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Dietary cosupplementation with curcumin and different selenium sources (nanoparticulate, organic, and inorganic selenium): influence on growth performance, body composition, immune responses, and glutathione peroxidase activity of rainbow trout (*Oncorhynchus mykiss*)

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Abstract The aim of this study was to investigate the effects of dietary selenium (nanoparticles, organic, and inorganic forms), curcumin (CUR), and their combination on survival, growth performance, body composition, innate immune responses, and glutathione peroxidase activity of rainbow trout (*Oncorhynchus mykiss*). CUR at level of 400 mg/kg dry diet and each of selenium nanoparticles (Se-NPs), organic selenium (Sel-Plax®), and sodium selenite at level of 1 mg/kg Se dry diet were added to basal diet. A total of 240 rainbow trout with mean initial weight of 14.65 ± 0.86 g were fed eight diets including control (basal diet), CUR, Se-NPs, Se-NPs + CUR, organic Se, organic Se + CUR, sodium Se, and sodium Se + CUR for 8 weeks. No significant increase in survival rate, growth performance, feed utilization, and body composition was observed in fish-fed CUR and Se included diets compared to control ($P > 0.05$). The highest lysozyme and alternative hemolytic complement activity was observed in fish-fed CUR and organic Se + CUR-supplemented diets ($P < 0.05$). Fish-fed Se-NPs and Se-NPs + CUR-supplemented diets had the highest glutathione peroxidase activity ($P < 0.05$). The results of the present study indicated that the combination of CUR and Se in nanoparticles and organic forms was more effective in promoting innate immune responses of rainbow trout compared to the other combined or separated Se and CUR forms.

Keywords Curcumin · Dietary supplements · Innate immunity · Glutathione peroxidase · *Oncorhynchus mykiss* · Selenium

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Introduction

Nowadays, aquaculture as a fast growing industry plays an important role in the production of aquatic animals in the world. By increasing the fish demand, intensified aquaculture systems have become more popular which in turn have magnified stressors for fish and thus enhanced the uprising of diseases with huge economic losses (Reverter et al. 2014). Aquaculture industry has experienced different solutions such as antibiotics and chemotherapeutics to control and prevent diseases (Ramesh and Souissi 2018). Excessive use of antibiotics and chemotherapeutics has caused antibiotic-resistant

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bacteria (Teuber 2001), environmental risks (Rico et al. 2012), and food safety issues (Kwon 2016) to increase in aquaculture. In recent years, demands for environmentally safe aquaculture have increased. Therefore, the application of natural growth promoters which stimulate growth and disease resistance has been promoted. Several medicinal plants with promising effects on growth performance and immune responses have been effectively used in aquafeeds (Reverter et al. 2014; Sahu et al. 2008). The immunostimulants increase the activity of phagocytosis cells and white blood cells, resistance to infectious diseases, and antibody production. They are found in two forms of plant extracts and synthetic form (Immanuel et al. 2009; Sakai 1999).

Curcumin (CUR) is a natural yellow acidic phenol derived from *Curcuma longa* L. (turmeric) rhizome (Mirzaei et al. 2017; Prasad et al. 2014). It is usually used as a spice, coloring agent, and food preservative. Several investigations have reported that CUR benefits from different pharmacological effects including anti-inflammatory, anti-oxidant, anti-viral, anti-stress, and immunomodulatory activities (Akdemir et al. 2017; Masuda et al. 2002; Negi et al. 1999; Yang et al. 2009). Fish growth and innate immunity increased significantly by elevated levels of dietary supplementation of turmeric powder in common carp, *Cyprinus carpio* (Abdel-Tawwab and Abbass 2017). Akdemir et al. (2017) also declared that feeding rainbow trout (*Oncorhynchus mykiss*) with CUR-supplemented diet at a level of 200 mg/kg diet under high stocking density conditions resulted in a significant increase in final body weight, weight gain, and feed intake of fish.

Selenium (Se), as an exogenous antioxidant, contributes indirectly to removing and preventing oxidative stress (Mansour et al. 2017; Rayman 2000). Selenium also is an essential part of the glutathione peroxidase (GPx), which directly protects the cells against drastic damages caused by hydrogen peroxide radicals. Previous studies have highlighted the advantages of dietary Se on growth and health of various species of fish (Le and Fotedar 2014; Liu et al. 2010; Wang et al. 2013). Selenium, as an immunostimulant, can be added to diet of cultured animals in aquaculture systems in organic and inorganic forms. Inorganic forms of Se include selenite and selenate; the organic forms involve selenomethionine and selenocystine (Wang and Lovell 1997).

Organic form of Se is more bioactive than the inorganic form by improving the growth, survival, and activity of antioxidant enzymes in aquatic animals (Ashouri et al. 2015; Saffari et al. 2017; Wang and Lovell 1997). In addition to those two common forms, selenium nanoparticles have recently been considered as a new form of selenium (Hu et al. 2012; Rezvanfar et al. 2013; Saffari et al. 2017). Nanoparticles are generally defined as particular materials with at least one dimension less than 100 nm with specific physical and chemical characteristics, including high surface-to-volume ratio and high reactivity (Christian et al. 2008). Despite the fact that the addition of some trace elements in their nanoparticulate form to the fish diet may cause some toxic effects in blood (Chupani et al. 2017), kidney (Chupani et al. 2018a), and intestine (Chupani et al. 2018b), selenium nanoparticles have recently been used in fish diets because of high bioavailability and low toxicity (Saffari et al. 2017). The results of a study showed that feeding common carp, *Cyprinus carpio*, with diet supplemented with selenium nanoparticles (Se-NPs) improved growth and antioxidant defense system of fish in comparison to other selenium sources of organic and inorganic (Saffari et al. 2017).

Some litreture have reported the response of various fish species to dietary selenium sources (Le and Fotedar 2014; Mansour et al. 2017; Naderi et al. 2017; Saffari et al. 2017), but information related to synergistic effects of different forms of dietary Se and CUR on growth and immunity of fish is limited. Thus, the aim of the present research was to investigate how dietary selenium (nanoparticulate, organic, and inorganic forms), CUR, and their combination influence survival, growth performance, feed utilization, body composition, innate immune responses, and glutathione peroxidase activity of rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Experimental diets

Se-NP suspension (1000 mg/L and purity of 99.95%) was purchased from Iranian Nanomaterials Pioneers Company (Mashhad, Iran). Organic selenium (Sel-Plax®, 1000 mg/kg Se) and sodium selenite (purity of 97%) were purchased from Alltech Co. and Sigma-Aldrich, respectively. CUR product was purchased from Merck. Particle size distribution and morphology of Se-NP suspension were determined using scanning electron microscopy (KYKY-EM3200). Axio Vision digital image processing software (Release 4.8.2.0, Carl Zeiss

Micro Imaging GmbH, Germany) was applied to evaluate the particle mean size distribution by measuring the diameters of 100 individual nanoparticles from three SEM images at random.

A commercial rainbow trout diet (FFT1, 3.3 ± 0.3 mm pellets, Faradaneh Co., Iran) was used as a basal diet in this study. To prepare the experimental diets, the supplements including selenium nanoparticles, organic selenium, sodium selenite (1 mg/kg Se dry diet), and CUR (400 mg/kg dry diet) were mixed with double-distilled water and sprayed over the basal diet. For this purpose, in each group, 1 kg of commercial feed was placed in a food mixer and gradually sprayed with the double-distilled water containing supplements and oven dried at 55 °C. Eight experimental diets including control (basal diet), CUR, Se-NPs, Se-NPs + CUR, organic Se, organic Se + CUR, sodium Se, and sodium Se + CUR were formulated. The basal diet without supplementation with Se sources and CUR was considered as the control treatment. In order to prevent the release of supplements from food into the water during the feeding, 10% of bovine gelatin solution was sprayed on prepared diets and oven dried again at 55 °C (Ramsden et al. 2009). The control diet was prepared in exactly the same way, except that the supplement solutions were replaced by an equal volume of double-distilled water. The inclusion levels of selenium nanoparticles, organic selenium, sodium selenite, and CUR were chosen based on a literature review on the requirements of selenium and CUR in rainbow trout (*Oncorhynchus mykiss*) (Akdemir et al. 2017; Hilton et al. 1980; Khan et al. 2017; Rider 2009). To measure selenium content of experimental diets, 1 g of dried diet was digested by 10 mL of nitric acid (65% suprapur grade, Merck, Germany) for 1 h at 40 °C and 2 h at 90 °C. Once completely dissolved, the contents were diluted to 50 mL with deionized water. The selenium contents in digested samples were determined by atomic absorption spectroscopy (PerkinElmer, Analyst 700, USA).

Fish culture and feeding trial

Fingerlings of rainbow trout with the average weight of 14.65 ± 0.86 g were received from a local fish farm in Chalus city, Northern Iran, and transferred to the University of Tarbiat Modares experimental hatchery. They were adapted to lab conditions for 2 weeks and were fed the control diet twice a day. Fish were distributed randomly into 24 tanks (capacity of 128 L) at a density of 10 fish per tank with 90% water exchange a day and at three replicates for each diet. For a period of 8 weeks, experimental diets were given to fish to apparent satiation three times a day. Daily water quality analysis showed a salinity of 2.22–2.52 ppt, pH of 8.23–8.64, temperature of 13–16.5 °C, and 9 mg/L for dissolved oxygen. A 12-h dark/12-h light regime was considered for fish.

Growth performance, survival rate, and nutritional efficiency indices

After 8 weeks of rearing, the fish of each tank were weighted and survival rate (%), growth performance, and nutritional efficiency indices including body weight gain (WG), specific growth rate (SGR), condition factor (CF), protein efficiency ratio (PER), feed conversion ratio (FCR), hepatosomatic index (HSI), and viscerosomatic index (VSI) were determined as follows (Mohanta et al. 2008; Ramos et al. 2015):

WG	W_f (g) − W_i (g)
SGR	$[(\ln W_f - \ln W_i)/t] \times 100$
FCR	Dry feed intake (g) / wet weight gain (g)
CF	$W_f / L_f^3 \times 100$
PER	W_{gain} / crude protein consumed
VSI	Weight of the whole digestive tract / body weight
HSI	Liver weight / body weight
Survival rate (%)	Final individual numbers / initial individual numbers × 100

where W_i, W_f, W_{gain}, L_f, and t are initial weight (g), final weight (g), weight increment (g), final length (cm), and experimental period (day), respectively.

Chemical analysis

Three fish were randomly collected at the end of the feeding trail from each tank for chemical analysis. Fish body and diet composition including crude fat, crude protein, moisture, and ash were measured using the standard methods (AOAC 1990). Crude fat was determined using the Soxhlet method, crude protein by nitrogen analysis after acid digestion using the Kjeldahl method, moisture content by drying at 105 °C until constant weight, and ash after 13 h combustion at 550 °C in an electric oven.

Biochemical analysis in blood and liver

To make six fish from each tank unconscious, the clove powder (500 mg/L) was used on the 57th day following a 24-h fasting. The blood samples were taken from the caudal vein. To store an aliquot of blood specimens, the heparinized tubes were utilized; then centrifuging was done at 3000×*g* for 15 min to collect plasma that was stored at − 80 °C up to the time of conducting the assay of activity of glutathione peroxidase (GPx) enzyme. The remaining blood samples were placed into non-heparinized tubes to determine the lysozyme (LYZ) and alternative hemolytic complement activity (ACH_{50}) in serum. Fish liver tissue was also kept at − 80 °C up to the time of measuring the GPx activity. By implementing the method described by Kumari et al. (2006), the lysozyme activity in serum was assayed on the lysis of the lysozyme-sensitive gram-positive bacterium, *Micrococcus lysodeikticus* (Sigma). For this purpose, 15 μL of serum sample was added to wells of a 96-well plate in triplicate. 0.02 M sodium acetate buffer with a pH of 5.8 (0.02 mg/L) was mixed with the prepared bacterial suspension (150 μL); then, it was added to each well. OD value was recorded at 450 nm using spectrophotometry. To measure OD reduction, the samples were kept for 1 h at room temperature. A standard curve made with the dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 U/mL in the same buffer and expressed as U/mL was used to determine the lysozyme activity. By implementing the method described (Amar et al. 2000), serum ACH_{50} was specified using rabbit hemolysis. To this aim, ethylene glycol tetra acetic acid-magnesium-gelatin veronal buffer (EGTA-Mg-GVB) was used to wash rabbit red blood cells (RaRBC) three times and the concentration was set to 2×10^8. The diluted serum samples (range, 50–250 μL) were put into test tube with a fixed total volume of 250 μL with EGTA-Mg-GVB. Then, 100 mL of RaRBC suspension was poured to each tube and incubation was done for 90 min at 20 °C with occasional mixing. After the adddition of 3.15 mL of NaCl to each tube, centrifuging was done at 1600×*g* for 5 min. The supernatant absorbance was read at 414 nm. The serum volume producing 50% hemolysis (ACH_{50}) and the number of ACH_{50} units per milliliter was determined for each group. ELISA reader (Stat Fax 3200, USA) at 405 nm and kit (ZellBio GmbH, Germany) was used to assay the glutathione peroxidase enzyme in plasma and liver; the results were expressed as specific activity (U/mg protein) in liver and as U in plasma.

Statistical analysis

This study was designed and implemented in a completely randomized design. All data were recorded as mean value with standard deviation (mean ± SD). The Kolmogorov-Smirnov test of normality was used to check data normal distribution. Data were analyzed statistically using the one-way analysis of variance (ANOVA). The Duncan multiple range test was also applied for post hoc analysis using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). A probability value of $P < 0.05$ was accepted as statistical significance.

Results

Characterization of nanoparticles and diets

Figure 1 shows the SEM image of nanoparticles in Se-NP stock suspension. This micrograph illustrates that Se-NPs have a spherical shape with size distribution ranging between 8.28 and 58.88 nm. The components of the basal diet and the final actual concentration of selenium in experimental diets are presented in Tables 1 and 2, respectively.

Survival, growth performance, and body composition

The data of growth performance, feed utilization, and survival rate of rainbow trout fingerlings fed diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR for 8 weeks are presented in Table 3. The results showed that the initial and final weight of fish in different treatments were not significantly different ($P > 0.05$). No significant differences were also observed in the survival rate, WG, SGR (%), FCR, CF, PER, and liver index (HSI) of fish-fed-supplemented diets and control group ($P > 0.05$). The visceral index (VIS) of fish fed with supplemented diets did not change significantly compared to control ($P > 0.05$). Among the supplemented treatments, the lowest and the highest VSI values were observed in sodium Se + CUR treatment and CUR treatment, respectively ($P < 0.05$).

In Table 4, body composition analysis of fish-fed diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR is presented. There were no significant differences in crude fat, crude protein, moisture, and ash contents of rainbow

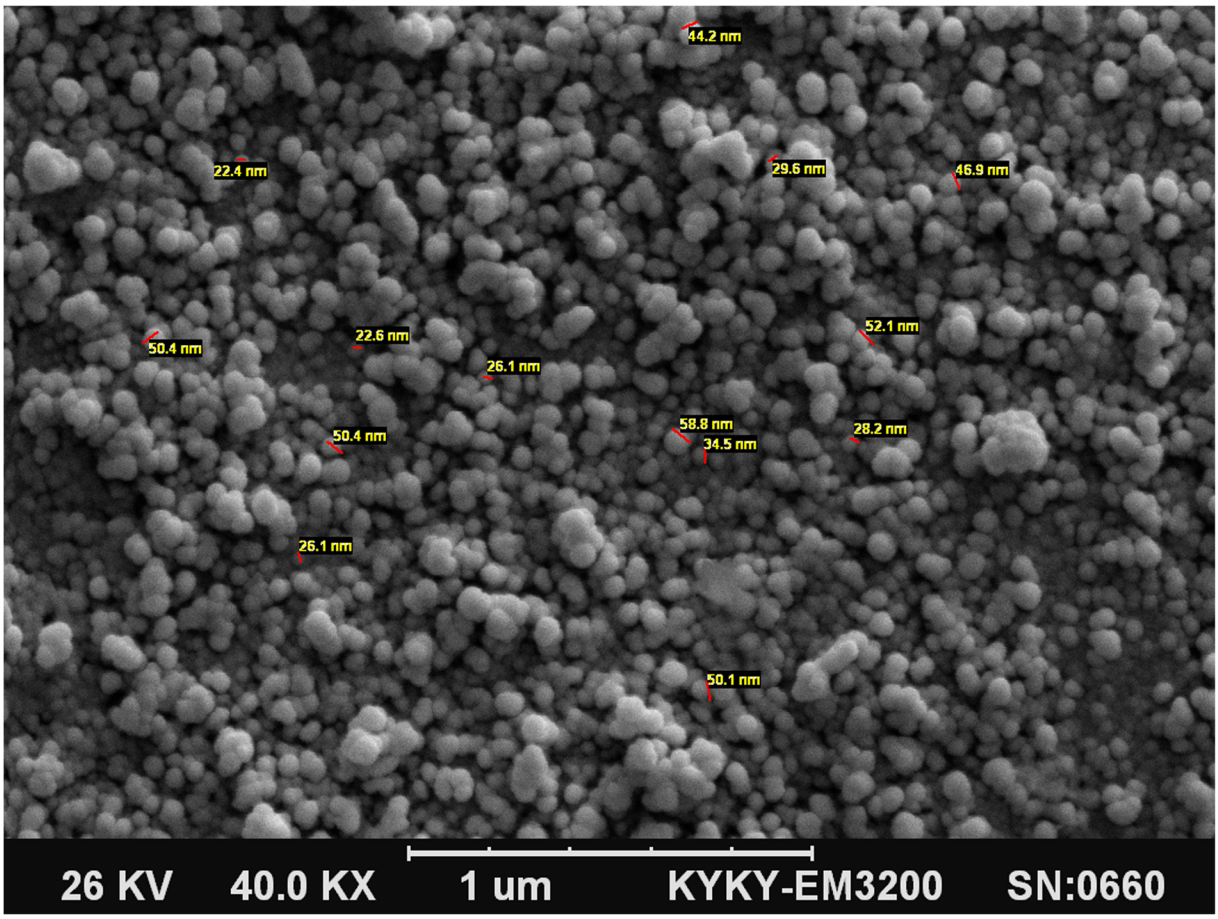

Fig. 1 SEM image of Se-NPs from stock suspension of 1000 mg/L

trout fingerlings fed supplemented and control diets ($P > 0.05$).

Biochemical analyses

Figure 2 shows the immunological responses of rainbow trout fingerlings fed diets supplemented with different selenium sources and CUR based on LYZ and ACH_{50} in serum. Lysozyme activity was significantly higher than control in all supplemented treatments ($P < 0.05$); sodium Se + CUR treatment was an exception. The highest LYZ activity was observed by CUR and organic Se + CUR treatments; the lowest activity of this enzyme was observed by sodium Se + CUR treatment ($P < 0.05$). In

Table 1 Average proximate composition of basal diets supplemented with different selenium sources and CUR (% dry weight) for rainbow trout fingerlings

Proximate chemical composition	%
Protein	47 ± 0.35
Lipid	11.33 ± 1.24
Ash	9.26 ± 0.11
Moisture	9.2 ± 0.21

Table 2 The final actual concentration of selenium (mg/kg) in experimental diets

Dietary treatment	Selenium concentration (mg/kg diet)
Basal diet (control)	0.35 ± 0.04
CUR	0.34 ± 0.05
Se-NPs	1.37 ± 0.05
Se-NPs + CUR	1.38 ± 0.03
Organic Se	1.33 ± 0.02
Organic Se + CUR	1.37 ± 0.04
Sodium Se	1.37 ± 0.05
Sodium Se + CUR	1.35 ± 0.06

Table 3 Growth, survival, and feed utilization of rainbow trout fingerlings fed diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR for 8 weeks (mean ± SD, $n = 3$)

Index	Dietary treatment							
	Control	CUR	Se-NPs	Se-NPs + CUR	Organic Se	Organic Se + CUR	Sodium Se	Sodium Se + CUR
Initial weight (g)	14.48 ± 0.76^{a}	14.45 ± 0.32^{a}	14.63 ± 0.81^{a}	14.39 ± 1.11^{a}	14.75 ± 0.87^{a}	14.64 ± 0.73^{a}	14.89 ± 1.05^{a}	14.96 ± 1.26^{a}
Final weight (g)	76.18 ± 0.99^{a}	74.58 ± 0.83^{a}	77.17 ± 1.14^{a}	78.46 ± 2.27^{a}	75.32 ± 0.77^{a}	75.44 ± 1.56^{a}	75.98 ± 4.06^{a}	77.54 ± 2.53^{a}
Weight gain (g)	61.91 ± 0.77^{a}	60.14 ± 0.85^{a}	62.53 ± 0.24^{a}	63.20 ± 1.80^{a}	60.56 ± 1.05^{a}	60.79 ± 0.94^{a}	62.08 ± 1.32^{a}	62.57 ± 1.74^{a}
SGR (%)	3.71 ± 0.01^{a}	3.64 ± 0.05^{a}	3.69 ± 0.14^{a}	3.47 ± 0.15^{a}	3.62 ± 0.13^{a}	3.64 ± 0.07^{a}	3.81 ± 0.43^{a}	3.67 ± 0.15^{a}
FCR	0.67 ± 0.005^{a}	$0.7.0 \pm 0.005^{a}$	0.67 ± 0.005^{a}	0.67 ± 0.03^{a}	0.69 ± 0.015^{a}	0.71 ± 0.049^{a}	0.68 ± 0.030^{a}	0.68 ± 0.024^{a}
CF (%)	0.86 ± 0.005^{a}	0.91 ± 0.075^{a}	0.84 ± 0.037^{a}	0.86 ± 0.035^{a}	0.95 ± 0.063^{a}	0.90 ± 0.032^{a}	0.89 ± 0.036^{a}	0.95 ± 0.047^{a}
PER (g)	2.29 ± 0.21^{a}	2.15 ± 0.095^{a}	2.24 ± 0.089^{a}	2.22 ± 0.090^{a}	2.12 ± 0.032^{a}	2.28 ± 0.058^{a}	2.21 ± 0.14^{a}	2.13 ± 0.029^{a}
HSI (%)	1.04 ± 0.16^{a}	1.11 ± 0.04^{a}	1.01 ± 0.045^{a}	0.97 ± 0.110^{a}	0.96 ± 0.023^{a}	1.04 ± 0.120^{a}	1.04 ± 0.072^{a}	1.03 ± 0.045^{a}
VIS (%)	8.46 ± 0.58^{abc}	9.32 ± 0.25^{a}	8.67 ± 0.55^{ab}	8.56 ± 0.54^{ab}	7.92 ± 0.65^{bc}	8.54 ± 0.48^{ab}	8.35 ± 0.67^{abc}	7.51 ± 0.31^{c}
Survival rate (%)	96.66 ± 0.57^{a}	100 ± 0.00^{a}	96.66 ± 0.57^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}

Values with different letters within a row are significantly different (ANOVA, $P < 0.05$)

Table 4 Approximate analysis of rainbow trout fingerlings' body composition after feeding with diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR for 8 weeks (mean ± SD, $n = 3$)

Index	Dietary treatment							
	Control	CUR	Se-NPs	Se-NPs + CUR	Organic Se	Organic Se + CUR	Sodium Se	Sodium Se + CUR
Crude protein (%)	64.05 ± 5.08^{a}	65.76 ± 3.07^{a}	65.82 ± 2.19^{a}	66.61 ± 1.20^{a}	67.10 ± 1.42^{a}	64.83 ± 2.33^{a}	65.86 ± 2.82^{ab}	67.65 ± 1.67^{a}
Crude fat (%)	28.04 ± 1.48^{a}	28.34 ± 0.87^{a}	28.69 ± 2.77^{a}	26.44 ± 3.89^{a}	25.59 ± 2.65^{a}	$26.350.96^{a}$	25.16 ± 1.83^{a}	26.49 ± 0.52^{a}
Ash (%)	5.73 ± 1.43^{a}	4.65 ± 0.91^{a}	4.14 ± 1.21^{a}	5.69 ± 0.38^{a}	5.98 ± 0.21^{a}	4.78 ± 2.86^{a}	6.03 ± 0.48^{a}	5.74 ± 0.50^{a}
Moisture (%)	73.44 ± 1.14^{a}	74.62 ± 1.19^{a}	73.76 ± 0.89^{a}	72.57 ± 1.02^{a}	74.85 ± 1.11^{a}	73.16 ± 1.47^{a}	74.84 ± 0.75^{a}	73.64 ± 1.01^{a}

Values with different letters within a row are significantly different (ANOVA, $P < 0.05$)

comparison with the control group, fish fed with the supplemented diets had higher ACH_{50} activity ($P < 0.05$). The highest ACH_{50} activity was observed in the organic Se + CUR treatment ($P < 0.05$). Figure 3 shows the effects of various selenium sources and CUR in experimental diets on plasma and liver glutathione peroxidase activity. The levels of glutathione peroxidase both in plasma and liver in all the supplemented treatments except CUR treatment increased significantly compared to the control group ($P < 0.05$). Se-NPs and Se-NPs + CUR treatments yielded the highest levels of glutathione peroxidase activity in plasma ($P < 0.05$). In comparison with other treatments, Se-NPs + CUR treatment yielded the highest level of glutathione peroxidase in liver ($P < 0.05$).

Discussion

The effects of different sources of Se including organic, inorganic, and nanoparticulate selenium on growth and survival of fish species are conflicting in literatures. Saffari et al. (2017) showed that Se-NPs supplemented in diet could improve the FCR and SGR% of common carp (*Cyprinus carpio*). The improvement of growth performance of crucian carp (*Carassius auratus gibelio*) fed diets supplemented with Se-NPs and organic Se (selenomethionine) was also demonstrated by Zhou et al. (2009). The results of the present study showed that the inclusion of different sources of selenium (nanoparticles, organic, and inorganic forms) at the level of 1 mg/kg and their combination with CUR in diets of

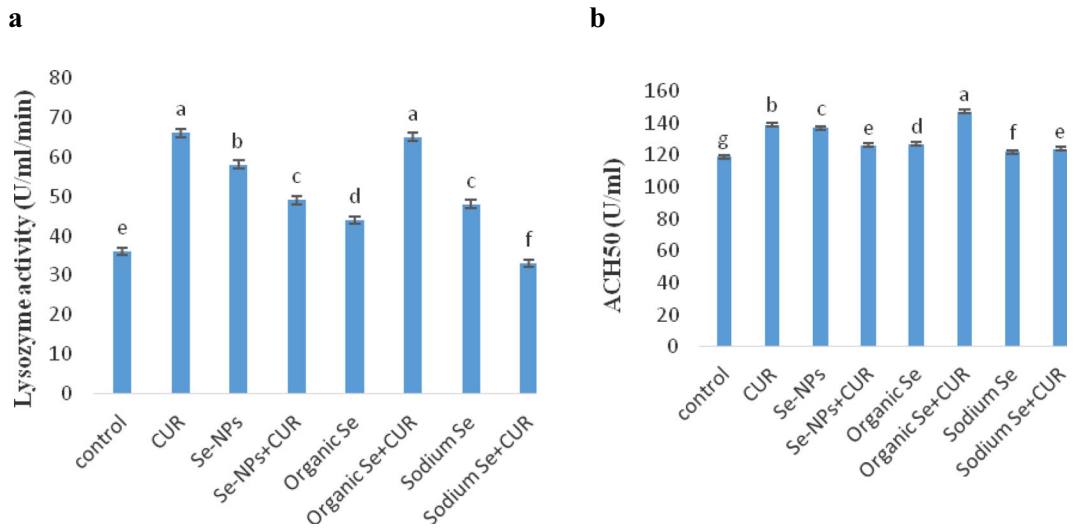

Fig. 2 Lysozyme (U/mL/min) (**a**) and alternative hemolytic complement (ACH_{50}) (U/mL) (**b**, activities in serum of fish-fed diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR for 8 weeks (mean ± SD, $P < 0.05$, $n = 3$)

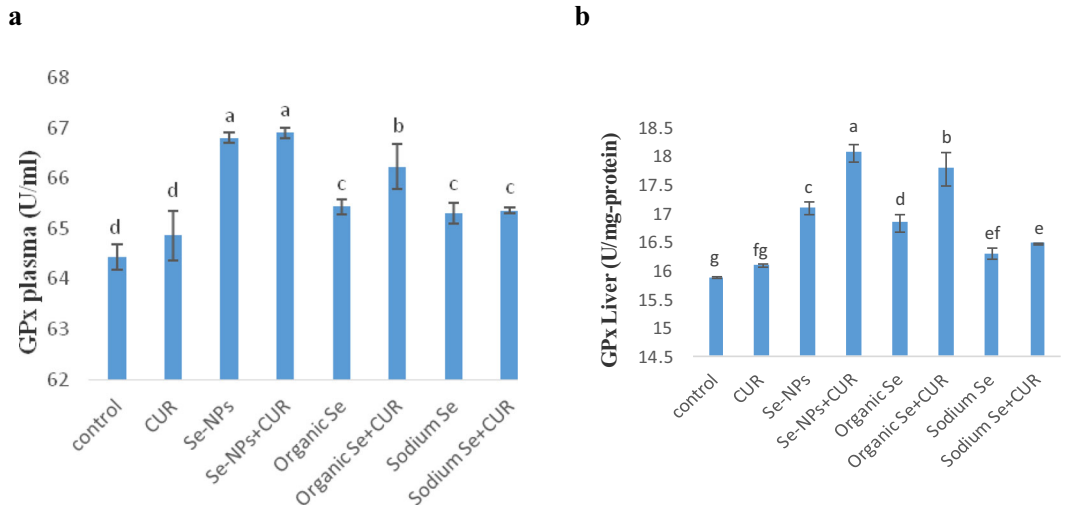

Fig. 3 Glutathione peroxidase (GPx) activity in plasma (U/mL) (**a**) and in liver (U/mg-protein) (**b**) of fish-fed diets supplemented with different selenium sources (nanoparticles, organic, and inorganic) and CUR for 8 weeks (mean ± SD, $P < 0.05$, $n = 3$)

O. mykiss did not significantly improve the survival rate, growth performance, and feed utilization of fish in comparison with the control group. This observation revealed that the Se requirement of rainbow trout fingerlings might not be met by inclusion of 1 mg/kg of Se in diet. This result is in accordance with Rider et al. (2009) who reported that the growth of *O. mykiss* was not affected by different levels of Se-yeast and selenite. The findings of another study indicated that the growth and survival of Senegalese sole (*Solea senegalensis*) larvae were not affected by feeding with live food supplemented with organic Se (Sel-Plex®, containing selenomethionine) (Ribeiro et al. 2012).

It has been described that improving the trypsin and lipase activity in intestine and hepatopancreas and the amylase activity in the hepatopancreas together with increasing the activity of Na^+/K^+-ATPase (NKA), intestinal alkaline phosphatase (AKP), gamma-glutamyl transpeptidase (γ-GT), and creatine kinase (CK) in the intestinal brush border section are the probable reasons that CUR enhances the growth performance and feed utilization (Jiang et al. 2016; Mahmoud et al. 2017). Mahmoud et al. (2017) showed that the inclusion of CUR at a level of 50 mg/kg in diet caused higher final weight, daily weight gain, and specific growth rate in Nile tilapia (*Oreochromis niloticus*). The finding of another study revealed an inverse relationship between level of turmeric incorporation in diet of juvenle Nile tilapia (*O. niloticus*) and growth performance. Turmeric supplementation at levels of 4 and 8 g/kg diet depressed weight gain and SGR% (Yusuf et al. 2017). Akdemir et al. (2017) also reported that 200 mg/kg of CUR in diet improved growth of rainbow trout (*O. mykiss*), but the level of 400 mg/kg did not significantly affect the growth of fish. According to the results of the present study, the growth performance, feed utilization, and survival of rainbow trout fingerling fed diet supplemented with 400 mg/kg of CUR were not improved significantly in comparison to the control group. This could be related to the reduction of feed intake resulting in decreased body weight gain. In fact, when CUR incorporates at high levels in fish diet, it produces a taste (Hosseini-Vashan et al. 2012) and would also act as phytoestrogen (Folwarczna 2013), and both of them could decrease feed intake by fish.

In the present study, fish proximate composition was not affected by dietary supplementation of Se and CUR after 8 weeks of feeding trial. This finding is similar to the results reported by Saffari et al. (2017) for common carpn (*C. carpio*) and Le and Fotedar (2014) for yellowtail kingfish (*Seriola lalandi*) fed diets supplemented with different sources of Se. Similar results were also reported by Jiang et al. (2016) for crucian carp (*Carassius auratus*) when CUR was supplemented to the fish diets.

Lysozyme and complement (classical and alternative pathways) as humoral components participate in the innate immune response of teloest fish (Magnadóttir 2006). Immunostimulants can increase serum lysozyme activity by either increasing the number of phagocytes secreting lysozymes or increasing the amount of lysozymes synthesized per cell (Pratheepa and Sukumaran

2014). Antioxidant and immunomodulatory activities of CUR have been well recognized (Akdemir et al. 2017; Sankar et al. 2010; Tilak et al. 2004). The mechanism of immunostimulation of CUR may be attributed to its effect on lymphoid cell populations, antigen presentation, humoral and cell-mediated immunity, and cytokine production (Gautam et al. 2007; Varalakshmi et al. 2008). The elevation of lysozyme activity has been demonstrated in Rohu, *Labeo rohita*, fed with 1 g/kg of turmeric (*Curcuma longa*) (Sahu et al. 2008) and Nile tilapia, *O. niloticus*, after feeding with 50 mg/kg CUR (Mahmoud et al. 2017). Xia et al. (2015) found that alternative pathway of complement hemolysis activity (ACH_{50}) in serum of juvenile Wuchang bream (*Megalobrama amblycephala*) increased with increasing dietary CUR levels up to 60 mg/kg diet and declined afterwards. In this study, the inclusion of 400 mg/kg CUR in rainbow trout diet resulted in significant elevation of serum lysozyme and ACH_{50} activities in comparison to fish-fed basal diet.

Selenium, as an essential nutrient for fish antioxidant defenses, contributes to preventing immunosuppression caused by oxidative stress (Takahashi et al. 2017). Se played a valuable role in stimulating immune system of goldfish (*Carassius auratus*) through the activation of plasma lysozyme and immunoglobulin (IgM) expression (Choi et al. 2013). Takahashi et al. (2017) reported that lysozyme activity increased in pacu fish (*Piaractus mesopotamicus*) fed diet supplemented with 1.15 mg/kg Se yeast, but ACH_{50} did not increase significantly compared to the control group. The increases in humoral innate immune parameters including lysozyme and ACH_{50} were reported in meager, *Argyrosomus regius*, juveniles fed diets containing Se-yeast at levels of 2 and 3 mg/kg (Mansour et al. 2017). The dietary Se-NP supplementation improved the lysozyme activity of juvenile *Tor putitora* (Khan et al. 2016).

In comparison with the control group, the highest lysozyme and ACH_{50} activities in the present study were observed in fish-fed diets supplemented with Se-NPs, organic Se, and sodium Se. In comparison with other Se sources, elevated activities of these humoral innate immune parameters were observed in fish-fed Se-NP-supplemented diet. These results were not consistent with the study by Naderi et al. (2017); they argued dietary selenium nanoparticles at the level of 1 mg/kg diet under high stocking density did not influence the serum ACH_{50} of rainbow trout.

While the combination of organic Se and sodium Se with CUR promoted the ACH_{50} activitiy of fish, the combination of Se-NPs with CUR reduced this parameter. The combination of organic Se with CUR significantly increasd the lysozyme activity. The highest lysozyme and ACH_{50} activities were observed in CUR and organic Se + CUR treatments. These resuts revealed that the combination of organic Se with CUR could enhance its function in improvement of the humoral immune response of rainbow trout.

Glutathione peroxidase (GPx) is one of the most important antioxidant enzymes (Ross et al. 2001), which directly provides protection to the cells from serious damages by decomposing hydroperoxide radicals (Silva-Brito et al. 2016; Takahashi et al. 2017). Se, as selenocysteine, is one of the components of GPx, which is effective in the antioxidant defense system and the expression of the GPx is dependent on selenium uptake (Kohrl et al. 2000; Silva-Brito et al. 2016). The results of this study showed the higher GPx activity in plasma and liver of rainbow trout in all Se treatments than the control. The GPx activity in plasma and in liver was recorded as Se-NPs > organic Se > inorganic Se, which shows more bioavailability of Se-NPs than other Se forms in rainbow trout. The higher GPx activity was observed in liver of common carp (*C. carpio*) fed on Se-NP diet than selenomethionine and selenite diets (Saffari et al. 2017). Ashouri et al. (2015) also reported the highest GPx activity in common carp (*C. carpio*) fed on 2 mg/kg Se-NPs. The more efficient effect of organic Se on raising hepatic GPx activity than inorganic Se has also been reported in common carp, *C. carpio* (Jovanovic et al. 1997), and channel catfish, *Ictalurus punctatus* (Wang and Lovell 1997). In contrast, selenite had higher hepatic GPx activity in hybrid striped bass than Se-yeast (Cotter et al. 2008) and selenite or selenocysteine was a better source of Se for plasma GPx activity than selenomethionine in Atlantic salmon (*Salmo salar*) (Bell and Cowey 1989). Le and Fotedar (2014) reported that there was no direct relationship between GPx activity in red blood cells of yellowtail kingfish (*S. lalandi*) and Se form.

It has been suggested that CUR may exert its protective effects by scavenging free radicals and stimulating antioxidant enzymes like SOD, CAT, and GPx (Manju et al. 2012; Reddy and Lokesh 1992). Our results revealed that the GPx activity in plasma and liver of fish-fed CUR-supplemented diet did not increase significantly compared to the control group. This result is consistent with Manju et al. (2012), who reported that the GPx activity in liver of *Anabas testudineus* remained

unaffected in CUR-fed groups compared to the control group, maybe due to the direct scavenging of free radicals by CUR. The increase of the GPx activity in plasma and liver of rainbow trout fingerlings fed diets supplemented with CUR + Se is probably related to the inclusion of Se at level of 1 mg/kg diet.

Conclusion

The results of this study revealed that the utilization of CUR and different sources of Se and their combination at the levels of 400 and 1 mg/kg in diet, respectively, had no positive effects on the survival, growth performance, feed utilization, and body composition of rainbow trout fingerlings. The effects of lower levels of CUR and higher levels of Se in diet on growth performance of rainbow trout need to be further evaluated. The highest lysozyme and ACH_{50} activity was observed in fish fed with CUR and organic Se + CUR-supplemented diets. The highest GPx activity was also observed in fish fed with Se-NPs and Se-NPs + CUR diets. These results indicate that the combination of CUR and Se in nanoparticulate or organic forms could act as a promising immunostimulant, which would improve the innate immune response of rainbow trout.

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Compliance with ethical standards

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

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