protein selenium (selenase). Importantly, Se is an integral constituent of the antioxidative enzyme GSH-Px, which promotes organic lipid peroxides (H_2O_2) and detoxifies hydrogen peroxide (GSH) (Look et al., 1997).

At present, Se sources used in animal feeds include inorganic Se, organic Se, and nano-selenium (NS) (Selim et al., 2015). Inorganic Se is the most common form used in dietary supplementation, though poor selenoprotein activity, high toxicity, and low absorption have been observed (Peng et al., 2007; Zhang et al., 2008; Hu et al., 2012). However, NS shows unique characteristics like large specific surface, high absorption efficiency, high surface activity, and low toxicity (Wang et al., 2007). In animal diets, NS is an additive increasingly used in animal production. A previous study showed that growth performance, immune function, and carcass characteristics of male Ross chicks were improved by dietary supplementation with NS (Ahmadi et al., 2018). Moreover, supplementation of NS in diets for chickens and pigs has been widely reported, and dietary NS had significantly positive influence on performance, immunity, and carcass and meat quality for chicks (Soliman et al., 2020). However, dietary supplementation with NS in geese diets is rarely reported. Therefore, in this study, the effect of dietary supplementation with different levels of NS on growth performance, antioxidant activity, biochemical parameters, and Se content in Landes geese (*Anser anser*) was effectively investigated.

2. Material and Methods

The methods used in this study were approved by the local Institutional Animal Care and Use Committee (case number G56/2018). The experiment complies with the approved guidelines and regulations of the regional Animal Ethics Committee. The experiment was performed in Changsha, China (28.1844° N, 113.0318 °E).

2.1. Animal and experimental procedures

A total of 120 80-week-old healthy male Landes geese with similar body weight (4.44±0.03 kg) were chosen and housed collectively during growth. Based on a completely randomized design, geese were individually divided into three groups, each with four replicates of 10 birds. Feed intake was progressively increased for one week to enlarge the volume of the digestive tract and initiate the metabolism to adapt to overfeeding. Before forced feeding, initial body weights (IBW) of the geese were measured separately.

After the end of the pre-overfeeding period, the basal diet without added selenium was formulated based on corn meal in compliance with the Chinese nutritional requirements of broiler geese (Table 1). The diet, provided by a commercial company, contains 98% corn, 1% plant oil, 0.5% salt, and 0.5% vitamins. The diet was prepared by grinding components in the Wiley mill (about 1 mm particle size) and slowly stirring in plant oil and vitamin. The experimental groups were as follows: the first group acted as the control, which was only fed the basal diet; the second group was given the basal diet supplemented with 0.2 mg/kg of NS (0.2 mg NS/kg); the third group was given the basal diet supplemented with 0.4 mg/kg of NS (0.4 mg NS/kg).

Table 1 - Formulation and calculated composition of the basal diet (as-fed basis)

Item	Amount
Ingredients	
Corn (%)	98
Plant oil (%)	1
Salt (%)	0.5
Vitamin (%)	0.5
Total (%)	100
Chemical analysis	
Crude protein (g/kg)	90
Crude fat (g/kg)	4.5
Metabolizable energy (kcal/kg)	3370

Forced feeding was carried out with the following procedure in compliance with approved guidelines and regulations of the regional Animal Ethics Committee. During the first week of the 28-day experiment, geese were overfed every eight hours with 450 g high-carbohydrate diet. From day 8 to 14, the geese were given four meals of 1000 g/d. For the last two weeks, geese were given five meals of 1,500 g/d. During the forced feeding, the temperature was 20-25 ℃, and the relative humidity was 70-80%. The geese were allowed free access to water.

2.2. Sample collection

After 28 d of overfeeding, the geese were subjected to a water-only fast overnight for 12 h. The next morning, geese were weighed, and blood was collected through jugular vein and placed at room temperature for 1 h. The serum supernatant was collected after centrifugation at 3000 g for 15 min at 4 ℃. Lastly, 1.0-1.5 mL of serum was transferred into a 1.5-mL centrifuge tube and stored below −20 ℃. The blood serum sample was divided evenly into two portions; one for analyzing antioxidation of serum and the second for performing biochemical tests (Liu et al., 2019).

After blood collections, the geese were sacrificed by exsanguination. The breast muscle, leg muscle, and liver were removed quickly, frozen in liquid nitrogen, and stored at −80 ℃ until analysis of antioxidation and Se tissue content analyses could be performed.

2.3. Animal growth performance

During the process of feeding, body weight was recorded weekly to calculate the average daily gain (ADG) and body weight gain rate. Forced feeding was terminated at 28 d, and the geese were starved for 12 h. The geese final body weight (FBW) was measured by digital balance.

2.4. Antioxidant activity

Catalase (CAT), GSH-Px, total superoxide dismutase (T-SOD), and malondialdehyde (MDA) in serum, breast, and liver were detected by using the corresponding kits in compliance with the instructions and according to the instructions of the manufacturer to operate the microplate reader.

2.5. Biochemical analysis

Alanine aminotransferase (ALT), globulin (GLB), aspartate aminotransferase (AST), ALB (albumin), total protein (TP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in serum were measured using an automatic biochemical instrument and matching kits produced by Mindray according to the method reported by Liu et al. (2021).

2.6. Selenium content

The connective tissues were removed from the breast muscle, leg muscle, and liver. The Se content in the tissue was determined by the fluorescence spectrophotometer. Homogenized tissue sample (5 g) in 25 mL of HNO_3-$HClO_4$ (4:1) was added into a 150 mL Erlenmeyer flask and heated at 100 ℃ until white fumes appeared. Hydrochloric acid (5 M; 8 mL) was added, and the mixture was again heated until white fumes were seen. The flask was removed from heat and left to cool. Once the mixture cooled to room temperature, 10 mL of 25% HCl was added, and the solution was boiled again. After reaching a boil, the flask was cooled to room temperature, and 50 mL ultrapure water was added. Finally, the supernatant was collected and measured by the spectrophotometer at the following parameters: 270 V negative high pressure, 30 mA hollow cathode lamp current, 7 mm electric heating atomizer height, high purity carrier gas Ar, 800 mL/min carrier, 1.0 inject samples.

2.7. Statistical analysis

The data obtained from the completely randomized design were analyzed with SPSS7.0 and general linear models (GLM). The difference among the groups was analyzed by one way ANOVA, and multiple comparisons were performed by Duncan's method (Chambers and Hastie, 1992). All the data were expressed as the mean and standard error of mean (SEM). The statistical model was analyzed as follows:

$$Y_{ij} = \mu + A_i + e_{ij},$$

in which Y_{ij} is the observation of traits, μ is the overall mean, A_i is the treatment effect, and e_{ij} is the experimental error. Orthogonal polynomial contrasts were used to determine linear and quadratic responses to NS. The significance between different groups was measured by a P-value <0.05.

3. Results

3.1. Growth performance

We found no significant difference (P>0.05) among IBW, FBW, ADG, and body weight gain rate. However, groups fed diets supplemented with NS (0.2 and 0.4 mg/kg) had higher ADG and body weight gain rate compared with the control (0.0 mg/kg) (P>0.05) (Table 2). Between both groups receiving NS-supplemented diets, geese receiving less NS/kg had the highest ADG and body weight gain rate (P>0.05).

Table 2 - Growth performance of geese fed diets with different levels of nano-selenium

Item	Dietary treatment			SEM	P-value		
	0.0 mg/kg	0.2 mg/kg	0.4 mg/kg		Treatment	Linear	Quadratic
IBW (kg)	4.45±0.03	4.42±0.08	4.44±0.07	0.03	0.948	0.915	0.780
FBW (kg)	8.02±0.07	7.87±0.12	7.93±0.12	0.05	0.491	0.558	0.403
ADG (kg)	0.18±0.00	0.23±0.00	0.21±0.00	0.01	0.192	0.455	0.163
Body weight gain rate (%)	80.76±2.33	85.92±4.32	82.90±2.42	1.75	0.541	0.652	0.399

IBW - initial body weight; FBW - final body weight; ADG - average daily gain; SEM - standard error of the mean.

3.2. Antioxidant activity

The effects of NS supplementation with different levels on activities of CAT, GSH-Px, T-SOD, and MDA in serum, breast, and liver was studied (Table 3). Supplementation of 0.2 and 0.4 mg NS/kg increased the activity of GSH-Px and T-SOD in serum (P<0.05). Similarly, supplementation of 0.2 and 0.4 mg NS/kg increased activities of CAT and T-SOD in breast muscle, while CAT and GSH-Px activities were increased in the liver (P<0.05). Compared with the control, MDA activity in breast muscle and liver was reduced in both experimental groups (P<0.05).

3.3. Serum biochemical parameters

The birds fed NS-supplemented diets had lower levels of ALT, TG, and LDL-C than the control birds (Table 4). Birds fed diets containing 0.4 mg NS/kg had the highest HDL-C counts (P<0.05) and lowest AST levels (P<0.05). Furthermore, the geese fed NS-supplemented diets had higher TC concentration than the birds in the control, though we observed no significant difference in TC concentration of the birds fed 0.4 mg NS/kg in the diets when compared with the other two groups.

Table 3 - Antioxidant enzyme activities of geese fed diets with different levels of nano-selenium in tissues

Item	Dietary treatment			SEM	P-value		
	0.0 mg/kg	0.2 mg/kg	0.4 mg/kg		Treatment	Linear	Quadratic
Serum							
CAT (U/mL)	11.81±1.80	11.97±2.35	11.49±3.47	1.52	0.992	0.934	0.929
GSH-Px (U/mL)	374.25±55.28b	697.42±48.07a	751.93±20.62a	50.28	<0.001	<0.001	0.028
T-SOD (U/mL)	94.02±5.62b	130.09±9.78a	144.99±2.74a	6.14	<0.001	<0.001	0.206
MDA (nmol/mL)	10.17±1.39	8.95±0.89	7.99±1.08	0.69	0.419	0.195	0.932
Breast muscle							
CAT (U/mg protein)	32.27±0.50b	34.62±0.96a	36.61±0.74a	0.58	0.005	0.001	0.851
GSH-Px (U/mg protein)	145.03±8.61	150.18±2.79	144.57±1.55	2.33	0.561	0.943	0.302
T-SOD (U/mg protein)	33.87±3.02b	118.47±2.29a	120.09±3.16a	11.47	<0.001	<0.001	<0.001
MDA (nmol/mg protein)	6.61±0.43a	2.30±0.11b	1.87±0.13b	0.59	<0.001	<0.001	<0.001
Liver							
CAT (U/mg protein)	37.96±0.46c	47.28±0.91b	54.34±1.14a	1.61	<0.001	<0.001	0.010
GSH-Px (U/mg protein)	563.16±7.08c	839.52±10.54b	1038.88±9.76a	47.58	<0.001	<0.001	<0.001
SOD (U/mg protein)	42.92±9.11	94.64±14.09	101.66±18.48	10.65	0.074	0.004	0.004
MDA (nmol/mg protein)	10.87±0.74a	5.38±0.06b	2.26±0.54c	1.07	<0.001	<0.001	0.167

CAT - catalase; GSH-Px - glutathione peroxidase; T-SOD - total superoxide dismutase; MDA - malondialdehyde; SEM - standard error of the mean.
Different letters in the same row were used to show different significance at P<0.05.

Table 4 - Biochemical indexes of geese fed diets with different levels of nano-selenium

Item	Dietary treatment			SEM	P-value		
	0.0 mg/kg	0.2 mg/kg	0.4 mg/kg		Treatment	Linear	Quadratic
ALT (U/L)	95.63±1.50a	65.42±0.96b	54.44±1.04c	4.34	<0.001	<0.001	<0.001
GLB (U/L)	36.33±0.88	33.88±0.81	36.30±0.82	0.52	0.086	0.979	0.030
AST (U/L)	234.98±3.69a	229.04±2.74a	153.90±2.86b	8.65	<0.001	<0.001	<0.001
ALB (U/L)	17.34±0.55	17.65±0.50	17.66±0.29	0.25	0.876	0.650	0.782
TP (U/L)	51.83±0.70	53.00±0.66	54.08±0.52	0.41	0.071	0.958	0.958
TG (mmol/L)	4.33±0.22a	3.60±0.18b	3.04±0.22b	0.17	0.001	<0.001	0.762
TC (mmol/L)	10.96±0.24b	12.09±0.18a	11.76±0.22ab	0.19	0.034	0.051	0.051
LDL-C (mmol/L)	11.43±0.15a	2.60±0.25b	2.72±0.28b	0.86	<0.001	<0.001	<0.001
HDL-C (mmol/L)	7.24±0.28b	7.43±0.18b	8.21±0.28a	0.16	0.027	0.014	0.291

ALT - alanine aminotransferase; GLB - globulin; AST - aspartate aminotransferase; ALB - albumin; TP - total protein; TG - triglyceride; TC - total cholesterol; LDL-C - low-density lipoprotein cholesterol; HDL-C - high-density lipoprotein cholesterol; SEM - standard error of the mean.
Different letters in the same row were used to show different significance at P<0.05.

3.4. Selenium content

The NS-supplemented diets increased Se content in tissues of the geese (Table 5). A significant increase of Se deposition in the liver (P<0.05) was observed in both groups receiving NS-supplemented diets, but Se content in breast muscle and leg muscle was not significantly different among all groups.

Table 5 - Selenium distribution in different tissues of geese fed diets with different levels of nano-selenium

Item	Dietary treatment			SEM	P-value		
	0.0 mg/kg	0.2 mg/kg	0.4 mg/kg		Treatment	Linear	Quadratic
Breast muscle	0.21±0.01	0.22±0.01	0.25±0.02	0.008	0.142	0.062	0.485
Leg muscle	0.16±0.00	0.16±0.00	0.17±0.00	0.002	0.412	0.203	0.843
Liver	0.20±0.01b	0.31±0.02a	0.31±0.02a	0.020	0.001	0.001	0.045

SEM - standard error of the mean.
Different letters in the same row were used to show different significance at P<0.05.

4. Discussion

The results of this study showed that supplementation with different levels of NS in the diet had no significant effect on geese growth performance. The effects of dietary supplementation with Se on growth performance in animals have varied conclusions. Not much is known about the NS influence on geese. Consistent with our findings, previous work has found no differences in growth parameters between broilers fed diets supplemented with varying levels of NS (Mohammadi et al., 2020; Bami et al., 2022). However, other studies have shown that dietary supplementation of NS had a significant effect on FBW, daily body weight gain, and feed conversion ratio (FCR). In Guangxi Yellow chickens fed NS-supplemented diets at 0.3 and 0.5 mg/kg, higher FBW and daily body weight gain (DWG) were observed (Zhou and Wang, 2011). Dietary supplementation with Se was also shown to improve gain:feed ratio and ADG in broiler chickens (Bakhshalinejad et al., 2018). El-Deep et al. (2016) verified that FCR was significantly improved with both NS, Se, and combinations of both under thermoneutral and high temperature conditions. Xia et al. (2005) reported that higher growth performance was observed in chickens fed diets supplemented with between 0.4-1.0 mg/kg NS when compared with chickens fed a Na_2SeO_3 supplemented diet. Therefore, supplementation of animal diets with Se remains unclear, and growth performance may depend on different levels and forms.

The antioxidant system has a diverse set of defense mechanisms, such as CAT, GSH-Px, T-SOD, and MDA among others. Selenium is an essential element required for components of the antioxidant defense mechanism. Glutathione peroxidase (GPx) works within the cytoplasm and plays a significant role in neutralizing reactive radicals (Yuan et al., 2012; Xu et al., 2016). The results of this study showed that the geese fed NS-supplemented diets had higher GSH-Px and T-SOD in serum than the birds on the control group. Birds fed 0.2 and 0.4 mg NS/kg had higher CAT and T-SOD in breast muscle compared with the control. Furthermore, geese fed diets containing 0.2 and 0.4 mg NS/kg had higher CAT and GSH-Px in liver and lower MDA in serum, breast, and liver when compared with the control. The study proved that the inorganic Se has higher bioavailability than the organic Se (Zhang et al., 2001). Supplementation of NS increased CAT, GSH-Px, and T-SOD levels to a greater degree than dietary supplementation of sodium selenite. The levels of GSH-Px in serum and liver were increased with NS-supplementation (Mohapatra et al., 2014a). Zhou and Wang (2011) reported that chickens fed diets containing NS had higher GSH-Px than control groups. Moreover, the results indicated that chickens fed diets supplemented with selenium yeast and sodium selenium had higher CAT, T-SOD, total antioxidant capacity, and lower MDA (Ahmad et al., 2012).

Selenium can increase TG, free fatty acids, and total cholesterol, but it decreases LDL-C in serum (Iizuka et al., 2001). Diagnostic evaluation of hepatocellular injury heavily relies on levels of ALT and AST, generally reflecting the physical condition of liver and other tissues (Wang et al., 2012). In this research, supplementation of NS in the diet has an influence on geese biochemical parameters. The birds fed diets containing 0.4 mg NS/kg had lower AST content and higher HDL-C concentration than the other two groups. Previous studies determined supplementation of inorganic and bacterial organic Se in the diet had a significant impact on ALT, lactate dehydrogenase (LDH), AST, and serum creatinine levels in broiler chickens (Dalia et al., 2017), which is in agreement with the results of this study. However, different kinds of Se show various effects on biochemical parameters. Emara et al. (2019) found that dietary supplementation with NS reduced the levels of triglycerides, phospholipid, TC, LDL, and very low-density lipoprotein cholesterol (VLDL) values. Sun et al. (2015) observed TP content and GLB were influenced when Yangzhou geese were fed diets supplemented with 0.35 mg/kg of Se. There were no differences in biochemical parameters between chickens fed diets supplemented with or without Se (Navas-Carretero et al., 2011).

This study also showed that Se content in geese muscle and liver can be improved by supplementing NS into the feed. We observed Se concentration in the liver was much higher than in breast or leg muscle. Similar results were reported by Cai et al. (2012), who investigated the effects of NS on tissue Se content in broilers. The Se content in laying hens fed NS-supplemented diets was higher than in birds given Se-free diets. Additionally, as the amount of NS increased in the diet, the Se content in the

chickens increased (Petrovič et al., 2006). Nano-selenium has excellent bioavailability, high catalytic efficiency, strong adsorption capacity, and low toxicity (Zhang et al., 2008). Broiler chickens fed diets supplemented with NS-Met had higher Se concentration in breast muscles and liver than those fed diets supplemented with organic and inorganic Se complexes (Mohammadi et al., 2019). The report showed that different levels and sources of dietary Se increased tissue Se concentration in broiler chickens. When fed the diet containing 0.25 mg/kg NS, Se content gradually increased in liver, breast muscle, and serum (Dukare et al., 2020). A dose-dependent response between tissue Se content and NS supplementation levels was observed. The higher tissue Se concentrations in broiler chickens were found when increasing supplementation level of NS in the dietary (Celi et al., 2014), which is consistent with the results of this study. Similarly, recent studies reported that diets supplemented with NS increased the Se content in Wister rats' serum (Mohapatra et al., 2014b), broiler chickens' liver (Petrovič et al., 2006), and hens' eggs (Attia et al., 2010).

5. Conclusions

This study suggests that nano-selenium-supplemented diets could improve the antioxidant performance and biochemical parameters in Landes geese. Although dietary supplementation with different levels of nano-selenium has little effect on geese growth performance, significant positive changes in other biochemical markers are observed in geese fed diets supplemented with 0.4 mg nano-selenium/kg. Furthermore, supplementing nano-selenium in the diet is effective in increasing meat selenium content.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Y. Liu. Data curation: Y. Liu. Formal analysis: Y. Liu and X. Liu. Funding acquisition: Y. Liu and X. Qu. Investigation: Y. Liu and X. Xiang. Methodology: Y. Liu and S. Guo. Project administration: X. Qu. Resources: Y. Liu and X. Qu. Software: Y. Liu and X. Liu. Supervision: Y. Liu and X. Liu. Validation: Y. Liu, X. Xiang and X. Qu. Visualization: Y. Liu and S. Guo. Writing – original draft: Y. Liu. Writing – review & editing: Y. Liu.

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