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# Effects of enriched artemia with selenium nanoparticles on growth, survival and biochemical factors of guppy (*Poecilia reticulata*)

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**Abstract**

This study was carried out to investigate the effects of enriched Artemia with selenium nanoparticles on growth and survival rates and biochemical factors in guppy *Poecilia reticulata* larvae over a six-week period. Guppy larvae with a mean weight of 33.55 mg in three treatments and one control group (each with three replicates) were fed with *Artemia franciscana* enriched with 5, 10, and 50 mg $L^{-1}$ selenium nanoparticles. In terms of growth indices, significant differences were observed among treatments in length increment, weight gain, specific growth rate and survival rate ($p<0.05$). There was no significant difference in condition factor ($p>0.05$). Regarding biochemical factors, significant differences were observed between treatments. Artemia - fed treatments enriched with 5 and 10 mg of selenium nanoparticles (treatment 1 and 2) showed the lowest and in the treatment fed with Artemia - enriched 50 mg nanoparticles (treatment 3) showed the highest levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine phosphokinase and lactate dehydrogenase. The highest total protein content was observed in treatment 2, which was significantly different from the other treatments ($p<0.05$). Overall, if the goal of using selenium-enriched Artemia in fish larval diet is to increase survival and growth rate and improve biochemical indices, the use of selenium nanoparticles at the levels of 5 and 10 mg $L^{-1}$ is appropriate for enriching Artemia to feed the fish larvae.

**Keywords:** Selenium nanoparticles, Guppy, Artemia, Growth, Enzyme

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## Introduction

Mass breeding of larvae is a major problem in aquaculture, and proper nutrition is especially important for the production of healthy larvae with high survival and optimal growth. Under these conditions, larvae can be brought to standard weight in the shortest possible time, along with their reasonable survival and health against stressful conditions (Lavens and Sorgeloos, 1996). Using the Artemia (dry cysts and Nauplius) to feed the aquarium fish larvae such as guppies, improves survival and growth (Lim *et al.*, 2002). *Artemia franciscana* cyst has high hatching percentage, small size, high nutritional value, high price, and high global demand. Compared to cladoceran, such as Moina, Artemia nauplius significantly improves growth and survival in guppy (Lim *et al*., 2002). In fish and shrimp hatcheries, the larval feeding performance depends on the characteristics of Artemia species as well as the feeding behavior of fish and shrimp (MacKenzie *et al.*, 1990; Lim *et al*., 2002, 2003). Artemia can also utilize as a carrier for certain nutrients such as long-chain unsaturated fatty acids, amino acids, vitamins and other substances such as drugs, vaccines, hormones and pigments (Agh and Sorgeloos, 2005). These compounds could be transferred to the predator and improve larval quality, increase survival and resistance to environmental stresses and various diseases; this is called biological encapsulation or enrichment (Browne and Wanigasekera, 2000).

Selenium is an essential micronutrient. The most well-known biological role of selenium is its presence as a functional component in the structure of selenoenzymes such as glutathione peroxidase and reductase. Selenium also participates in the structure of proteins as selenocysteine and prevents oxidative damage to the body tissues and plays an important role in the antioxidant defense system, regulating thyroid hormone metabolism and cell growth (Patching and Gardiner, 1999; Eisler, 2000). With the advent of nanotechnology, the element of nano selenium has shown new and different characteristics than the other sources of selenium and has recently attracted much attention due to its high bioavailability and low toxicity because these nanoparticles have new features including high specificity, high activity level, numerous active surface centers, high catalytic efficiency, and strong adsorption ability and low toxicity (Pelyhe and Mezes, 2013). Moreover, the use of selenium nanoparticles reduces the risk of elemental toxicity. Selenium nanoparticles have only 1.7% toxicity compare to selenium compounds. The size of selenium nanoparticles has a very important effect on the biological activity of this element. The high biochemical activity of these particles compared to standard selenium compounds is remarkable as 5–200 nm selenium directly contributes to removal of free radicals in the

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laboratory environment (Albrecht *et al*., 2006).

Many experiments and studies have been done to study the effects of different forms of selenium including selenium nanoparticles on rainbow trout, common carp, Malabaris grouper, red drum, and yellow catfish (Ashouri *et al.*, 2015; Hu *et al*., 2016; Juhasz *et al*., 2017; Nazari, 2017; Safabakhsh, 2020). The results of these studies confirm the positive role of selenium in the diet on biochemical and biological parameters in aquatic animals.

Guppy is one of the most beautiful ornamental fish and highly resistant to changes in physicochemical properties. Guppy is ease to reproduce (Gideon *et al*., 2006) and is used to control the eggs and newborns of insects, especially malaria. The same benefits of guppy have made it as a useful species in laboratory research.

According to the above, in this study, the effect of selenium on biochemical parameters, growth and survival rates of guppy larvae were investigated using selenium nanoparticles enriched Artemia.

## Materials and methods

Implementation and analysis of the indices of the present study were carried out for six weeks at Khatam Al-Anbia University of Technology, Behbahan, Iran.

*Pilot system management*

In this study, 4 aquaria (40×30×60 cm) which were divided into 3 equal sections using glass blades were used for maintenance and culture of guppy larvae. For this experiment, 350 10-day-old guppy larvae were purchased from Shiraz Agricultural Jihad Research Center and transferred by oxygenated plastic bags to the Behbahan Khatam Al-Anbia University of Technology. To adapt to the rearing conditions, the fish were kept in an 80-liter aquarium for 72 hours. After the adaptation period, 20 guppy's larvae with an average weight of and length of 33.55 mg and 1.5 mm were randomly distributed to each aquarium. During the fish maintenance period, the water was siphoned to 35 percent of the volume in each aquarium every three days and the water needed to replace was provided from a 355-liter tank that was dechlorinated by full-time aeration.

*Proliferation and enrichment of Artemia franciscana with selenium nanoparticles*

Artemia hatching was performed according to Lavens and Sorgeloos (1996). One-liter conical containers (inside a 72-liter aquarium) were used for hatching *Artemia franciscana*. The required illumination (1800 lux) provided by using a fluorescent lamp for 24 hours and the required temperature (35°C) supplied via inserting the conical containers into a water bath equipped with a 100 W electric heater. The saltwater needed for Artemia cultivation was provided by adding 35 g of sodium chloride without iodine to one liter of distilled water. For

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Artemia cultivation, 2 grams of Artemia cyst was added into a one-liter container and after 24 hours, the nauplii were collected by siphoning. They were then used to prepare the desired density and enrichment during the experiment. Selenium nanoparticles were obtained from Pishgaman Nanomaterials Co (Mashhad, Iran), with a purity of 99% and the average particle size of 35 nm in solution and after preparing the desired doses (According to Ziaei-nejad *et al*., 2015), the nauplii were exposed to the desired amounts (see experimental treatments and feeding section) of nanoparticles for 24 hours (Juhasz *et al*., 2017). Enriched nauplii were harvested by siphoning in a 300 µm mesh and they were kept in a refrigerator at 4° C with gentle aeration until use (Juhasz *et al*., 2017).

*Experimental treatments and feeding*

This study was performed as a completely randomized design with three experimental treatments containing Artemia enriched with 5, 10 and 50 mg selenium nanoparticles per liter of water and one control treatment. The fish were fed with enriched Artemia three times daily at 8, 14 and 20. Siphoning was also done once every two days and food residues and feces of larvae and other wastes and losses were removed from the aquariums and their amounts were recorded.

*Sampling*

For this purpose, feeding was stopped 24 h before sampling. Weight of all fish in each replicate was measured individually after anesthesia using the digital balance. Fish of each replicate were thoroughly washed with biological serum and were homogenized in 9 volumes of 0.05 M Tris (hydroxymethyl) amino-methane hydrochloride buffer, pH 7.8, with 0.011 M CaCl2 in a glass homogenizer (Ziaei-nejad *et al*., 2006) and were centrifuged at 7000 rpm for 10 minutes at 4°C. The supernatant of each sample was assayed in triplicate.

*Analysis of growth, survival and blood biochemical parameters*

The following formulas were used to analyze growth indices (De Silva and Anderson, 1995; Helland *et al*., 1996):

Survival percentage = (Final Number of Larvae/ Primary Number of Larvae) ×100
Length increment= Final Total Length (mm) - Primary Total Length (mm)
Weight gain = final weight (g) - initial weight (g)
Specific growth rate = [(Ln Final Weight (g) - Ln Initial Weight (g)) / Number of days of trial period] × 100
Condition factor = [Body Weight (g) / Body Length (cm)3] × 100

All biochemical parameters were determined using standard biochemical reagents (Pars Azmun Company, Tehran, Iran) and a UV-visible

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spectrophotometer. The total plasma protein was measured at 540 nm by the Biuret reaction. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined by NADPH consumption and its conversion to NAD+ at 340 nm. Lactate dehydrogenase (LDH) was determined based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) based on converting nitrophenolphosphate into nitrophenol and phosphate at 405 nm, creatinine phosphokinase (CPK) based on the conversion of creatinine phosphate into creatinine at 340 nm and based on optical density (OD) absorption and the formula presented in the kits' manual (Moss and Henderson, 1999).

*Statistical analysis*

Statistical analysis of data was performed using SPSS (version 22, SPSS IBM) software and charts were drawn using the software. Data normality were tested using the Kolmogorov-Smirnov and then were analyzed with one-way ANOVA test. The means were compared with Duncan's multiple range tests with a 95% confidence level.

## Results

*Absorption rate of selenium nanoparticles in Artemia and guppy larvae*

According to the results, the highest uptake of selenium by Artemia and guppy larvae was observed in 50 mg $L^{-1}$ selenium nanoparticles which showed a significant difference with the other doses. Also, the selenium nanoparticle uptake in 5 and 10 mg $L^{-1}$ treatments showed a significant difference compared to the control treatment (Fig. 1).

*Growth and survival factors*

According to the results of this study, survival rate was 100% not only in treatments 5 and 10 mg/L nano-selenium but also in the control group, while mortality was observed in treatment 50 mg $L^{-1}$ nano-selenium which showed a significant difference with the other treatments. The highest length gain was observed in treatment 10 mg $L^{-1}$ nano-selenium and showed significant differences compared to the others. Also, the lowest weight gain and specific growth rate were related to treatment 50 mg $L^{-1}$ nano-selenium and the highest were found in treatment 10 mg $L^{-1}$ nano-selenium. Regarding the condition factor, no significant differences were observed between treatments (Fig. 2).

*Biochemical parameters*

The highest amount of lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and creatinine phosphokinase enzymes were observed in treatment 50 mg $L^{-1}$ nano-selenium and the lowest were found in treatment 10 mg $L^{-1}$ nano-selenium and significant differences were observed among treatments.

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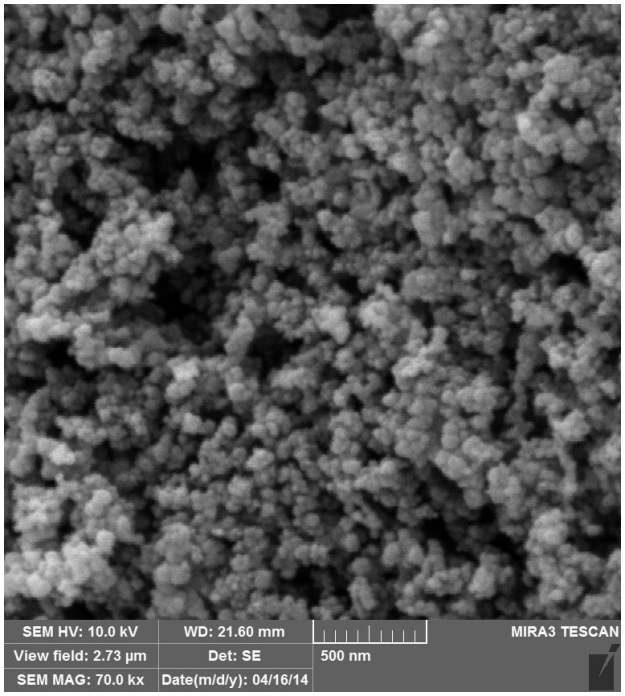



**Figure 1: Electron microscopy image of nano-Selenium that used in the research.**

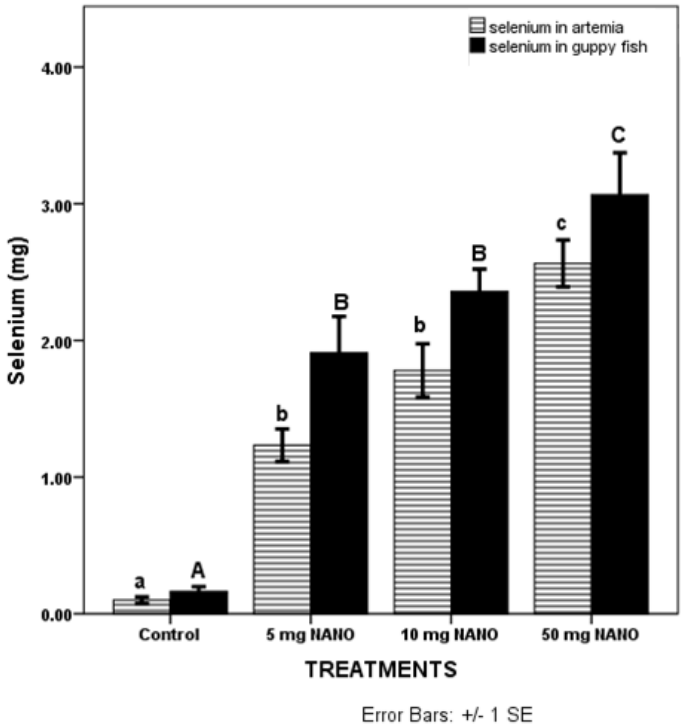



**Figure 2: Selenium accumulation in Artemia nauplius and guppy larvae carcass at different treatments (Mean±S.E.). Means with the same superscript are not significantly different (*p*>0.05).**

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Total protein content in treatment 10 mg $L^{-1}$ nano-selenium was significantly different from the other treatments and control group (Figs. 3 and 4).

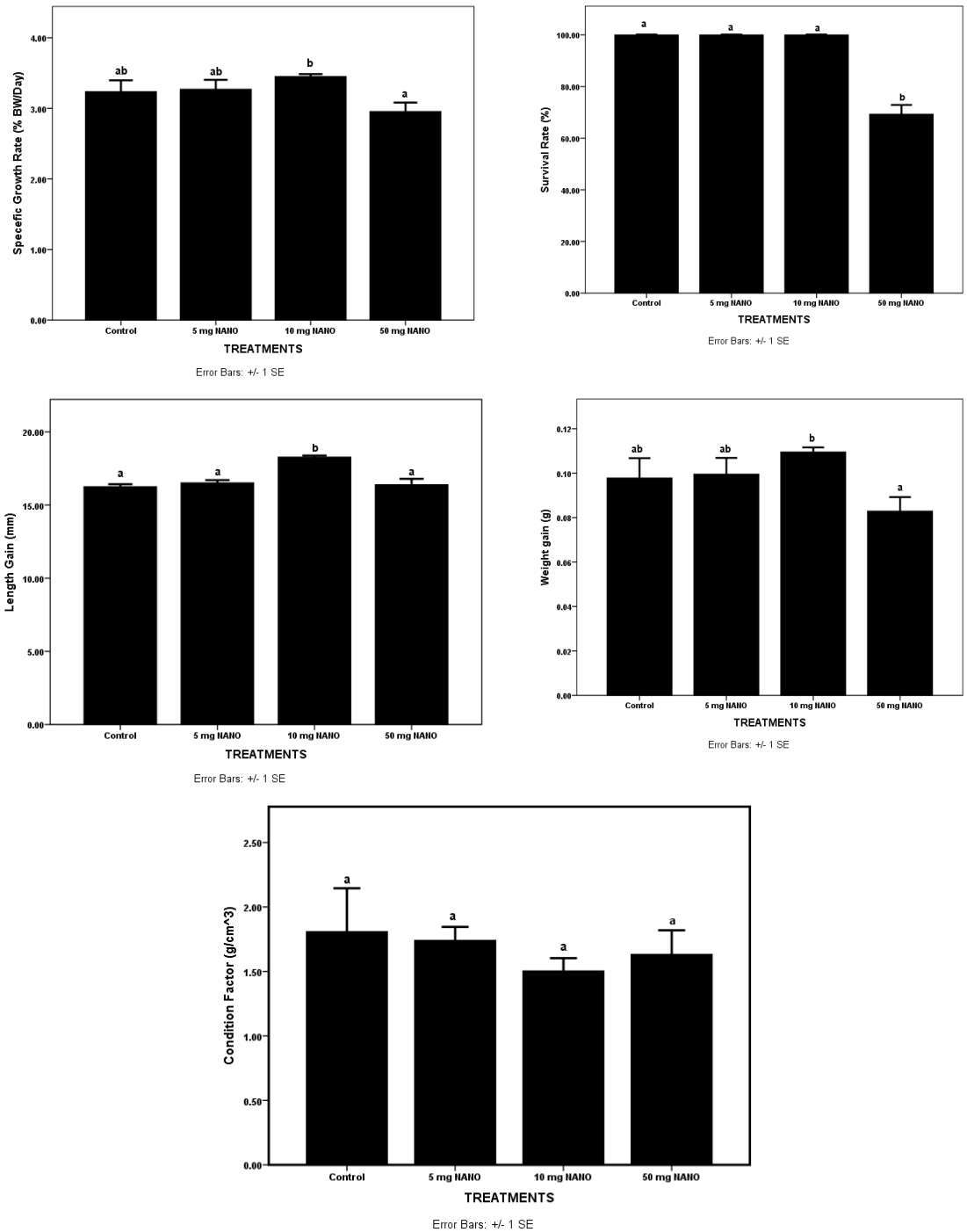



**Figure 3: Growth factors and survival rate of guppy fed with different levels of selenium nanoparticles (Mean±S.E). Means with the same superscript are not significantly different ($p$>0.05).**

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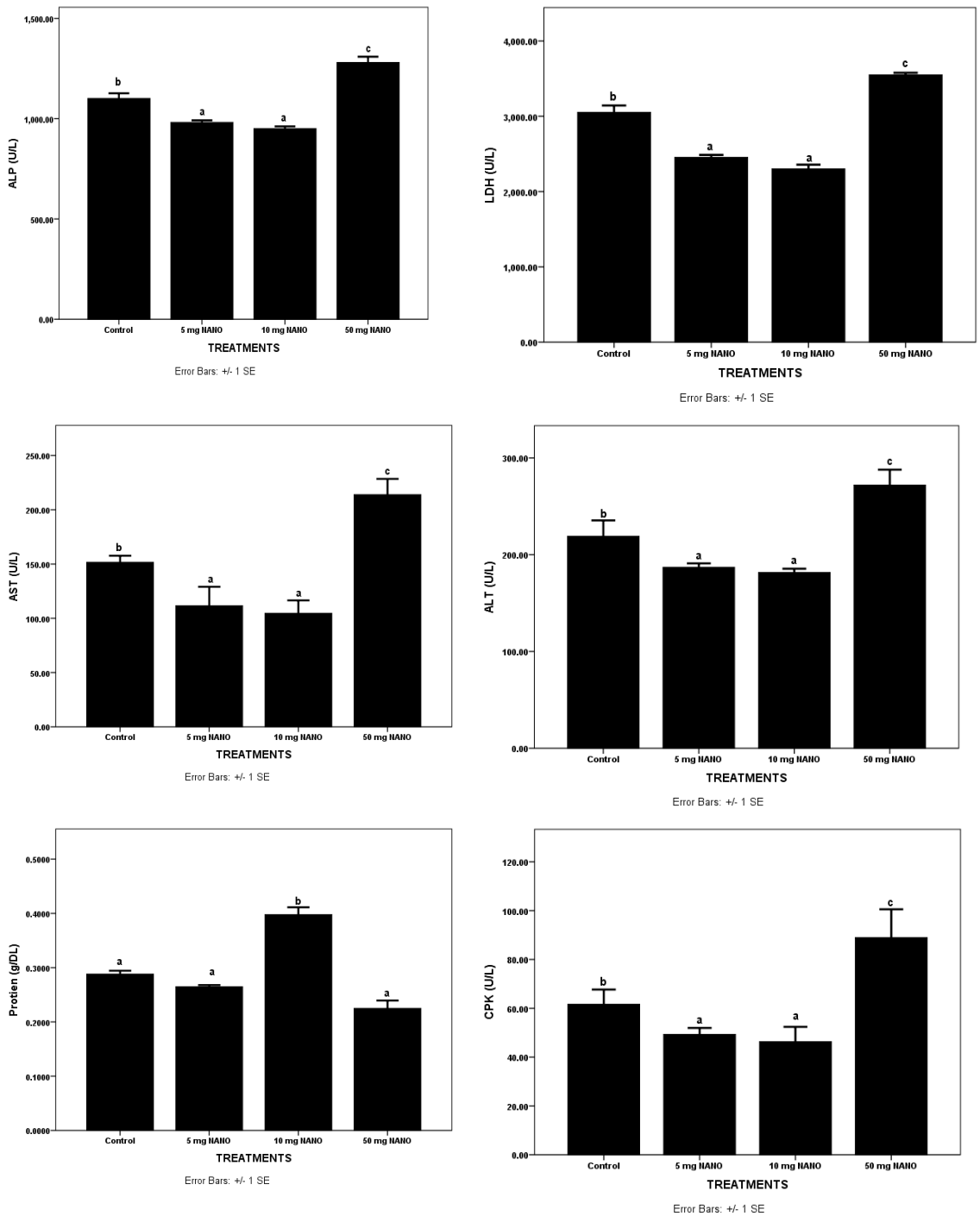



**Figure 4: Biochemical parameters of guppy fed with different levels of selenium nanoparticles (Mean±S.E). Means with the same superscript are not significantly different ($p$>0.05).**

## Discussion

The results of selenium nanoparticle uptake in the treatments showed that the amount of selenium in the fish carcass is strongly affected by the amount of nanoparticle absorbed by the Artemia used in fish nutrition. The fish fed 50 mg $L^{-1}$ nano-selenium enriched Artemia had the highest selenium accumulation in the carcass. In treatments 5 mg $L^{-1}$

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and 10 mg $L^{-1}$, there was no significant difference between the levels of selenium accumulation in the fish carcass. In a study by Ashouri *et al*. (2015), the increasing uptake of selenium into the tissues and liver of common carp was also reported with increasing levels of selenium nanoparticles. The mechanism of selenium transfer from the gastrointestinal tract and its accumulation in the liver and body tissue is well known. Selenium nanoparticles are absorbed by active transport from the intestinal duodenum and are transported to the liver via the portal system and then accumulate in this tissue (Zhang *et al*., 2001).

It was determined that the length gain increased significantly in fish fed Artemia enriched with 10 mg $L^{-1}$ selenium nanoparticles (treatment 2). This difference may be related to the improvement of growth conditions due to the effect of selenium nanoparticles on growth factors in fish larvae. This improvement in growth factors may be related to the important role of selenium in the antioxidant defense system, regulating thyroid hormone metabolism and cell growth (Patching and Gardiner, 1999; Eisler 2000). Weight gain, specific growth rate and condition factor showed no significant differences between control, 5 mg $L^{-1}$ and 10 mg $L^{-1}$ treatments. This result is in line with the results of Juhasz *et al.* (2017) which found that using enriched-Artemia with selenium nanoparticles at doses of 5 and 10 mg positively affected the growth of *Sciaenops ocellatus* larvae and increased their growth.

The survival rate in fish larvae in treatments 1 and 2 and the control group was 100% but in treatment 3 survival rate was significantly reduced and more than 30% mortality was observed in this treatment. Also, the growth indices in treatment 3 showed a dropping trend compared to the other treatments and the control group. This difference may be due to the high accumulation of selenium nanoparticles in body tissues and their negative effect on growth indices and survival rate in fish larvae. Also, Juhasz *et al*. (2017) reported the death toll in fish fed 50 mg of selenium-enriched Artemia. However, when the amount of selenium consumed exceeds the fish's nutritional requirement, selenium toxicity may also occur (Han *et al*., 2011).

In the guppies fed Artemia enriched with 5 and 10 mg selenium nanoparticles (treatment 1 and 2), the levels of lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, and creatinine phosphatase decreased significantly. Lactate dehydrogenase depletion has been shown to improve the activity of body tissues, especially the heart, liver, red blood cells, kidneys, skeletal muscles, and lungs (Murray *et al*., 2003). Aspartate aminotransferase is present in the cytoplasm and mitochondria of cells, at the time of mild tissue injury, the predominant amount of aspartate aminotransferase is its cytoplasmic type and a small amount

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of mitochondrial aspartate aminotransferase, resulting in severe damage to the tissues. High levels of transaminase can cause symptoms of malnutrition and organ damage, so a decrease in this enzyme indicates an improvement in these factors in the treated fish larvae.

Alkaline phosphatase is an enzyme found in bile duct epithelium, liver cells, and in the intestinal mucosa and kidney. In the liver, this enzyme is found in Kupffer cells. These cells cover the biliary tract. Thus, an increase in alkaline phosphatase activity causes cholestasis, especially jaundice, which is often seen in diseases related to the skeletal system and hepatic disorders such as hyperparathyroidism and osteoporosis, as well as intra and extrahepatic biliary obstruction. This study showed that the amount of this enzyme was significantly reduced in fish fed with Artemia enriched with 5 and 10 mg of selenium nanoparticles.

Alanine aminotransferase is an enzyme that most commonly found in liver and kidney cells. Much smaller amounts of this enzyme are also found in the heart and muscles. In healthy animals, alanine aminotransferase levels are low in the blood. When the liver is damaged, alanine aminotransferase is released into the bloodstream before the more severe symptoms of a liver injury such as jaundice occur. This makes alanine aminotransferase as a useful test for the diagnosis of liver injury. Alanine aminotransferase is often combined with aspartate aminotransferase either as part of a liver panel for screening or to aid in the diagnosis of liver disorders. Alanine aminotransferase and aspartate aminotransferase are considered to be the most important indicators to detect liver injury, although alanine aminotransferase is more specific than the aspartate aminotransferase. The value of alanine aminotransferase is often compared with the results of other tests such as alkaline phosphatase, a protein that is used to help determine the type of liver disease (Hao *et al*., 2014; Ashouri *et al*., 2015). Alanine aminotransferase is used to monitor and evaluate the effectiveness of liver disease treatment (Bhattacharya *et al*., 2008). The significant increase in the levels of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase enzymes can be considered as responses to stressful conditions in animals (Bhattacharya *et al*., 2008). A study by Ashouri *et al*. (2015) showed that the use of 1 mg selenium nanoparticles had a positive effect on growth indices in common carp, but with increasing levels of selenium nanoparticles in the diet, levels of aspartate aminotransferase, alkaline phosphatase, and alaninephenase enzymes were increased. It can be due to the toxic effects of selenium nanoparticles (Han *et al*., 2011).

Abdel-Tawwab *et al*. (2007) conducted a study on African catfish and found that 0.5 g of organic selenium per kg diet could increase the

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activity of aspartate aminotransferase and alanine aminotransferase. Xie *et al*. (1995) reported that grass carp fed with sodium selenium hydrogen showed the reduction of ALT, AST, and LDH release from hepatocytes, which reduced cellular damage as well as decreased activity of these enzymes in the blood. In addition, Ozardah *et al*. (2004) reported that levels of AST and ALT were decreased in fish treated with carbon tetrachloride following selenium injection, which showed that selenium reduced liver injury. In this experiment, the levels of these enzymes showed a significant decrease in treatments 1 and 2, and it can be concluded that hepatic parameters were in good condition. These enzymes depletion in this study can be due to the strengthening of tissue cells and their protection when fish were fed Artemia enriched with 5 and 10 mg nano-selenium, as previously reported by Hao *et al*. (2014).

Creatine phosphokinase is an enzyme found in skeletal muscle (Grzyb and Skorkowski, 2005), heart (Haagensen *et al*., 2008), gill (Gong *et al*., 2004), and brain (Dickmeis *et al*., 2001). This enzyme is one of the factors affecting the immune system that is involved in producing energy in anaerobic conditions. Increased creatine phosphokinase is due to vigorous physical activity and increases in the blood, indicating tissue damage, heart failure, muscular dystrophy, renal failure, seizures, and inflammatory conditions. In other words, increasing the activity level of this enzyme can be a clinical marker in diagnosis of damage to muscle fibers or other tissues (Ozawa *et al*., 1999).

Considering that in this study the level of this enzyme was significantly decreased in treatments 1 and 2 compared to control, it can be concluded that the immune system in fish larvae of these treatments improved and selenium nanoparticles were able to strengthen the immune system in these fish. In treatment 3, this enzyme significantly increased and showed a difference with other treatments and control.

The amount of total protein in treatment 2 was significantly higher than the other treatments. The level of total protein in treatment 1 and treatment 3 showed a significant difference compared to the control group but these differences were not significant for the other one. Decreases in total protein concentration are common in many diseases and may be due to impaired synthesis, decreased protein uptake or loss (Bernet *et al*., 2001).

According to the results of this study, it can be concluded that using Artemia enriched with 5 and 10 mg selenium nanoparticles in fish larval feeding can improve biochemical parameters and increase growth indices. Larvae of fish fed Artemia enriched with 5 and 10 mg of selenium nanoparticles showed the lowest levels of AST, ALT, LDH, ALP and CPK, which indicates the positive effect of this nanoparticle on biochemical factors. Therefore, it is

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suggested that feeding guppy with selenium nanoparticles of 10 to 5 mg can improve growth indices and blood biochemical parameters.

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