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Effects of Nano-Selenium on Antioxidant Capacity in Se-Deprived Tibetan Gazelle (*Procapra picticaudata*) in the Qinghai–Tibet Plateau

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Abstract

Tibetan gazelle (*Procapra picticaudata*) is an endangered ungulate in the Qinghai–Tibet Plateau, China. This study aimed to determine the influence of nano-Se on antioxidant system in Se-deprived *P. picticaudata.* We analyzed contents of mineral elements in soil, forage, and animal tissue. Blood parameters and antioxidant indexes were also determined. The results showed that Se concentrations in the soil and forage from affected pasture were significantly lower than those in healthy area ($P < 0.01$). Se concentrations in blood and hair from affected *P. picticaudata* were also significantly lower than those in healthy animals ($P < 0.01$). Meanwhile, the levels of Hb, RBC, and PCV in affected gazelle were significantly lower than those in healthy animal ($P < 0.01$). The activities of AST, ALT, LDH, CK, and UA content in affected animal were significantly lower than those in healthy gazelles ($P < 0.01$). The levels of SOD, GSH-Px, CAT, and T-AOC in serum were significantly lower and the MDA content was significantly higher in affected compared with healthy gazelle ($P < 0.01$). Affected *P. picticaudata* were treated orally with nano-Se, Se concentration in blood significantly increased and serum antioxidant indexes greatly returned to within the healthy range. Consequently, nano-Se could not only markedly increase the Se content in blood in Se-deprived *P. picticaudata* but also much improves the antioxidant capacity.

Keywords Selenium deprivation · Nano-selenium · ***Procapra picticaudata*** · Antioxidant capacity · The Qinghai–Tibet Plateau

Introduction

Tibetan gazelle (*Procapra picticaudata*) belongs to Antilopinae, Procapra, and is endemic and a well-known ungulate in the Qinghai–Tibet Plateau, China [1, 2]. The *P. picticaudata* are widespread throughout Qinghai, Tibet, and open grassland of Inner Mongolia, inhabiting terrain between 3000 and 5100 m in elevation. Alpine meadow, high elevation steppe, and desert steppe are the primary habitats of *P. picticaudata*, preferring broad valley with sufficient water supply and gentle slope [3]. In recent years, with the increased human activities and the changed environment, the distribution area has been shrinking, and the number of *P. picticaudata* has been decreasing, which has been listed as the class II protected animals in China [4, 5].

Selenium (Se) plays important roles in growth and development in animals. It occurs in selenoproteins in the form of selenocysteine, which is involved in antioxidant activity, immune modulation, endocrine function, calcium (Ca) metabolism, iodine (I) metabolism, and reproductive processes [6, 7]. Se element is an integral part of the enzyme, glutathione peroxidase (GSH-Px), which can reduce lipoperoxides and hydrogen peroxide by catalyzing reduced glutathione (GSH) to oxidized glutathione (GSSG), and thereby protecting the organism against oxidative damage [8, 9]. Animals with insufficient Se intake also exhibit different signs, including anorexia, diarrhea, attenuation of production performance,

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allotriophagia, growth retardation, fecundity depression, and emaciation [10]. Chen found that broiler fed Se-deficient forage were prone to white muscle disease, presenting as muscular dystrophy and myocardial necrosis [11]. In addition, Se deficiency can lead to developmental disorders, reproductive disease, and sperm abnormalities in rats, whereas these conditions can be reversed somewhat if Se is incorporated into the diet [12]. Se is an essential trace element for wildlife and livestock [13]. Adding Se to the feed can improve the antioxidant capacity and immune function and promote the growth and development of animals [14, 15]. Nano-selenium (nano-Se) is a common artificial nano material. It can not only be used as animal feed additive, antiosteoporosis and antiaging nano medicine, and intrauterine device in biomedical field, but also as lubricant additive, high-efficiency catalyst, and surface coating of electronic products in industrial field, bringing technological revolution to the development of animal husbandry and industry [16–20]. Nano-Se uses nanotechnology to attract the element Se by using the amide plane of protein to form nano particles, which has the advantages of high absorption efficiency, low toxicity, strong biological activity, and low environmental pollution [21, 22].

In order to find the best way to supplement Se in Se-deprived *P. picticaudata*, we supplement different levels of nano-Se or sodium selenite (Na_2SeO_3). Changes of Se concentration in blood were observed in this experiment, and antioxidant indexes were analyzed.

Materials and Methods

Study Area

The study sites are Tianjun County (36°53′–48°39′N, 96°49′–99°41′E) and Gonghe County (35°46′–37°10′N, 98°54′–101°22′ E) in the northeast of Qinghai Province, China. The average elevation is 3000 m, the average atmospheric temperature is − 5.1 to 9.0 °C, and the annual precipitation is 400 mm. The main grassland species include *Poa annua*, *Kobresia humilis*, *Thermopsis lanceolala*, *Potentilla fulgens*, *Trifolium repens*, *Lobularia*, *Saussurea eopygmaea*, *Astragalus floridus*, etc.

Experimental Design

Thirty-five healthy *P. picticaudata*, body weight (BW) 15.31 ± 1.32 kg, were selected from the Gonghe Area. Thirty-five affected *P. picticaudata* from the Tianjun Area were selected. All affected *P. picticaudata* had emaciation, anorexia, allotriophagia, and growth retardation. The affected *P. picticaudata* randomly divided into 7 groups. Se (nano-Se and Na_2SeO_3) was given orally, once/5 days for 6 consecutive times. Dose of nano-Se are 0.5, 1, and 1.5 mg/kg BW for group 1, group 2, and group 3, respectively. Dose of Na_2SeO_3 is 0.5, 1, and 1.5 mg/kg BW for group 4, group 5, and group 6, respectively. Group 7 was not supplemented Se (Control). Se contents and the antioxidant level in blood were detected every 5 days.

Sample Collection

Ten soil samples were collected from the surface layer (0 to 20 cm) in each pasture, marking sampling points. Ten samples of mixed herbage were collected from each pasture. In order to reduce soil pollution, forage were cut 0.1 cm above ground level.

The hair samples were taken from the animal necks. Each sample was washed with acetone, rinsed five times with deionized water, and then kept on a silica gel in a desiccator until analyses. The blood samples were obtained from the jugular vein using a vacuum blood collection tube without anticoagulant for biochemical analysis and a vacuum blood collection tube containing 1% sodium heparin as an anticoagulant for hematological and mineral element analyses. The serum biochemical samples were separated by centrifugation at 13,000×*g* for 10 min, and stored in vials at − 4 °C.

Sample Treatment and Analysis

The sample solvent was prepared using a microwave digestion system (Touchwin 4.0, APL Instrument Co., Ltd., Chengdu, China). Soil, forage, and animal tissues were dried in a drying chamber at 60–80 °C for 48 h. Soil samples were dissolved by microwave heating with nitric acid (HNO_3), perchloric acid ($HCLO_4$), and hydrofluoric acid (HF) mixture (5:2:5). Herbage and animal tissue samples were dissolved by microwave heating with HNO_3 and $HCLO_4$ mixture (4:1).

Mineral concentrations in soil, forage, and animal tissue were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Elements analyzed were copper (Cu), Se, iron (Fe), manganese (Mn), zinc (Zn), and molybdenum (Mo). Levels of hemoglobin (Hb), erythrocyte count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC) were determined using an automatic blood cell analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan). Biochemical analyses, which included lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), creatine kinase (CK), uric acid (UA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidant capacity (T-AOC), and catalase (CAT) were determined by automatic biochemical analyzer (SF-1, Shanghai Medical Apparatus and Instruments Factory (Shanghai, China).

Table 1 Mineral concentrations in soil and forage (mg/kg)

Elements	Soil		Forage	
	Affected area	Healthy area	Affected area	Healthy area
Mn	258.38 ± 46.72	256.64 ± 45.78	62.72 ± 15.72	60.64 ± 15.57
Zn	43.35 ± 7.62	42.54 ± 6.57	33.59 ± 6.35	35.64 ± 7.54
Se	0.03 ± 0.01b	0.09 ± 0.02a	0.02 ± 0.01b	0.09 ± 0.02a
Cu	6.54 ± 1.42	7.75 ± 1.64	4.64 ± 0.45	4.47 ± 0.64
Fe	7168.75 ± 626.22	7172.39 ± 625.35	725.27 ± 215.52	722.65 ± 216.72
Mo	1.93 ± 0.23	1.96 ± 0.22	1.77 ± 0.13	1.75 ± 0.14

Different little letters indicate significant difference ($P < 0.01$)

Mn manganese, *Zn* zinc, *Cu* copper, *Fe* iron, *Mo* molybdenum, *Se* selenium

Statistical Analyses

Experimental data were analyzed by using the statistical package (SPSS, version 23.0, Inc., Chicago, Illinois, USA) and presented in the form of mean ± standard deviation (SD). Significant differences between groups were assessed using Student's *t* test with least significant differences of 1% ($P < 0.01$).

Results

Mineral Concentrations

Concentrations of Se in soil and forage from affected area were significantly lower than those in healthy area ($P < 0.01$), and there were no significant differences in other elements, as shown in Table 1. Se contents in the blood and hair from affected *P. picticaudata* were significantly lower than those in healthy *P. picticaudata* ($P < 0.01$), and there was no significant difference in other elements, as shown in Table 2.

Effects of Se Deficiency on Physiological and Biochemical Parameters

As shown in Table 3, the levels of Hb, RBC, and PCV from affected *P. picticaudata* were significantly lower than those in healthy animals ($P < 0.01$).The activities of LDH, AST, ALT, and CK from affected *P. picticaudata* were significantly lower than those in healthy animals ($P < 0.01$), and UA content was also significantly lower than those in healthy animals ($P < 0.01$).

Effects of Se Deficiency on Antioxidant Function in Serum

As shown in Table 4, the activities of GSH-Px, CAT, SOD, and T-AOC from affected animals were significantly lower than those in healthy *P. picticaudata* ($P < 0.01$), and MDA content was significantly higher than those in healthy *P. picticaudata* ($P < 0.01$).

Effect of Nano-Se on Se Contents in Blood

Different Se sources have different therapeutic effects on *P. picticaudata* (see Fig. 1). From Fig. 1, we can find that the Se content in blood had no significant change in the control group. Among the three groups (nano-Se), the third group (1.5 mg/kg BW) had the fastest effect. Nano-Se supplementation for up to 5 days, the Se content in blood significantly increased. After 20 days of continuous supplement, Se content in blood is basically stable. Compared with nano-Se group, the effect of Na_2SeO_3 was slower. It can be found that supplementing nano-Se (1.5 mg/kg BW) is the best way to solve Se deprivation in *P. picticaudata*.

Effect of Nano-Se on Antioxidant Capacity

As shown in Table 5, compared with the control group, the activity of GSH-Px in the experimental group was significantly increased ($P < 0.01$). In group 3, GSH-Px activity was increased by 30.26%, which was significantly different from other experimental groups. Among the six experimental groups, group 3 and group 6 had the most significant effect on SOD activity, which increased SOD activity by 21.03% and 18.01%, respectively ($P < 0.01$), and there was significant difference between the other groups ($P < 0.01$). Compared with the control group, the activity of SOD in group 1 and group 4 increased by 9.36% and 7.16%, respectively ($P < 0.01$). Compared with the control group, the activity of SOD in group 2 and group 5 increased by 16.23% and 13.34%, respectively ($P < 0.01$). Compared with the control group, the activity of CAT in the nano-Se group was significantly increased ($P < 0.01$). Dose of 1.5 mg/kg BW (nano-Se) had the best effect, increasing CAT activity by 2.29%.

Table 2 Mineral concentrations in tissues (mg/kg)

Element	Blood		Hair	
	Affected animals	Healthy animals	Affected animals	Healthy animals
Mn	0.47 ± 0.04	0.43 ± 0.08	5.33 ± 0.45	5.31 ± 0.39
Zn	7.61 ± 1.42	7.57 ± 1.41	109.94 ± 25.23	107.62 ± 24.57
Cu	0.63 ± 0.19	0.64 ± 0.14	5.45 ± 0.24	5.17 ± 0.24
Se	0.04 ± 0.01b	0.13 ± 0.02a	0.05 ± 0.01b	0.11 ± 0.01a
Fe	510.35 ± 52.68	499.57 ± 52.34	330.57 ± 23.52	334.68 ± 25.45
Mo	0.34 ± 0.02	0.35 ± 0.01	0.51 ± 0.05	0.53 ± 0.06

Different little letters indicate significant difference ($P < 0.01$)

Mn manganese, *Zn* zinc, *Cu* copper, *Fe* iron, *Mo* molybdenum, *Se* selenium

Content of MDA in group 3 and group 6 decreased by 8.18% and 7.22%, respectively ($P < 0.01$). In terms of T-AOC, the experimental group and the control group had significant difference ($P < 0.01$), and the T-AOC was significantly increased after Se supplementation.

Discussion

The alpine meadow ecosystem in the Qinghai–Tibet Plateau has an important gene pool in wildlife. Mineral nutrients are of great significance to the evolution, growth, development, and species reproduction in animals [21–23]. In general, the Se contents in soil and forage higher than 0.1 mg/kg DM should be sufficient for animal [24, 25]. However, in our study, Se concentrations in the soil and forage from the Tianjun area were much lower than those in the sufficient levels ($P < 0.01$). Se content in the blood is the most direct indicator of the nutritional status in animals [26, 27]. At the same time, Se contents in the blood and hair in affected animals from the Tianjun area were also significantly lower than those in healthy animals from the Gonghe area ($P < 0.01$). Results were consistent with criteria of Se deprivation illness in horses, camels, lambs, and pigs [28–31]. Supplement of nano-Se can significantly improve the blood Se content in Se-deprived *P. picticaudata* and maintain the Se content in blood at a relatively stable level, which is similar to the previous research results. Compared with nano-Se group, the effect of Na_2SeO_3 group was slower.

Se element is an essential nutrient for animals and performs numerous biological functions in organisms [32, 33]. Some reports showed a significant relationship between Se deficiency and anemia, which was associated with an increased generation of reactive oxygen species and exposure of erythrocytes to a high degree of oxidative stress [34]. Hematological parameters are the diagnostic indicators used for assessing anemia degree in animals [35, 36]. In our study, Hb, PCV, and RBC in the affected *P. picticaudata* significantly

Table 3 Effects of Se deficiency on physiological and biochemical values

Items	Affected animals	Healthy animals
Hb(g/L)	91.56 ± 8.72b	125.43 ± 9.64a
RBC(10^{12}/L)	10.58 ± 1.41b	15.56 ± 2.36a
PCV(%)	39.53 ± 2.56b	51.55 ± 1.75a
MCV(fl)	36.67 ± 2.25	38.72 ± 2.86
MCH(pg)	14.75 ± 1.67	13.63 ± 1.89
MCHC(%)	31.63 ± 8.67	30.78 ± 9.86
WBC(10^9/L)	8.77 ± 0.62	8.53 ± 0.79
LDH(U/L)	297.75 ± 25.83a	109.47 ± 20.96b
AST(U/L)	152.35 ± 33.63a	111.75 ± 30.26b
ALT(U/L)	65.67 ± 5.86a	39.86 ± 7.36b
BUN (mmol/L)	3.85 ± 0.31	3.82 ± 0.29
CK (U/L)	279.64 ± 10.64a	193.51 ± 7.87b
UA (μmol/L)	32.27 ± 2.78b	83.57 ± 2.65a

Different little letters indicate significant difference ($P < 0.01$)

Hb hemoglobin, *RBC* erythrocyte count, *PCV* packed cell volume, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *WBC* white blood cell count, *LDH* lactate dehydrogenase, *AST* aspartate aminotransferase, *ALT* alanine transaminase, *BUN* blood urea nitrogen, *CK* creatine kinase, *UA* uric acid

Table 4 Effects of Se deficiency on serum antioxidant function

Items	Affected animals	Healthy animals
GSH-Px(U/mL)	33.45 ± 4.56b	63.52 ± 5.63a
SOD (U/mL)	69.58 ± 9.37b	91.63 ± 12.68a
CAT (U/mL)	3.63 ± 0.25b	5.63 ± 0.54a
MDA (nmol/mL)	11.74 ± 2.35a	3.38 ± 1.22b
T-AOC (U/mL)	3.67 ± 0.23b	7.53 ± 1.26a

Different little letters indicate significant difference ($P < 0.01$)

SOD superoxide dismutase, *GSH-PX* glutathione peroxide, *MDA* malondialdehyde, *CAT* catalase, *T-AOC* total antioxidant capacity

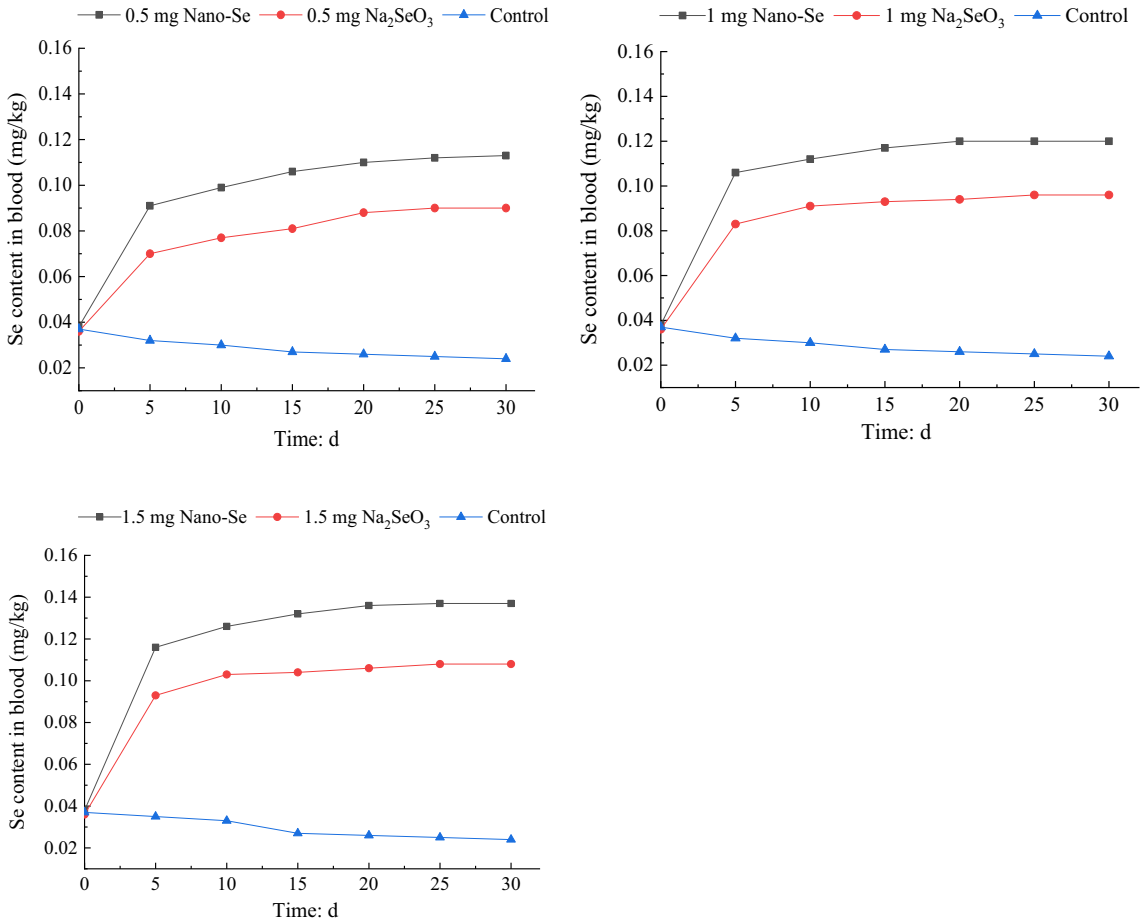

Fig. 1 Effect of nano-Se on blood Se content in *P. picticaudata*

decreased ($P < 0.01$), indicating that the affected animals had subclinical anemia. The activity of ALP significantly increased suggests that the affected *P. picticaudata* may have cholestasis. When muscular dystrophy and liver necrosis occur, ALT activity increased, which is a sign of liver cell damage. AST activity can also reflect the degree of liver injury. When the kidney, myocardium, and skeletal muscle are damaged, LDH activity in serum was significantly increased [37, 38]. In our study, Se deficiency resulted in liver cell damage and increased cell membrane permeability, and the

Table 5 Effect of nano-Se on serum antioxidant capacity

Items	Control	Nano-Se			Na_2SeO_3		
	0	0.5 mg	1 mg	1.5 mg	0.5 mg	1 mg	1.5 mg
GSH-Px (U/mL)	32.57 ± 4.45e	56.47 ± 3.54c	60.43 ± 3.71b	65.33 ± 4.45a	47.31 ± 3.39d	55.33 ± 3.21c	56.31 ± 3.22c
SOD (U/mL)	69.43 ± 9.46e	79.61 ± 10.54d	85.57 ± 11.37c	91.94 ± 11.23a	76.62 ± 11.57d	82.94 ± 12.76c	87.62 ± 11.43b
CAT (U/mL)	3.52 ± 0.25e	5.03 ± 0.29c	5.34 ± 0.15b	5.76 ± 0.24a	4.37 ± 0.33d	4.42 ± 0.24d	4.41 ± 0.26d
MDA (nmol/mL)	11.74 ± 2.35a	6.04 ± 1.08b	5.13 ± 1.02c	3.40 ± 1.11e	5.89 ± 1.21b	5.92 ± 1.20b	4.36 ± 1.19d
T- AOC (U/mL)	3.77 ± 0.23b	7.35 ± 1.38a	7.46.57 ± 1.24a	7.57 ± 1.52a	7.40 ± 1.45a	7.33 ± 1.22a	7.29 ± 1.35a

Different little letters indicate significant difference ($P < 0.01$)

SOD superoxide dismutase, *GSH-PX* glutathione peroxide, *MDA* malondialdehyde, *CAT* catalase, *T-AOC* total antioxidant capacity

activities of AST and LDH significantly increased, which was consistent with results of Dong et al. [39]. Wang et al. showed also that LDH, AST, and ALT activities in Se-deprived carp significantly increased [40]. The much increased CK activity reflects the damage of kidney, myocardium, and skeletal muscle. The greatly decreased UA content suggests that Se deprivation may cause kidney damage in *P. picticaudata*.

The antioxidant system of the organism includes enzymatic system and non-enzymatic system. The enzymatic system mainly includes SOD, CAT, and GSH-Px, and the non-enzymatic system mainly includes Vitamin C, carotene, and lactoferrin. Se element plays an important role in antioxidant system and can play a role through selenoproteins and selenicnucleic acids with enzyme function [41, 42]. GSH-Px is the main antioxidant enzyme in the organism, which can reduce the toxic peroxide to non-toxic hydroxyl compounds, promote the decomposition of H_2O_2, and ultimately reduce the number of free radicals and generate products that are easily metabolized through organism, so as to protect the structure and function in cell membrane from the damage and interference of peroxides [43, 44]. When Se is deprived, the decreased activity of GSH-Px will lead to increased lipid free radicals and peroxides, the destruction of cells, and tissue damage [45]. In our study, the activity of GSH-Px in nano-Se group was significantly higher than that in Na_2SeO_3 group ($P < 0.01$). It showed that nano-Se was easily absorbed in the digestive tract. Selenomethionine was converted into selenocysteine in vivo, which promoted the synthesis of GSH-Px, thus improving the activity of GSH-Px [46]. SOD can eliminate superoxide radicals (O_2^-) in biological cells through disproportionation reaction and generate H_2O_2 and O_2. H_2O_2 is catalyzed by CAT to generate H_2O and O_2, thus reducing the toxicity of free radicals to organisms [47]. Wu et al. showed that Se deprivation could reduce the activities of SOD and CAT in of lactating cows [48]. In our study, serum SOD and CAT activities in *P. picticaudata* were significantly increased by supplement nano-Se, which was consistent with the results of Qu et al., Jing et al., and Wang [49–51], and nano-Se (1.5 mg/kg BW) had the most significant effect. The activities of SOD and CAT inNa_2SeO_3 groups were also significantly increased ($P < 0.01$), and the effect of nano-Se is better than that of Na_2SeO_3. MDA is the most common product of lipid peroxidation, and its level can directly reflect the degree of lipid oxidative injury [52–54]. Li et al. reported that adding Se to the diet of rats can significantly reduce the serum MDA content [55]. Gao et al. reported that adding organic Se to the diet of pigs can significantly decrease the serum MDA content [56]. The results showed that compared with the control group, the content of MDA in nano-Se group (1.5 mg/kg BW) and Na_2SeO_3 group (1.5 mg/kg BW) significantly decreased by 8.18% and 7.22%, respectively ($P < 0.01$). T-AOC can reflect the compensatory capacity to external stimuli and the metabolism capacity of free radicals in organisms [57]. Decreased function of the T-AOC defense system cannot keep the antioxidant system active, leading to the abundance of lipid peroxides and free radicals. Sun et al. showed that compared with Na_2SeO_3, the effect of dietary supplement of nano-Se on the levels of GSH-Px and T-AOC of laying hens was better [58]. It is consistent with the results of this study. From the above five antioxidant indexes, the antioxidant effect of nano-Se groups in *P. picticaudata* was higher than that in Na_2SeO_3 groups ($P < 0.01$), and nano-Se (1.5 mg/kg BW) was better. The main reason may be the different metabolic pathways in the body. Nano-Se not only has an efficient absorption mode, but also can directly remove free radicals in the body.

Conclusion

According to the results, nano-Se could not only markedly increase the Se content in blood in Se-deprived *P. picticaudata* but also much improve the antioxidant capacity, and its effect is better than that of Na_2SeO_3.

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Compliance with Ethical Standards

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

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