Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (Cyprinus carpio)

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A B S T R A C T
The aim of this study was to assess the effects of different doses of selenium nanoparticles (nano-Se) on growth performance, feed conversion, tissue composition, Se accumulation in muscle and liver, antioxidant response and biochemical blood parameters of common carp, Cyprinus carpio. Nano-Se was added to the experimental basal diets at the rates of 0 (control), 0.5, 1 and 2 mg/kg dry feed weight. Four groups of fish with an average weight of about 10 g were fed one of the experimental diets for 8 weeks. Fish that were fed diet supplemented with 1 mg Se/kg displayed improved (P < 0.05) growth performance, including final weight and weight gain (WG). No significant differences (P > 0.05) in feed conversion ratio (FCR) and survival were observed in fish fed different diets for 8 weeks. Dietary nano-Se had no effects on proximate composition of carp. Fish fed with nanoseelenium-supplemented diets showed a higher (P < 0.05) Se content in the muscle and liver tissues compared to that of the control. Liver malondialdehyde (MDA) level was significantly (P < 0.05) lowered in fish fed on diet supplemented with 2 mg nano-Se/kg, whereas the activity of glutathione peroxidase (GPx) and catalase (CAT) in livers of carp fed on diet supplemented with 2 mg nano-Se was significantly (P < 0.05) higher as compared to other experimental diets. The highest superoxide dismutase (SOD) activity was observed in fish fed on 2 mg nano-Se, but it was not significantly (P > 0.05) different from the enzyme activity in the 1 mg nano-Se group. Fish fed on diet supplemented with 2 mg nano-Se showed significantly (P < 0.05) higher total protein and globulin contents but lower albumin level. The serum cholesterol level was significantly (P < 0.05) decreased in fish fed diets containing 2 mg nano-Se/kg. No significant difference (P > 0.05) in serum triglycerides was observed in fish fed different diets. A significant (P < 0.05) decrease in low-density lipoprotein (LDL) was observed in groups receiving 1 and 2 mg nano-Se/kg, whereas serum high-density lipoprotein (HDL) was higher in carp fed on diet with 2 mg nano-Se/kg. Fish fed on 1 and 2 mg nano-Se/kg diets exhibited the highest values of aspartate transaminase (AST) activity as compared to other treatments. The activity of alanine transaminase (ALT) was significantly (P < 0.05) higher in fish fed on 2 mg nano-Se/kg as compared to other test groups. No significant differences (P > 0.05) were found in serum alkaline phosphatase (ALP) activity among the experimental groups. Therefore, the data of the present study recommend adding 1 mg nano-Se per kg diet to improve fish growth and antioxidant defense system.

Statement of relevance
The data of the present study recommend adding 1 mg nano-Se per kg diet to improve carp growth and antioxidant defense system.

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1. Introduction
Selenium (Se) is an essential trace element for animals including fish, and has been considered as an excellent essential nutrient for aquaculture product enhancement. Se is a component of the enzyme glutathione peroxidase (GPx), which protects cell membrane from oxidative stress (Rotruck et al., 1973; Watanabe et al., 1997). Administration of dietary selenium has been reported to enhance growth performance and immunological function in various fish species (Jaramillo and Gatlin, 2004; Zhou et al., 2009), although no positive effects have been observed in other studies (Gatlin and Wilson, 1984; Kim et al., 2003; Lorentzen et al., 1994).
As the chemical form in which Se is supplemented is an important consideration (Han et al., 2011), various forms of Se supplementation have been studied in fish species including hybrid striped bass (Cotter et al., 2008), common carp (Jovanovic et al., 1997), crucian carp (Wang et al., 2007a) and common barbel (Kouba et al., 2014). The above-mentioned studies demonstrated that organic forms of Se are more digestible, better accumulated, and more biologically active than inorganic forms. Recently, Zhou et al. (2009) showed that selenium nanoparticles (nano-Se) are more efficient in increasing muscle Se content compared to organic Se (selenomethionine, SeMet) in the crucian carp. Since materials at the nanometer dimension exhibit novel properties (Wang et al., 2007b), nano-Se as the novel form of Se has attracted attention. However, the effect of different levels of nano-Se on common carp, Cyprinus carpio is not studied. Therefore, the aim of this study was to assess the effect of different doses of nano-Se on growth performance, feed conversion, tissue composition, Se accumulation in muscle and liver, antioxidant response and biochemical blood parameters.

2. Materials and methods

2.1. Diet preparation

Diet formulation and proximate composition of the basal diet were shown in Table 1. Nano-Se (Iranian Nanomaterials Pioneers, Mashad, Iran) was used as the Se source and was added to the experimental basal diets at the rates of 0 (control), 0.5, 1 and 2 mg/kg dry feed weight. The sizes of the elemental nano-Se particles ranged from 30 to 45 nm. The ingredients and different Se sources were mixed, extruded and air-dried at room temperature. After drying, diets were kept at −20 °C until used. The final actual concentration of Se in each diet was determined (Younglin AAS 8020A, Korea) and shown in Table 2.

2.2. Fish and experimental design

Juveniles of common carp obtained from a local hatchery (Ahvaz, Iran) were used in the study. Upon arrival, the fish were acclimated to laboratory conditions for 2 weeks, and were fed with basal diet. At the beginning of the experiment, 14 fish with an average weight of about 10 g were stocked in each of 12 fiber glass 300 l tanks. Each experimental diet was assayed in triplicate (4 experimental diets × 3 tanks). The fish were fed three times daily at 8:00, 13:00 and 19:00 with each feed over an 8-week period. Daily feeding rate was about 3% of total body weight. Every day, each tank was cleaned and the water partially exchanged. Water temperature, dissolved oxygen and pH were monitored during the trial.

Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>(g/kg)</th>
<th>Proximate compositiona (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>300</td>
<td>Protein: 32 ± 1.11</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>160</td>
<td>Lipid: 10.2 ± 0.31</td>
</tr>
<tr>
<td>Corn meal</td>
<td>200</td>
<td>Moisture: 9.2 ± 0.21</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>180</td>
<td>Ash: 11.1 ± 0.91</td>
</tr>
<tr>
<td>Rice bran</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Se-free premixc</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

a Nanoparticle of selenium was added to the basal diet at 0, 0.5, 1 and 2 mg Se kg⁻¹ diet (Iranian Nanomaterials Pioneers, Mashad, Iran).

b Values are presented as mean ± SE, n = 3.

c Supplied by Damloran Razak Pharma. Co. Iran, and contains the following (as mg/kg of premix): vitamin A, 50,000 IU; vitamin D₃, 10,000 IU; vitamin E, 30; vitamin B₁₂, 20; vitamin B₆, 10; vitamin B₉, 3; vitamin K₃, 15; nicotinamide, 150; calcium pantothenate, 40; copper (Cu²⁺), 30; iron (Fe²⁺), 100; zinc (Zn²⁺), 150; manganese (Mn²⁺), 200.

daily. The mean water quality parameters were recorded as follows: temperature 28.3 °C, dissolved oxygen 6.4 mg/ml and pH 7.8. A photoperiod of 14 h light (06:00–20:00 h) and 10 h dark was used.

2.3. Sampling and analytical methods

All fish in the different experimental groups were weighed at the end of 8-week feeding trial for estimation of growth. Growth performance parameters were calculated according to the following formulae: weight gain (WG) = 100 × (final weight-initial weight) / (initial weight), and feed conversion ratio (FCR) = feed given (dry weight) / total wet weight gain. In addition, survival rate was calculated at the end of experiment: survival = 100 × (final fish number / initial fish number).

After the final weighing, two fish were randomly sampled from each tank and used for the determination of proximate composition. Protein, lipid, moisture and ash were analyzed according to the standard methods: crude protein by analysis of nitrogen using the Kjeldahl method; crude lipid by petroleum ether extraction using the Soxhlet method; moisture by drying at 105 °C to a constant weight and ash by combustion at 550 °C for 24 h.

At the end of feeding trial, two fish from each tank were randomly selected for measurement of selenium bioaccumulation in muscle and liver tissues. Selenium was estimated according to the method described by Elia et al. (2011) by an atomic absorption spectrophotometer equipped with transversely heated graphite atomizer system (Younglin AAS 8020, Korea).

Two other fish were selected randomly from the remaining fish in each tank for blood enzyme determination. The blood was drawn from the caudal vein of individual fish to get the serum samples using standard procedures. Isolated sera were stored at −80 °C until further analysis. Following the blood sampling, portions of liver were dissected from each fish and stored at −80 °C until used for enzyme assays.

Glutathione peroxidase (GPx) activity was assayed using the method described by Noguchi et al. (1973). Activity of liver superoxide dismutase (SOD) was measured according to the method of McCord and Fridovich (1969). Liver catalase (CAT) activity was determined following the method of Abei (1984). Malondialdehyde (MDA) concentration, also known as thiobarbituric acid reactive substances (TBARS), was measured colorimetrically using the method of Buege and Aust (1978).

Serum total protein and albumin were estimated by Biuret and bromocresol green (BCG) dye binding method (Dumas et al., 1971). Serum globulin was calculated by subtracting serum albumin from total protein content. Serum total cholesterol was determined following the method of Wybenga et al. (1970). Concentrations of triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured using commercial kits (ZistChimi Chemical Company, Tehran, Iran). Serum enzymatic activity of aspartate transaminase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT) were measured as described previously (Peyghan and Takamy, 2002).

2.4. Statistical analysis

The data (means ± SE, standard error) were analyzed by one way analysis of variance (ANOVA) followed by Duncan to compare the means between individual treatments with SPSS at P < 0.05 level.
3. Results

The growth performance of common carp fed diets supplemented with varying levels of dietary nano-Se is presented in Table 3. Fish that were fed diet supplemented with 1 mg Se/kg displayed improved \((P < 0.05)\) growth performance, including final weight and weight gain (WG). No significant differences \((P > 0.05)\) in feed conversion ratio (FCR) and survival were observed in fish fed different diets for 8 weeks (Table 3). Proximate compositions of carps including moisture, crude protein, crude fat and ash were not affected by the dietary supplementation after 8 weeks of culture (Table 4). With the increased dietary Se supplementation concentrations, Se content in the muscle and liver tissues showed an increasing trend (Table 5).

Effects of different levels of dietary nano-Se on oxidative stress and antioxidant responses of common carp are presented in Table 6. Liver MDA level was significantly \((P < 0.05)\) lower in fish fed on diet supplemented with 2 mg nano-Se as compared to other test groups. Activity of GPx and CAT in livers of carp fed on diet supplemented with 2 mg nano-Se was significantly \((P < 0.05)\) higher than those of fish fed with other experimental diets. The highest SOD activity was observed in fish fed on 2 mg nano-Se, but it was not significantly \((P < 0.05)\) different from the enzyme activity in the 1 mg nano-Se group (Table 6).

Effects of varying doses of dietary nano-Se supplementation on biochemical blood parameters are shown in Table 7. Fish fed on diet supplemented with 2 mg nano-Se showed significantly \((P < 0.05)\) higher total protein and globulin contents but lower albumin level. The serum cholesterol level was significantly \((P < 0.05)\) decreased in fish fed diets containing 2 mg nano-Se/kg. No significant difference \((P > 0.05)\) in serum triglycerides was observed in fish fed different diets. A significant \((P < 0.05)\) decrease in LDL was observed in groups receiving 1 and 2 mg nano-Se/kg, whereas serum HDL was higher in carp fed on diet with 2 mg nano-Se/kg. Fish fed on 1 and 2 mg nano-Se/kg diets exhibited the highest values of AST activity as compared to other treatments. The activity of ALT was significantly \((P < 0.05)\) higher in fish fed on 2 mg nano-Se/kg as compared to other test groups. No significant differences \((P > 0.05)\) were found in serum ALP activity among the experimental groups.

4. Discussion

Selenium is an essential element for fish normal growth and development (Hamilton, 2004). In this study, fish were fed diets supplemented with 0, 0.5, 1 and 2 mg nano-Se/kg for 8 weeks. With the increasing dietary nano-Se levels, final weight and weight gain (WG) of fish significantly increased at the levels of ≤1 mg nano-Se/kg diet and then showed no significant difference above 1 mg nano-Se/kg level. As the basal diet contained 0.43 mg Se/kg, doses of Se delivered in 2 mg nano-Se/kg group equated to 2.51 mg Se/kg, indicating that changes in growth seem to be related to the actual concentration of Se in the diets. The optimum Se requirement for common carp determined in this study (1 mg nano-Se/kg) is similar to that reported for gibel carp (1.18 mg Se/kg; Han et al., 2011), but higher than that determined for loach (0.48-0.50 mg Se/kg; Hao et al., 2014) and grouper (0.77 mg Se/kg; Lin and Shiau, 2005). Thus, the difference in Se requirement could also be related to species and age of fish, chemical forms of Se and dietary factors (Hao et al., 2014).

Proximate composition of fish was not affected by the dietary treatment after 8 weeks of culture, indicating that the carp muscle proximate composition is not sensitive to dietary Se treatments. This observation is in agreement with the results obtained by Zhou et al. (2009) for crucian carp and Le and Fotedar (2014) for juvenile yellowtail kingfish. The muscle and liver Se concentrations were significantly \((P < 0.05)\) affected in fish fed with nanoselenium-supplemented diets. Carp that were fed the 2 mg nano-Se/kg diet showed the highest Se content in muscle and liver tissues compared to other test groups. The Se concentrations in liver tissues were higher than those of muscle tissues, showing the higher capacity of liver to accumulate Se. These data are in agreement with the results obtained for juvenile carp (Elia et al., 2011) and for other fish species (Hamilton, 2004).

Se plays an important role in activating antioxidant defense system as it forms selenocysteine, which is part of the active center of the glutathione peroxidase (Kohrle et al., 2000). Under oxidative stress, antioxidant defense systems including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are essential for scavenging the reactive oxygen species (ROS) (Atencio et al., 2009). TBARS test is considered as an index of lipid peroxidation products and lower MDA level is related to longer shelf life and better meat quality (Gatta et al., 2000). In the present study, the activities of liver GPx and SOD were significantly increased in the 1 and 2 mg nano-Se/kg groups. In addition, significantly higher activity of CAT and lower level of MDA were observed in fish receiving 2 mg nano-Se/kg. These data suggest that supplementation of 2 mg nano-Se/kg seems to be more effective than lower levels of nano-Se in strengthening the antioxidant system against oxidative stress. Similar to our results, Hao et al. (2014) reported that GPx and SOD activities were significantly increased in liver tissues of loach fed on 0.5 mg Se/kg, and the lowest MDA level was observed in the 0.48 mg Se/kg group.

Total protein and globulin contents were significantly higher and albumin concentration was significantly lower in fish that received 2 mg nano-Se/kg as compared to other groups. Measurement of globulin is of

<table>
<thead>
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<th>Table 3</th>
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<tr>
<td>Growth performance and survival of common carp fed different levels of nano-Se.</td>
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<tr>
<td>Basal diet</td>
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<tr>
<td>0.5 mg nano-Se</td>
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<tr>
<td>1 mg nano-Se</td>
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<tr>
<td>2 mg nano-Se</td>
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</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other (significance level is defined as \(P < 0.05\)).

<table>
<thead>
<tr>
<th>Table 4</th>
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<tr>
<td>Proximate composition of muscles of common carps fed different levels of nano-Se.</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
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<tr>
<td>Lipid (%)</td>
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<td>Moisture (%)</td>
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<td>Ash (%)</td>
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</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other (significance level is defined as \(P < 0.05\)).

<table>
<thead>
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<th>Table 5</th>
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<tbody>
<tr>
<td>Se content in muscle and liver tissues of common carps fed on different levels of nano-Se.</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>Liver</td>
</tr>
</tbody>
</table>

Values are mean ± SE of three replicate groups. Mean values with different superscripts are significantly different from each other (significance level is defined as \(P < 0.05\)).
remarkable diagnostic value in laboratory animals and increased serum globulin and the lowered albumin/globulin ratio levels are indicative of immunity status (Bunglavan et al., 2014). Therefore, dietary nano-Se at the rate of 2 mg/kg exerted positive effects on the immune status of the carp. Similarly, Abdel-Tawwab et al. (2007) reported the increased total protein, globulin and albumin contents in African catfish fed with 0.5 g organic Se/kg diet.

Based on our data, 2 mg nano-Se/kg supplementation caused significant decrease in serum total cholesterol level. Also, a significant decrease in LDL concentration was observed in 1 and 2 mg nano-Se/kg groups and HDL level remarkably increased in carp fed on 2 mg nano-Se/kg diet. Similar to our results, Bunglavan et al. (2014) demonstrated that Se can significantly reduce serum total cholesterol in Wistar rats. Also, it was reported that organic Se could significantly reduce LDL and increase HDL (Hao et al., 2014). Several authors have reported that HDL has antioxidant effects due to its antioxidant proteins and enzymes (Bandeali and Farmer, 2012; Gbandjaba et al., 2012). Apolipoprotein-Al is considered as the main antioxidant factor in HDL, which inhibits lipid peroxidation in LDL (Podrez, 2010). The increased level of serum HDL in the present study was accompanied with decreased concentrations of LDL and MDA. These results may indicate that carp were made stronger and protected when they were fed diets containing 2 mg nano-Se/kg.

While there was no significant difference in ALP activity among the experimental groups, the fish fed on 1 and 2 mg nano-Se/kg diets showed the highest values of AST activity compared to other treatments. Moreover, the activity of ALP was significantly higher in fish fed on 2 mg nano-Se/kg diet as compared to other treatments. Significant increase in the activities of serum ALP, AST and ALT is considered as the response of organism to stressors (Bitiren et al., 2004). The increased levels of ALT and AST in serum at the highest dose (2 mg nano-Se/kg) may be a sign of toxic effect. Therefore, the data of the present study recommend adding 1 mg nano-Se per kg diet to improve carp growth and antioxidant defense system.

In conclusion, effects of different levels of nanoselenium on common carp were investigated. This research demonstrated that supplementation of nano-Se in diet at the rate of 1 mg/kg could enhance growth performance of common carp. Carps fed on 2 mg nano-Se/kg diet showed the highest muscle and liver Se concentration. Moreover, the lowest MDA level, and the highest activities of antioxidant enzymes were observed in fish fed on 2 mg nano-Se/kg diet. While the supplementation of Se nanoparticles at the rate of 2 mg/kg exerted positive effects on the immune status of the carp, the increased levels of ALT and AST in serum at the highest dose (2 mg nano-Se/kg) may be a sign of toxic effect. Therefore, the data of the present study recommend adding 1 mg nano-Se per kg diet to improve carp growth and antioxidant defense system.

Acknowledgment

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