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Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile *Tor putitora*

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Abstract: A 70-day feeding trial was conducted to evaluate the effects of dietary supplementation of selenium nanoparticles (Se-NPs) on physiological and biochemical aspects of juvenile mahseer (*Tor putitora*) fish. Maintaining a 40% protein level, 2 different experimental diets supplemented with 0% Se-NPs and 0.68 Se-NPs mg kg^{-1} were fed to triplicate groups of fish. The experiment was conducted in a semistatic condition consisting of 6 fiber tanks. The fish were first acclimatized, and then 30 fish per tank were randomly put into each fiber tank. At the end of the feeding trial, samples were collected from the fish and analyzed. Results obtained showed that the diet supplemented with Se-NPs significantly increased ($P < 0.05$) physiological aspects like red blood cell count, hemoglobin level, hematocrit values, and lysozyme activity as compared to the basal diet. Similarly, biochemical parameters including serum growth hormone levels, tissue total protein content, and glutathione peroxidase activity in liver and muscle tissues of *T. putitora* also significantly ($P < 0.05$) increased. Thus, dietary Se-NP supplementation at the rate of 0.68 mg kg^{-1} diet has very beneficial effects on the physiobiochemical health aspects of juvenile *T. putitora.*

Key words: *Tor putitora*, physiological aspects, biochemical aspects, selenium nanoparticles

1. Introduction

Selenium is an important dietary micronutrient (Dare et al., 2001) required for the normal body functions and metabolism of animals (Hamilton, 2004). It plays a significant role in the catalytic processes within the enzyme system that consist of a variety of enzyme activities linked with the metabolic, endocrine, and immune systems (Keen et al., 2004). Selenium supplementation prevents cell damage and plays an important role in the development, fertility, and immune functions of humans and other vertebrates, including fish (Hoffmann and Berry, 2008; Schrauzer and Surai, 2009). However, its deficiency causes many metabolic disorders and diseases such as calf pneumonia, white muscle disease in calves and beef cows, exudative diathesis, and infertility in fish and other vertebrates; thus, a lack of selenium exerts a negative influence on the immune function (Muller et al., 2002; Ekiz et al., 2005).

Selenium plays an important role in the formation of several important types of selenoproteins such as glutathione peroxidase (GSH-Px) and thioredoxin reductase (Yu et al., 2005) and provides protection to the body against oxidative stress (Burk and Hill, 1993). GSH-Px is the most important selenoenzyme that plays an important role in all animal cells, because when it is in a reduced state, it decreases the production of lipid hydroperoxides and hydrogen peroxides near the cytosol and mitochondrial milieu (Dong, 2000). Thus, it provides protection to the organism against oxygen free radicals produced during stressful conditions or when an animal is exposed to certain kinds of toxicity (Beckett and Arthur, 2005; Castellano et al., 2005).

Previous studies have shown that selenium is indirectly involved in growth hormone secretion, as selenium is an important constituent of the deiodinase enzyme, which is necessary for the proper functioning of the thyroid hormones and takes part in the conversion of T_4 to T_3, the biologically more active form of thyroid hormone (Kohrle and Gartner, 2009). In consequence, thyroid hormones (T_3 and T_4) are required for the proper secretion of growth hormone from the pituitary gland in all vertebrates including fish (Valcavi et al., 1992; Muller et al., 1999); thus, selenium is indirectly involved in growth hormone secretion.

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Selenium also plays a valuable role in the physiology of fish by improving the physiological status of the animal; its deficiency weakens the fish's physiological system. In hybrid tilapia, proper dietary selenium supplementation improved the red blood cell (RBC) count and percentage of hematocrit (Hct%), while its deficiency caused a decrease in hematocrit value and RBC count (El-Hammady et al., 2007). Its role in immunity depends upon its concentration. Its supplementation improves the immunity of fish by increasing the lysozyme activity and RBC count of the fish (Khalafalla et al., 2011; Sadeghian et al., 2012; Le et al., 2013).

Materials of less than 100 nm in size (Roco, 2003; Masciangioli and Zhang, 2003; SCENIHR, 2007) are called nanoparticles. When converted into nanoform, the materials show unique physical and chemical properties. These properties are different from those of both isolated atoms and bulk material in shape, size, conductivity, and surface area to volume ratio (Oberdorster et al., 2005; Nel et al., 2006). Fish accept and eat feed containing nanomaterials; therefore, nanotechnology can be used for improving the physiological and biochemical status of fish and thus fisheries and the aquaculture industry can be advanced by the use of nanoparticles of micronutrients and constituents in fish feed (Roco and Bainbridge, 2005; Handy, 2012). Selenium is an important dietary micronutrient; when delivered in nanoform, it shows important biological activities and is of lower toxicity than selenomethionine, which accumulates in muscle tissue (Zhou et al., 2009). Many research studies have reported the higher efficiency of selenium nanoparticles (Se-NPs) compared to sodium selenite or selenomethionine as a dietary source (Wang et al., 2007; Zhou et al., 2009; Jamil, 2013).

Tor putitora (Hamilton), also known as golden mahseer, is a fish of the family Cyprinidae and is found all along the Himalayas, including Pakistan, Kashmir, and Bangladesh. Mahseer reduction has been described by many researchers (Islam and Tanaka, 2004). The population of this fish has been declining because of overfishing, loss of habitat, introduction of exotic species, and human changes to the surrounding waters (Joshi, 1987). Moreover, the construction of dams creates physical blockades for this migratory species, preventing its entry to its normal breeding and feeding grounds (Pathani, 1979), thus negatively influencing the propagation of this important fish.

The potential of mahseer fish for aquaculture has become known recently. It is considered a commercially important game fish by anglers, as well as being highly valued as a food fish (Shrestha, 1990). As selenium has several important biological functions in animals including fish (Kohrle et al., 2000), research is ongoing to differentiate the bioavailability and efficiency of various dietary sources of Se (sodium selenite, selenomethionine, and Se-NPs). The present study was thus designed to study the effects of dietary Se-NPs on physiological and biochemical aspects of *T. putitora*. The main aim of this study is to improve the physiobiochemical health status of *T. putitora* and to increase its production rate at the hatchery level to prevent the complete elimination of this important fish from Pakistani waters and to protect it from diseases and stressful conditions.

2. Materials and methods

2.1. Se-NP synthesis

Se-NPs at the rate of 0.68 mg kg^{-1} as reported by Jamil (2013) were synthesized at the National Center for Physics, Quaid-i-Azam University, Islamabad, Pakistan. They were prepared through the precipitation method. Selenium dioxide (SeO_2) and hydrazine hydrate (NH_2NH_2) were used for the synthesis of the Se-NPs. Selenium dioxide solution was used as a precursor and hydrazine hydrate acted as a reducing agent, reducing selenium dioxide into elemental selenium particles. The precursor solution of SeO_2 (50 mM) was prepared by dissolving 5.54 g of SeO_2 in 1000 mL of distilled water. A 50-mM solution of hydrazine hydrate was then prepared by dissolving 6.25 mL of hydrazine hydrate in 500 mL of distilled H_2O. After this, under constant stirring, 50 mM hydrazine hydrate solution was added drop by drop to the precursor solution until precipitates formed. The reaction mixture was then kept at room temperature for 2 days or until all the precipitates (nanoparticles) were settled; the mixture was strained through the filtration process, and particles were washed twice with distilled water so that all residues were removed. The particles were kept in a furnace overnight and were dried at 100 °C. The dried particles were ground and filtered until a fine powder was made.

The fine powder was characterized by the X-ray diffraction (XRD) technique; the XRD pattern of Se-NPs is shown in Figure 1. The crystalline size of Se-NPs was determined by using Scherrer's formula; $D = K\lambda/\beta\cos\theta$, where D indicates the crystallite size; K is Scherer's constant, which shows the unity order for a normal crystal; β signifies the full width at half maximum, and θ is the diffraction angle.

2.2. Experimental diets

The formulation of the 40% protein basal diet for *T. putitora* is shown in Table 1. To the experimental diet, Se-NPs were added at the optimum level, 0.68 mg kg^{-1}, as reported by Jamil (2013) for *T. putitora*. All dried feed ingredients were ground in a grinder and mixed with oil, water was added, and then dough was made. It was passed through a meat grinder and pellets were made, which were dried at room temperature, saved in Ziploc bags, and kept at a low temperature.

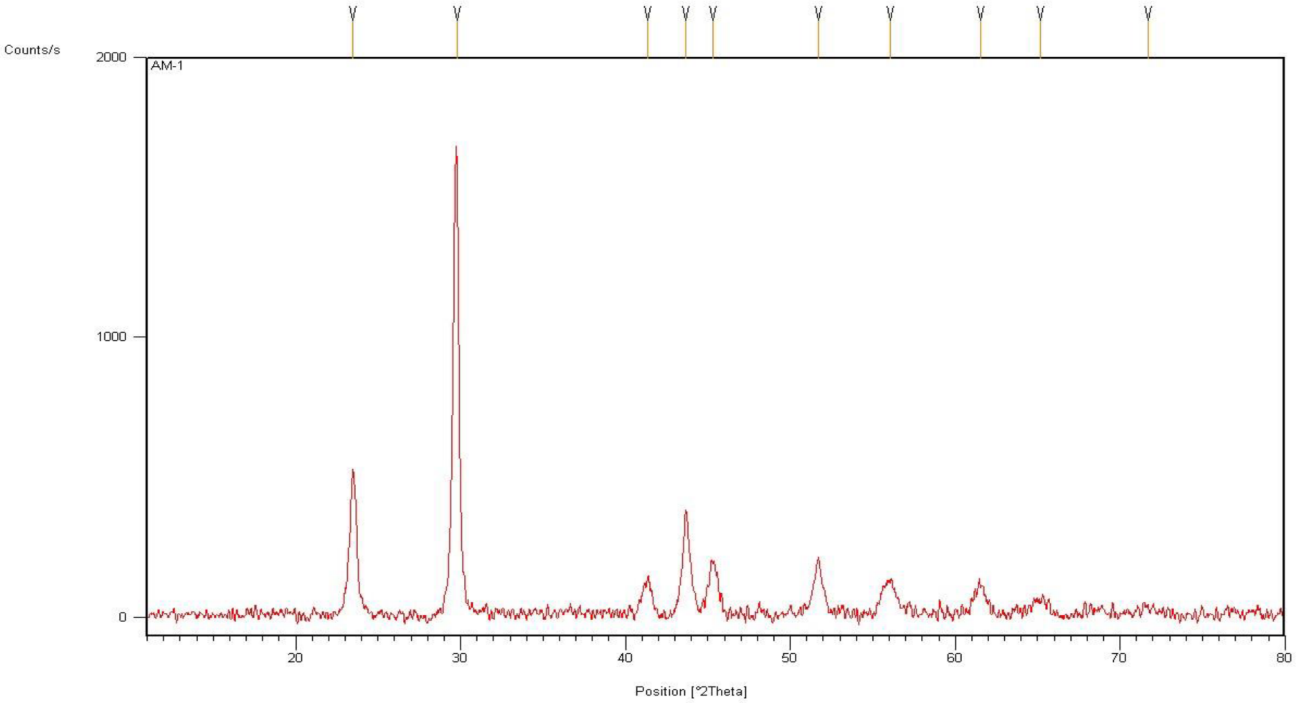

Figure 1. XRD pattern of Se-NPs.

Table 1. Formulation of 40% protein basal diet.

Ingredients	Amount (g kg^{-1})
White fish meal	250
Soybean meal	130
Sunflower meal	50
Gluten 60%	500
Wheat bran	10
Rice bran	10
Wheat flour	10
Premix*	8
Canola oil	20
Vitamin C	2
Di-calcium phosphate	10
Total	1000 g

*Vitamin premix contains vitamins, amino acids, minerals premix kg^{-1}. Vitamin A BP 40,000,000 IU; vitamin D3 BP 820,000 IU; vitamin E BP 6200 mg; vitamin K3 BP 800 mg; vitamin B2 BP 2500 mg; vitamin B3 BP 5100 mg; vitamin B12 BP 1000 mg; vitamin PP BP 10,500 mg; L-lysine BP 10,500 mg; DL-methionine BP 50,500 mg; choline chloride USP 125,500 mg; manganese USP 30,000 mg; zinc USP 17,555 mg; copper BP 1000 mg; cobalt BP 50 mg; iodine BP 300 mg.

2.3. Experimental procedure and feeding trial

The feeding trial consisted of 70 days and was conducted in 6 fiber tanks in semistatic conditions provided with a flow-through system receiving oxygenated freshwater. Juvenile *T. putitora* were obtained from the Attock Mahseer Seed Nursery and were transported to the Laboratory of Fisheries and Aquaculture, Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Uniform-sized fish regardless of sex, each of an average body weight of 2.27 ± 0.01 g, were divided into 2 replicated groups. Thirty fish per tank at the rate of 2.0 g L^{-1} (stocking density) were stocked. One group of fish was fed the basal diet, while the other group of fish was fed a diet supplemented with Se-NPs at the rate of 0.86 mg kg^{-1}. Initially, the fish were fed their respective diets at a rate of 6% of wet body weight, twice a day, and then the feeding rate was adjusted fortnightly. Daily feed intake was recorded by removing undigested feed and feces through siphoning. Tanks were cleaned every 15 days in order to prevent fungal and algal growth in the tanks. During the feeding trial, water quality parameters were measured regularly, such as water temperature and pH, which ranged from 21 to 23 °C and from 7 to 8, respectively. The ammonia recorded was less than 0.20 mg L^{-1}, while the DO_2 level was kept at 6–6.5 mg L^{-1}.

2.4. Sample collection and analysis

At the end of the feeding trial, all fish from each tank were anesthetized immediately with MS222 (60 mg L^{-1}) and

blood was collected in Eppendorf tubes by tail ablation. Subsequently, the fish were decapitated on the ice box and liver, muscle, gill, and brain tissues of each fish were removed and deep-frozen in liquid nitrogen. The samples were kept at –20 °C for the determination of total protein content and GSH-Px enzyme activity.

2.5. Hematology

The hematological study was carried out according to the method followed by Khan et al. (2015). Blood drawn by tail ablation was collected in VACUETTE EDTA K3 tubes for the analysis of hematological indices. The RBC (10^6 μL^{-1}), hemoglobin (Hb; g dL^{-1}), and Hct% values were determined by using a hematology analyzer (Sysmax KX-21, Japan).

2.6. Lysozyme activity

Lysozyme activity (μg mL^{-1}) in blood serum was determined by a method described by Anderson and Siwicki (1994) with some modifications. Fresh blood was taken in an Eppendorf tube and centrifuged at 1500 rpm for 15 min. Serum was then separated and stored in a freezer at –20 °C. For lysozyme activity, 100 μL of serum was placed in test tubes, and 900 μL of a 0.75 mg mL^{-1} *Micrococcus lysodeikticus* (Sigma, St. Louis, MO, USA) suspension (in phosphate buffered saline; pH 6.2) was added and shaken well. The absorbance was noted with the help of a spectrophotometer at 450 nm after 1-min intervals for 10 min, and lysozyme activity was calculated by using hen egg white lysozyme (Sigma-Aldrich) as a standard.

2.7. GSH-Px enzyme activity

GSH-Px activity in liver, muscle, gill, and brain tissues of mahseer fish was determined by the method described by Mohandas et al. (1984). First, 90 mg of each tissue was homogenized in 3 mL of 0.1 M KH_2PO_4 buffer. Next, 1.49 mL of phosphate buffer (0.1 M, pH 7.4), 50 μL of glutathione reductase (1 U mL^{-1}), 100 μL of sodium azide (1 mM), 100 μL of EDTA (1 mM), 100 μL of NADPH (0.1 mM), 50 μL of GSH (1 mM), 10 μL of H_2O_2 (0.25 mM), and 100 μL of homogenate (sample) were briefly mixed. The total volume was 2 mL. The disappearance of NADPH at 340 nm was recorded at 25 °C. Enzyme activity was calculated as nM NADPH oxidized min^{-1} mg^{-1} protein using a molar extinction coefficient of 6.22×103 M^{-1} cm^{-1}. The homogenized mixture was centrifuged at 12,000 × *g* for 30–35 min at 4 °C. The supernatant was decanted into a separate tube and stored at –20 °C for further analysis.

2.8. Total tissue protein content

For the determination of total protein content in liver and muscle tissues, the method described by Lowry (1951) was followed, while bovine serum albumin (BSA) was used as a standard. The values obtained were then used for the calculation of GSH-Px activity in different tissues of *T. putitora*.

2.9. Growth hormone assay

The method followed by Khan et al. (2015) was used for growth hormone (GH) analysis. Before centrifugation, blood was allowed to clot, then centrifuged at 3500 rpm for 10 min; blood serum was collected in separate tubes. Serum GH concentrations in all groups of mahseer were estimated by using a Micro ELISA GH kit (Amgenix MicroLISA, USA). All samples were run in duplicate. The GH concentrations obtained from the kit were validated by verifying that the slope of the curve obtained by serial dilution of the sample (0%, 20%, 40%, 60%, and 80%) matched the standard curve ($P = 0.94$). The intra- and interassay coefficients of variation were found to be <12%.

2.10. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) by using STATISTICA 8.1 analytical software. When a significant effect was observed, a least significant difference (LSD) test was used to compare means. Treatment effects were considered with the significance level at $P < 0.05$.

3. Results

3.1. Hematology

The effect of dietary Se-NPs on RBC count, Hb level, and Hct% of *T. putitora* is shown in Table 2. A significant ($P < 0.05$) increase in RBC count, Hb, and Hct% was observed in fish fed the Se-NP-supplemented diet compared to the control group of fish.

Table 2. Effects of dietary Se-NPs on hematological aspects and immunity parameter lysozyme activity of juvenile *T. putitora* after 70 days of supplementation.

Parameters/ Diets	RBCs (10^6 μL^{-1})	Hb (g dL^{-1})	Hct (%)	Lysozyme activity (μg mL^{-1})
Control	2.26 ± 0.08^a	7.3 ± 0.20^a	32.92 ± 0.01^a	0.88 ± 0.08^a
Se-NPs	2.6 ± 0.11^b	7.8 ± 0.08^b	33.46 ± 0.03^b	1.73 ± 0.08^b

Data are represented as mean ± SE (n = 30). Means followed by a different letter within a column are significantly different ($P < 0.05$) (ANOVA followed by LSD test).

3.2. Lysozyme activity

The dietary Se-NP supplementation significantly ($P < 0.05$) improved the lysozyme activity of *T. putitora*; the results are shown in Table 2.

3.3. Total protein content

The diet supplemented with Se-NPs significantly increased ($P < 0.05$) the protein content in the liver and muscle tissues of *T. putitora* as compared to the control diet; the results are shown in Table 3.

3.4. GSH-Px enzyme activity

Results for the GSH-Px enzyme activity determined in different tissues of *T. putitora* are shown in Table 4. The GSH-Px activity in liver tissues was significantly higher ($P < 0.05$) with the Se-NP-supplemented diet as compared to the control diet. In muscle tissues, the group of fish fed the Se-NP-supplemented diet had significantly higher GSH-Px activity in comparison with fish reared on the control diet.

The same trend was shown with GSH-Px enzyme activity in gill and brain tissues, followed by these enzymes in liver and muscle tissues. In gill and brain tissues, the fish fed the Se-NP-supplemented diet showed significantly ($P < 0.05$) higher GSH-Px enzyme activity than with the control diet.

3.5. Serum growth hormone levels

GH concentration of *T. putitora* was significantly ($P < 0.05$) increased with dietary Se-NP supplementation. GH results are shown in Figure 2. The GH concentration in response to the Se-NP-enriched diet showed an increasing trend, and significantly ($P < 0.05$) higher serum GH level was observed in the group of fish fed the Se-NP-supplemented diet. The lowest serum GH level was observed in the group of fish reared on the basal diet.

4. Discussion

When supplied in feed at an optimal level, selenium has many beneficial effects on the physiological and biochemical health of animals, including fish; however, at a high level, it can become toxic and produce harmful effects (Hamilton, 2004; Jaramillo and Gatlin, 2004; Raza,

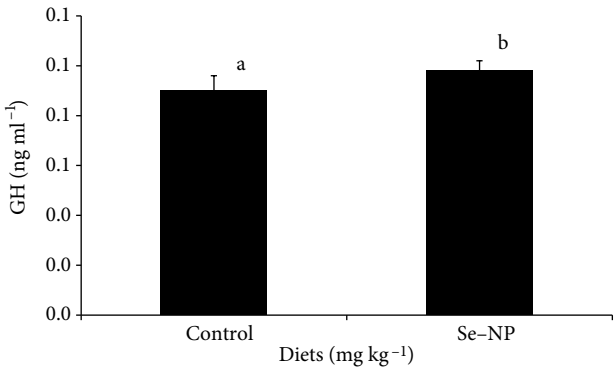

Figure 2. Serum growth hormone (ng mL^{-1}) levels of juvenile *T. putitora* after dietary Se-NP supplementation for 70 days.

Table 3. Effects of dietary Se-NPs on total protein content in liver and muscle tissues of juvenile *T. putitora* after 70 days of supplementation.

Parameter/diets	Total protein content (mg g^{-1})	
	Liver	Muscle
Control	6.47 ± 0.35^a	8.54 ± 0.23^a
Se-NPs	14.92 ± 0.20^b	27.91 ± 0.38^b

Data are represented as mean ± SE (n = 30). Means followed by a different letter within a column are significantly different ($P < 0.05$) (ANOVA followed by LSD test).

Table 4. Effects of dietary Se-NPs on GSH-Px enzyme activity in liver, muscle, gill, and brain tissues of juvenile *T. putitora* after 70 days of supplementation.

Parameter/diets	GSH-Px (μmol min^{-1} mg^{-1} protein) enzyme activity			
	Liver	Muscle	Gills	Brain
Control	1.22 ± 0.07^a	1.10 ± 0.01^a	0.46 ± 0.03^a	1.10 ± 0.04^a
Se-Np	2.20 ± 0.01^b	1.72 ± 0.01^b	1.14 ± 0.02^b	1.97 ± 0.01^b

Data are represented as mean ± SE (n = 30). Means followed by a different letter within a column are significantly different ($P < 0.05$) (ANOVA followed by LSD test).

2012; Jamil, 2013). Raza (2012) observed that selenium at a rate of 1.5 mg kg^{-1} in the diet is optimum for improving the growth performance of *T. putitora* fish. Jamil (2013), working on the comparative study of inorganic selenium and the nanoform of selenium, observed that the nanoform of selenium is much better than inorganic selenium and documented that nanoform selenium at the rate of 0.68 mg kg^{-1} is optimal for the growth performance of *T. putitora*.

Selenium is a strong antioxidant micronutrient; this strong antioxidant property of Se might provide stability and integrity of cells inside the animal's body and protect them from hemolysis (Ong and Packer, 1992). Several research studies have proved the role of selenium in improving the hematological indices of fish (El-Hammady et al., 2007; Molnár et al., 2011). Hematocrit value was significantly reduced when hybrid tilapia were reared on a diet not supplemented with Se or with a decreased level of Se (El-Hammady et al., 2007). In another study conducted by Molnár et al. (2011), it was noticed that RBC count was higher in the blood of Nile tilapia fed graded levels of an Se-enriched diet (0.5 to 4 mg kg^{-1} diets) as compared to those raised on an Se-free diet. Thus, it has been proved that selenium supplementation in fish feed increases the stability of hematological parameters. In accordance with these results, the present research study has also revealed that a diet supplemented with Se-NPs significantly ($P < 0.05$) increased RBC count, Hb, and Hct% values of *T. putitora* as compared to the basal diet.

It is well known that a deficiency of selenium greatly affects the reproductive potency of lymphocytes by altering the receptors of transferrin (Pighetti et al., 1998). This might be due to the antioxidant ability of selenium. According to Combs and Combs (1986), selenium takes part in antibody production through proliferation and protects the B lymphocytes by activation of GSH-Px. Zhou et al. (2009) documented that a diet supplemented with selenium nanoparticles or selenomethionine significantly increased ($P < 0.05$) GSH-Px activity in plasma and tissue, which in turn beneficially affected white blood cell production in crucian carp (*Carassius auratus gibelio*). Thus, B-lymphocyte production plays a significant role in the improvement of the lysozyme activity of fish and other animals, which in turn increases the animal's immune status (Burk et al., 2003; Kumar et al., 2008). Choi et al. (2013) observed that the immune system in goldfish (*Carassius auratus*) was stimulated with the treatment of selenium at adequate concentrations, i.e. 1–2 mg L^{-1}, through the activation of plasma lysozyme and IgM expression. Similarly, Lee et al. (2009) also observed the positive effect of a selenium-supplemented diet (1.07 mg kg^{-1} feed) on lysozyme activity of juvenile olive flounder (*Paralichthys olivaceus*). We have also noticed in the present study that the lysozyme activity of *T. putitora* significantly ($P < 0.05$) increased with Se-NP supplementation (0.68 mg kg^{-1}). Moreover, Le et al. (2013) documented considerably higher ($P < 0.05$) mean lysozyme enzyme activity in yellowtail kingfish (*Seriola lalandi*) fed a diet supplemented with selenium at the rate of 2 mg kg^{-1} feed as compared to a selenium-free diet.

Selenium is also associated with protein in fish and other vertebrates (Burk and Hill, 1993). According to Tinggi (2008), selenium has been used as a substitute for sulfur in protein synthesis, and the resultant selenoprotein shows better biological activity. The groups of proteins that contain selenium as an integral part of their polypeptide chain are called selenoproteins. They are present in all lineages of life. They are generally few in number; however, the largest series is present in fish, with 30 individual selenoproteins (Castellano et al., 2005). Moreover, Evan (1976) noticed that growth rate depends on the secretion of growth hormone; hence, an increase in growth hormone concentration will in turn enhance the protein content.

Selenium is involved in the formation of several types of selenoproteins, among which GSH-Px is the most important component of the immune system of the body (Rotruck et al., 1973). It provides protection to the cells against oxidant materials by increasing the production of selenoenzymes and selenoproteins (GPx system, thioredoxin reductase (TrxR), selenoprotein P, etc.). Thus, an increased amount of these proteins will show a more protective role and thus enhances the immunity status of fish and other animals (Burk et al., 2003; Kumar et al., 2008), while low concentrations of selenoproteins increase oxidative stress, which then leads to viral infection (Sritunyalucksana et al., 2011). These selenoproteins participate actively in the antioxidant defense system and prevent the harmful effects of reactive oxygen species (Tapiero et al., 2003). They provide protection to cell membranes against oxidative stress by using reduced glutathione, which catalyzes the reduction of peroxides and hydroperoxides (Arteel and Sies, 2001; Rider et al., 2009).

Previous research has proved that a diet enriched with Se-NPs enhanced the activities of GSH-Px (Zhou et al., 2009; Jamil, 2013). Sunde (2001) noticed that Se intake affects the activity of GSH-Px enzyme and selenium deficiency rapidly decreases cellular GSH-Px activity. In accordance with these findings, in the present study a significant increase in GSH-Px activity was observed when *T. putitora* fish were fed a diet supplemented with Se-NPs at a rate of 0.68 mg kg^{-1} compared with the basal diet.

GH is a pluripotent hormone produced by the pituitary gland in teleosts as in other vertebrates (Einarsdóttir et al., 2007). The physiological role of GH as a growth-promoting hormone has been well established (Pérez-Sánchez and Bail, 1999; Cao et al., 2011). Chen et al. (1994)

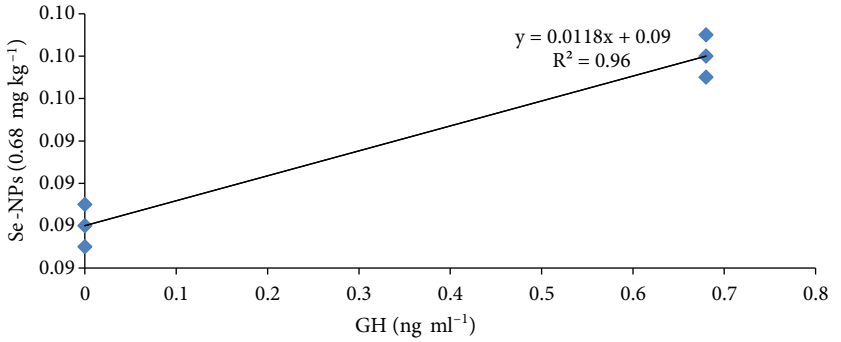

Figure 3. Positive correlation between Se-NPs and serum GH level of *T. putitora*.

observed that GH regulates growth in fishes as well as in other vertebrates. Furthermore, in fish, GH participates in almost all major physiological processes that occur inside the body such as the regulation of ionic and osmotic balance; lipid, protein, and carbohydrate metabolism; skeletal and soft tissue growth; reproduction; and immune function. The latest research studies have showed that GH affects a number of characteristics of behavior such as hunger, foraging behavior, aggression, and escaping from predators, which in turn has environmental significance (Björnsson et al., 2004; Reinecke et al., 2005).

According to Kohrle et al. (2000), selenium is a constituent of the deiodinase enzyme, which is necessary for the conversion of T_4 to the biologically more active T_3. It is well established that thyroid hormones (T_3 and T_4) are required for normal pituitary GH secretion in man and several other mammalian species, as well as fish (Valcavi et al., 1992; Muller et al., 1999). It has been documented that thyroid hormone (T_3) increases the levels of GH messenger RNA in the pituitary cells of teleosts (Moav and McKeown, 1992; Farchi-Pisanty et al., 1995). In another study, it was observed that when thyroxin was injected into female *Oreochromis niloticus*, it greatly increased activin bA production in the developing tissues of its larvae (Mousa, 2004). Similarly, treatment of young rainbow trout (*Oncorhynchus mykiss*) fish with thyroid hormone (T_3) for 1 week increased the steady-state levels of mRNA for growth hormone in the pituitary 3–4 times more than in the control groups (Moav and McKeown, 1992). In vertebrates like rats, administration of a selenium-supplemented diet significantly increased the size of the anterior pituitary gland and the secretion of GH (Evan, 1976). In the present study, we also observed increased plasma GH levels in response to a diet supplemented with Se-NPs (0.68 mg kg^{-1}) in *T. putitora*. The group of fish with higher GH levels also showed a significant increase in weight as compared to the group of fish with low levels of GH; in turn, low GH levels led to reduced growth.

Moreover, in the present study, a close relationship was observed between serum growth hormone level and selenium nanoparticles (Figure 3). Thus, this finding is in strong agreement with that of the study of Collipp et al. (1984), who also observed a significant relationship between growth hormone level and selenium nutritional status and confirmed the view that Se supplementation significantly ($P < 0.05$) affects the serum growth hormone concentration and the subsequent growth performance of fish.

In conclusion, serum GH levels, GSH-Px activity, total protein content, RBC count, Hct%, Hb level count, and lysozyme activity of *T. putitora* showed close association with dietary Se-NP supplementation. Therefore, feeding juvenile *T. putitora* with an Se-NP-supplemented diet at a rate of 0.68 mg kg^{-1} significantly increases ($P < 0.05$) the physiological and biochemical parameters of the fish. The results clearly indicated the positive effects of dietary Se-NPs, thus suggesting that Se-NPs at the rate of 0.68 mg kg^{-1} in the diet are adequate for the improvement of the physiobiochemical health of juvenile *T. putitora*.

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