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Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*)

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ABSTRACT

An experiment was conducted to compare the effects of selenium nanoparticle (T-1) and selenomethionine (T-2) on crucian carp, *Carassius auratus gibelio*. There were significant differences ($P<0.05$) in relative gain rate and final weight of T-1 and T-2 compared with the control. Survival rate and feed conversion ratio (FCR) were not affected by the dietary treatments. Fish fed the basal diet (control) showed lower ($P<0.05$) selenium content in muscle (6.10 ± 0.78 μg g^{-1}) compared to T-1 and T-2 (16.42 ± 1.07 μg g^{-1} and 13.52 ± 1.31 μg g^{-1}, respectively). Furthermore, the highest value ($P<0.05$) of selenium content in muscle was observed in T-1. Glutathione peroxidase (GSH-Px) activities in carps plasma and liver of T-1 and T-2 were significantly different ($P<0.05$) with that of the control. However, no significant differences were observed in GSH-Px activities both in plasma and liver between T-1 and T-2.

1. Introduction

Selenium (Se) is an essential trace element for human and animal health. It was found to be an integral part of the glutathione peroxidase enzyme (Rotruck et al., 1973). Glutathione peroxide takes part in the cellular defence against oxidative damage of cytoplasmic structures by catalyzing the reduction of hydrogen peroxide and lipid peroxides (Watanabe et al., 1997). Selenium has been studied in rainbow trout (Hilton et al., 1980, 1982; Bell et al., 1985; Vidal et al., 2005), catfish (Gatlin and Wilson, 1984), Atlantic salmon (Lorentzen et al., 1994) and juvenile grouper (Lin and Shiau, 2005). Selenomethionine is a predominant chemical form of organic selenium in feedstuffs due to their excellent bioavailability and has been reported to have higher bioavailability than inorganic Se (sodium selenite) for Atlantic salmon (Bell and Cowey, 1989; Lorentzen et al., 1994) and channel catfish (Wang and Lovell, 1997).

Elemental Se powder in the redox state of zero is not water soluble and generally considered to be biologically inert (Zhang et al., 2005). Nanotechnology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties different from those of both isolated atom and bulk material (Albrecht et al., 2006; Wang et al., 2007). Selenium was also associated with protein in animal tissues (Burk and Hill, 1993). Consequently, muscle meats and seafood were dependable dietary sources of the mineral (Gibson, 1990). Deteriorative oxidative reactions in meat led to losses of both nutritional value and food quality. To increase the oxidative stability of meat, antioxidants such as selenium had been added to the feed of farm animals, leading to an improved meat quality (Mahan et al., 1999; Downs et al., 2000; Gatellier et al., 2004).

However, little had been done to incorporate Se nanoparticle (Nano-Se) into crucian carp, *Carassius auratus gibelio*, based on the growth performance, feed conversion, tissue composition and glutathione peroxidase activities. Thus, this study was designed to evaluate the application of different Se sources, including Nano-Se and selenomethionine, as feed additives in diets for crucian carp, which was one of the most valuable freshwater fish species cultured in China. Furthermore, the biochemical analysis and Se concentration in carp muscles were also investigated.

2. Materials and methods

2.1. Materials and diets

Nano-Se was provided by the Nano-biology Laboratory of Feed Science Institute of Zhejiang University, China. In this study, nano-Se was the particles of red elemental Se in the redox state of zero and prepared by adding bovine serum albumin (BSA) to the redox system of selenite and glutathione according to Zhang et al. (2001). The sizes of elemental Se particles ranged from 60 to 80 nm, with an average size of 69 nm. Ingredient and chemical composition of the basal diets used in the experiment were according to Lovell (1998). The basal diet formulation and proximate composition were shown in Table 1.

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Table 1
Formulation, proximate composition and gross energy of experimental diets.

Ingredients (%)		Proximate composition (% dry weight)	
Casein	32	Crude protein	38.75
Gelatin	8	Crude fat	3.74
Dextrine	28	Crude ash	13.20
Cellulose	19	Gross energy (MJ Kg^{-1})	15.13
Fat[a]	6	Moisture	6.73
Carboxy methyl cellulose	2		
Mineral premix[b]	4		
Vitamin premix[c]	1		

[a] The mixture of fish oil and lard (1:1).
[b] Mineral premix (%): $KAl(SO_4)_2$, 0.159; $CaCO_3$, 18.101; $Ca(H_2PO_4)_2$, 44.601; $MgSO_4$, 5.216; $CoCl_2$, 0.07; KCl, 16.553; ferric citrate ($5H_2O$), 1.338; $MnSO_4 \cdot H_2O$, 0.07; KI, 0.014; $ZnSO_4$, 0.192; NaH_2PO_4, 13.605; and $CuSO_4 \cdot 5H_2O$, 0.075.
[c] Vitamin premix: thiamin hydrochloride, 10 mg kg^{-1}; riboflavin, 20 mg kg^{-1}; calcium pantothenate, 40 mg kg^{-1}; nicotinic acid, 50 mg kg^{-1}; pyridoxine hydrochloride, 10 mg kg^{-1}; folic acid, 5 mg kg^{-1}; inositol, 400 mg kg^{-1}; choline chloride, 2000 mg kg^{-1}; menadione, 10 mg kg^{-1}; cholecalciferol, 1500 IU; biotin, 1 mg kg^{-1}; vitamin B_{12}, 0.02 mg kg^{-1}; vitamin A, 3000 IU; vitamin E, 50 IU; and vitamin C, 200 mg kg^{-1}.

Nine tanks (250 l) with three replicates for treatments and control were used. Three treatments were carried out with crucian carp. In treatment 1 (T-1) and treatment 2 (T-2), nano-Se and selenomethionine were added to the experimental basal diets at the rate of 0.5 mg kg^{-1} dry feed weight, respectively. The control groups were basal diet only. The ingredients and different selenium sources were mixed, extruded and air-dried at room temperature. Then this diet was kept at −20 °C until used. In order to mix adequately, nano-Se or selenomethionine was slowly applied to the diet ingredients, mixing part by part in a drum mixer. The final actual concentration of Se in each diet was determined (AA6501, Shimadzu Ltd, Japan) and shown in Table 2. All ingredients and chemicals were purchased from Sangon and East China Pharmaceuticals Company, Shanghai, China.

2.2. Fish and experimental design

Healthy juveniles of crucian carp provided by the Fish Hatchery of Hangzhou, China, were acclimatized in two concrete tanks (each measuring 400 × 150 × 100 cm^3), and were fed with basal feed twice daily for two weeks. Then the crucian carps were distributed into 9 tanks ($r = 3$) with initial stocking density of 20 crucian carps per tank for a 30-day culture. All crucian carps had similar initial weights (13.08–15.12 g). The experiment was conducted as a completely randomized design with three treatments (T-1, T-2 and Control). The feeding trial was conducted under the supervision of the Animal Care and Use Committee of the university.

Crucian carps were fed three times daily at 6:00, 12:00 and 18:00 with each feed. Daily feeding rate was about 3% of total body weight and properly regulated according to actual intake of common carps. Every day the diet remains of each tank were collected by siphoning before the second daily feeding. A daily record was kept of feed offered and remains. Every third day, each tank was cleaned and the water partially changed (about 50%).

The tanks were supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 l min^{-1}), then passed successively through a tungsten heater and degassing column packed with plastic rings (Zhenhua Electric Industrial Co., Ltd., China). Temperature range of tanks water was 25–26 °C with a photoperiod of 12 h light and 12 h darkness. For water quality control, temperature and dissolved oxygen (DO) were measured daily, and weekly analyses were conducted for total ammonium, nitrite and pH levels using the Hach kit model DREL 2400 (Hach Co., USA). Dissolved oxygen level was maintained above 6 mg l^{-1} by setting the air pump (ADP-2200, Jinlai Pump Factory, China).

Table 2
The actual concentration of selenium in diet.

Group/treatment	T-1	T-2	Control
Selenium sources	Nano-Se	selenomethionine	/
Actual concentration (mg kg^{-1})	0.550 ± 0.008	0.551 ± 0.009	0.053 ± 0.005

Table 3
Growth performance and feed utilization of crucian carp supplemented with different Se sources (selenium nanoparticle and selenomethionine, T-1 and T-2, respectively) and without Se (control).

Group/treatment	T-1	T-2	Control
Initial weight (g)	14.05 ± 0.44a	14.10 ± 0.60a	14.45 ± 0.50a
Final weight (g)	25.07 ± 0.95a	24.98 ± 1.12a	22.07 ± 0.93b
Survival rate (%)	100a	100a	100a
RGR (%)	78.58 ± 7.40a	77.52 ± 13.41a	52.75 ± 5.66b
FCR	1.65 ± 0.11a	1.63 ± 0.10a	1.73 ± 0.10a

RGR, Relative gain rate; FCR, Feed conversion ratio. Results were presented as means ± S.E. of triplicate observations. Means in the same row with different letters were significantly different ($P<0.05$).

2.3. Sampling and analytical methods

The proximate composition including crude protein, crude fat, crude ash, gross energy and moisture of basal diets was determined using the standard procedures of China according to Zhang and Zhu (1998). Crude protein was determined using the Kjeltec Analyzer Unit (2300, Sweden) and crude fat was measured using the Soxtec Auto Extraction Unit (2050, Sweden). Gross energy was determined with an adiabatic bomb calorimeter (PARR 1281, USA). Weights of all collected crucian carps from each tank were determined at initial and the end of experiment, which were treated as initial weight and final weight, respectively. At the same time, crucian carp survival was also determined by counting the individuals in each tank. The relative gain rate (%) (RGR) used the following formula: (final weight − initial weight) / initial weight × 100%. And the feed conversion ratio (FCR) was expressed as: (total feed casting − total feed residue) / (total final weight − total initial weight + total mortality weight).

For the biochemical analysis of crucian carp muscles and glutathione peroxidase analysis, 6 carps starved for 24 h were collected randomly from each tank at the end of the trial and anesthetized in diluted MS-222 (Ethyl 3-aminobenzoate methanesulfonate, Tricaine; Sigma) solution with a concentration of 1:2500. The concentration of Se was determined according to the method described by Tinggi (1999) by hydride generation atomic absorption spectrophotometer (AA6501, Shimadzu Ltd, Japan). The proximate composition of crucian carp muscles including crude protein, crude fat, crude ash and moisture was also determined using the forenamed standard procedures. Blood samples were taken from the caudal vein into tubes containing sodium EDTA as anticoagulant and the liver were removed and pooled. Total protein content of supernatant was assayed according to Bradford (1976) using bovine albumin as a standard. The enzyme activity of glutathione peroxidase was measured according to Bell et al. (1986) and expressed as specific activity (U mg^{-1} protein) in liver and as U in plasma.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to determine the significant ($P<0.05$) difference between the tested groups. All

Table 4
Biochemical analyses and selenium contents in crucian carp muscles.

Group/treatment	T-1	T-2	Control
Moisture (%)	74.47 ± 0.80a	74.64 ± 0.62a	74.54 ± 1.09a
Crude protein (%)	20.04 ± 0.28a	19.71 ± 0.36a	19.93 ± 0.39a
Crude fat (%)	2.42 ± 0.19a	2.47 ± 0.17a	2.45 ± 0.21a
Crude ash (%)	1.24 ± 0.12a	1.20 ± 0.15a	1.24 ± 0.14a
Se (μg g^{-1} dry weight)	16.42 ± 1.07a	13.52 ± 1.31b	6.10 ± 0.78c

Results were presented as means ± S.E. of triplicate observations. Means in the same row with different letter were significantly different ($P<0.05$).

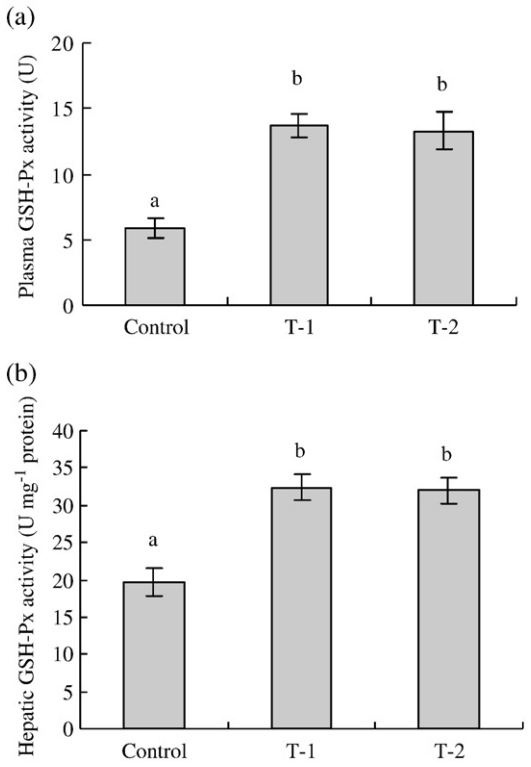

Fig. 1. Glutathione peroxidase (GSH-Px) activity of crucian carps in plasma (a) and liver (b) supplemented with selenium nanoparticle (Nano-Se, T-1) and selenomethionine (T-2) and without any Se (Control) in basal diet. Means with different letter were significantly different ($P<0.05$).

statistics were performed using SPSS for Windows version 11.5 (SPSS, Chicago, USA).

3. Results

3.1. Water quality and growth performance

There was no effect of different Se sources on water quality. Total ammonium (0–0.2 mg l^{-1}), nitrite (0–0.1 mg l^{-1}) and pH (7.4–7.8) were stable and within acceptable ranges (Boyd and Tucker, 1998).

At the beginning, no significant difference was observed in the initial weight between T-1, T-2 and the control (Table 3). There were significant differences ($P<0.05$) in RGR and final weight of T-1 and T-2, as compared with the control. However, there were no significant differences ($P>0.05$) in RGR and final weight between T-1 and T-2. Survival rate and FCR were not affected by the dietary treatments after 30 days culture.

3.2. Biochemical analysis

After 30 days culture, fish fed with the nano-Se diet (T-1) showed a higher ($P<0.05$) Se content in the muscle tissue (16.42 ± 1.07 μg g^{-1}) compared to that of T-2 and the control (13.52 ± 1.31 μg g^{-1} and 6.10 ± 0.78 μg g^{-1}, respectively) (Table 4). Moreover, T-2 showed the higher value Se content in the muscle tissue ($P<0.05$) than the control. However, there were no significant differences ($P>0.05$) in other muscles components, including moisture, crude protein, crude fat and crude ash, of crucian carps among T-1, T-2 and the control.

3.3. Enzyme activity

Glutathione peroxidase (GSH-Px) activities in crucian carps plasma and liver of all Se treatment groups (T-1 and T-2) were significantly different ($P<0.05$) with that of the control (Fig. 1). GSH-Px activity both in plasma and liver was higher in T-1 (13.65 ± 0.94 U and 32.35 ± 1.76 U, respectively) compared with T-2 (13.29 ± 1.43 U and 32.02 ± 1.74 U, respectively). However, there was no significant difference between T-1 and T-2.

4. Discussion

Dietary Se supplementation is necessary and Gatlin & Wilson (1984) demonstrated that the growth of channel catfish (*Zctalurus punctatus*) was affected by dietary Se level. Similar results were observed in the present study. It clearly indicated that Se supplemented diet could improve the final weight and relative gain rate of crucian carp, *Carassius auratus gibelio*. Selenium deficiency generally results in growth depression. Mortality noted in salmon fry fed with a Se-deficient diet was prevented by administration of a diet containing 0.1 mg Se kg^{-1} and 500 IU vitamin E kg^{-1} (Poston et al., 1976). The results from this study proved this point from the opposite side and it might be associated with the quantity of Se in diet.

It was obvious that the Se contents in crucian carps muscle were markedly changed with the addition of dietary Se and higher content of Se was found in T-1. The results indicated that nano-Se and selenomethionine had different metabolic pathways, although both inorganic and organic forms cross the intestinal barrier. In general, animal study trials demonstrate that bioavailability of organic forms of Se (selenomethionine) was higher than that of inorganic forms (sodium selenite) (Levander, 1983; Smith and Picciano, 1987), as was also observed in human studies (Favier, 1993; Thomson and Robinson, 1993). The selenomethionine can be stored in a protein pool when the methionine is limited or catabolized with the release of Se which passes to another pool. Thus Se bioavailability depended not only on its absorption by the intestine but also on its conversion to a biologically active form (Foster and Sumar, 1995). Nano-Se had similar bioavailability in terms of inducing seleno-enzymes in cultured cells and in Se-deficient rats (Zhang et al., 2001). However, nano-Se could have a special metabolism pathway and deposition mechanism in crucian carps according to our results.

Selenium has a number of biological functions in animals including fish (Kohrle et al., 2000) and the most important action is its antioxidant effect because it forms selenocysteine, part of the active center of the GSH-Px (Levander and Burk, 1994). Thus, the amount of Se intake affects the expression of GSH-Px and Se deficiency rapidly decreases all cellular GSH-Px activity (Sunde, 2001). Thus, increased diet Se level tends to elevate GSH-Px activity in plasmid and tissues. The results of T-1 and T-2 in this study also showed higher GSH-Px activity in plasma and liver of crucian carps as compared with the control. Furthermore, Se as the active core of GSH-Px in crucian carps should be presumed from the results of this research (Bell et al., 1986). On the other hand, in the cell GSH-Px plays an important function, because the reduced form of this enzyme reduces the hydrogen peroxide and lipidic hydroperoxides at the level of the cytosol and mitochondrial matrix (Dong, 2000). This element is also included in other functionally active selenoproteins as the type 1 iodothyronine 5′-deiodinase which interacts with iodine to prevent abnormal hormone metabolism (Foster and Sumar, 1997). The effect of Se on some growth performances of carps might be associated with these forenamed functions of Se.

However, in the present study, the activity of GSH-Px was similar at the same Se additive concentration ($P>0.05$) regardless of Se sources. These results coincided with that of growth performance observed in this research, likely due to the difference in metabolic pathway of Se source conversion to selenoprotein (Thomson et al., 1982). Activated selenomethionine is converted to selenocysteine and the selenocysteine is degraded further in liver to serine and selenide (Schrauzer, 2000). Sodium selenite is converted initially to selenoglutathione trisulfide, and then degraded in liver to form selenide (Kim and

Mahan, 2003). The selenide is finally used for selenoprotein synthesis, such as GSH-Px (Favier, 1993). The discrepancy of muscle Se concentration and GSH-Px activity indicated that only a part of nano-Se was adopted for selenoprotein synthesis and another metabolic pathway of nano-Se differed from sodium selenite and selenomethionine may exist.

In conclusion, this research had demonstrated that different Se source (nano-Se and selenomethionine) supplemented in basal diet could improve the final weight, relative gain rate, GSH-Px activities and muscle Se concentration of *C. gibelio*. Moreover, nano-Se appeared to be more effective ($P<0.05$) than that of organic selenomethionine in increasing muscle selenium content.

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