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Effects of Selenium Nanoparticles Supplemented Feed on Biochemical Indices, Growth and Survival of Yellow-Tail Seabream (*Acanthopagrus latus*)

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ABSTRACT

The aim of this study was to evaluate the effects of feed supplemented with different levels of Selenium Nanoparticles (SeN) on growth, survival, and biochemical indices of *Acanthopagrus latus*. Yellow-tail seabream fish with a mean weight of 52±3 gwasfed with four experimental diets containing 0, 0.5, 1, and 2 mg kg^{-1} SeN for 8 weeks. At the end of the experiment, the fish fed 0.5 mg kg^{-1} SeN showed a significant improvement of weight gain compared to the control (P< 0.05). Specific growth rate, feed conversion ratio, and survival were not significantly different between treatments (P> 0.05). The lowest activity of alkaline phosphatase, alanine amino transferase, creatinine phosphokinase and cholesterol were observed in 0.5 mg kg^{-1} SeN treatment, which was significantly lower than the control (P< 0.05). Although aspartate amino transferase, lactate dehydrogenase, triglyceride and creatinine were not significantly different among experimental treatments (P> 0.05), total protein and albumin levels were significantly increased in the 1 and 2 mg kg^{-1} SeN treatments compared to the control group. The highest level of globulin was observed in the 2 mg kg^{-1} SeN treatment. According to the results, the addition of 0.5 mg kg^{-1} SeN to fish feed could improve growth and biochemical parameters.

Keywords: Aquaculture, Blood metabolites, Feed supplementation, Fishery product, Nanotechnology.

INTRODUCTION

The development of nanotechnology has created widespread applications for nanomaterials in the nutritional and medical sciences due to their new features compared to other materials (Albrecht *et al*., 2006) and, with the increasing development of nanotechnology, more and more trace elements are being transformed into nanoparticles every day. In addition, nanoparticles are used in aquaculture for aquatic animal growth improvement, as well as fishery product processing (Ziaei-nejad *et al*., 2015; Ziaei-nejad *et al*., 2018).

Selenium is a trace element and at the same time an essential nutrient for both human and animals (Kacjan Maršič *et al*., 2019) and plays an important role in the antioxidant defense system, regulating thyroid hormone metabolism and cell growth (Eisler, 2000). In addition, selenium, as a component of selenoproteins, has a metabolic activity to prevent oxidative tissue damage (Yan and Johnson, 2011). Selenium also plays an important role in glutathione peroxidase enzyme activation (Lin and Shiau, 2005) and enhances the cellular antioxidant system. This enzyme increases resistance to oxidative damage that destroys fatty acids by reducing the amount of

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hydrogen and lipid peroxides in different cells. The level of activity of this enzyme in the liver or plasma indicates the level of selenium (Lin and Shiau, 2005).

Selenium Nanoparticles (SeN) have been used in the aquaculture industry in recent years due to their high availability and reduced toxic effects (Zhang *et al*., 2001); and various studies on the nutritional effects of these particles are done. Impact of different sources of selenium on Goldfish (*Carassius auratus*) (Zhou *et al*., 2009), Malabar grouper (*Epinephelus malabaricus*) (Lin and Shiau, 2005), largemouth bass (*Micropterus salmoide*) (Zhu *et al*., 2012) and Atlantic salmon (*Salmo salar*) (Lorentzen *et al*., 1994) have been studied. Previous researches on organic and inorganic selenium compounds and their results indicate that different species of fish need different chemical forms of selenium (Zhou *et al*., 2009; Ashouri *et al*., 2015). However, few studies have been conducted on the effects of SeN on fish growth, nutrition, survival, and biochemical parameters. Therefore, the present study was conducted with the aim of using SeN to improve the growth and biochemical status of yellow-tail seabream (*Acanthopagrus latus*).

MATERIALS AND METHODS

Experimental Treatments

The research was conducted at the Marine Fishes Research Station of Imam Khomeini Port. The experiment was conducted in a completely randomized design with four treatments and three replications, for 8 weeks. For this purpose, 300-liter tanks with a storage density of 15 yellow-tail seabream (*A. latus*, average weight of 52±3 g and initial length of 12±1 cm) per tank were used. Treatments included application of different amounts (mg kg^{-1}) of nano selenium: T1 (0), T2 (0.5), T3 (1), and T4 (2) (Ashouri *et al*., 2015). Water quality was monitored every day for the following parameters: Temperature 28°C, salinity 30%, and pH 8–8.5.

Selenium Nanoparticles

The used SeN (particle size ranging from 30 to 45 nm) with purity of 99.95% were purchased from Iranian Pishgaman Nano Materials Company (Mashhad, Iran) (Figure 1).

Preparation of Diets and Feeding

The feed was purchased from Faradaneh Co. (Iran). Proximate composition of the diet is shown in Table 1. In each treatment, the calculated amount of SeN was first dissolved in 9 volumes of sterilized distilled water and then sprayed separately on the feed. In order to equalize the conditions for the control treatment (no SeN), an equal amount of distilled water was sprayed on the feed. The prepared feeds were then dried for 3 hours at 45°C. In order to protect the feed and prevent the leaching of nanoparticles into the water, the feeds were covered with a layer of bovine gelatin (10%) (Ramsden *et al*., 2009). Selenium is present in fish feed ingredients due to the presence of Se in marine fisheries by-products used by the aquaculture fish industry. For that reason, the final actual concentration of Se in each experimental diet was determined by an atomic absorption spectrophotometer as described by Elia *et al*. (2011) (Table 2). During the experiment, the feeding was done 2 times daily at 10 am and 5 pm.

Growth and Nutritional Indicators

At the end of the study, all treatments were bio-assayed for growth, survival and nutritional parameters according to the following formulas (Buyukcapar *et al*., 2011):

Weight Gain (WG)= Final body weight (g)-Initial body weight (g)

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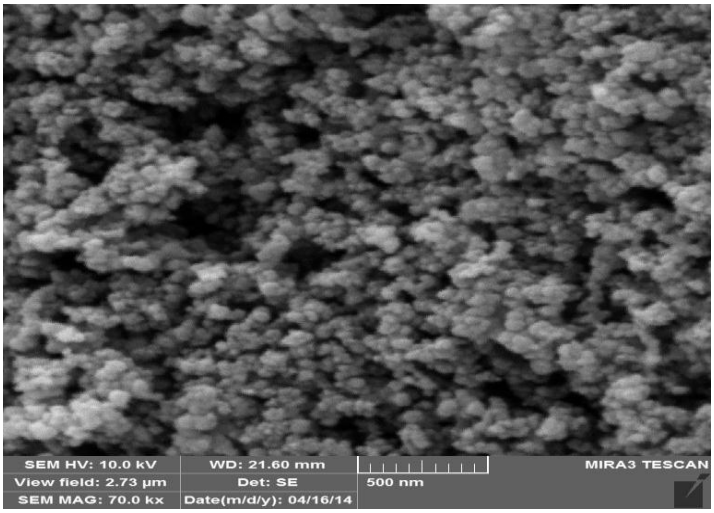

Figure 1. Scanning Electron Microscope (SEM) image of selenium nanoparticles.

Table 1. Proximate composition of the basal experimental diet (mean±SE, n= 3).

Proximate composition	%
Crude Protein	50.0±2.0
Crude fat	11.0±1.0
Crude fiber	1.5±0.5
Moisture	6.0±1.0
Ash	9.0±4.0

Table 2. The final actual concentration of Se (mg kg^{-1}) in experimental diets (mean±SE, n= 3).

Treatments (mg nano-Se kg^{-1})	Actual Se concentration (mg nano-Se kg^{-1})
0 (control)	0.35 ± 0.07
0.5	0.85 ± 0.10
1	1.32 ± 0.14
2	2.38 ± 0.22

Specific Growth Rate (SGR) = 100×[(Ln Final weight-Ln Primary weight)]/Number of culture days

Survival percentage (SR) = 100×(Final number of fish/Initial number of fish)

Feed conversion Rate (FCR) = Feed consumed (g)/Body weight gain (g)

Blood Collection for Biochemical Studies

After anesthesia, blood samples were taken from 5 fish from each reservoir with phenoxyethanol (500 µL L^{-1}). For serum isolation, blood samples were first incubated at laboratory temperature for one hour without shaking; and then centrifuged at 3,000 rpm for 10 minutes (Acerete *et al*., 2004). Sera were stored at -80°C until biochemical tests were performed. All blood biochemical indices were assayed with Pars Test kits (Pars Test Co., Iran) using UV/Vis spectrophotometer (UNICO, Model 2100, USA).

Total serum protein was determined according to Lowry *et al*. (1951) using the standard protein kit (Zistchem Diagnostics,

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Iran). Soluble extraction method was used to measure albumin (Annino and Giese, 1976). Globulin levels were also calculated from the difference between total protein and albumin.

Aspartate aminotransferase (AST) activity was measured based on NADPH consumption and its conversion to NAD^+. The optical absorption intensity was measured at 340 nm in 3 minutes (Moss and Henderson, 1999).

Alkaline Phosphatase (ALP) activity was measured by converting nitrophenylphosphate to nitrofenol and phosphate. The optical absorption intensity was measured at 405 nm for 3 minutes (Moss and Henderson, 1999).

The activity of Alanine aminotransferase (ALT) was measured based on NADPH consumption and its conversion to NAD^+. Optical absorption intensity was measured at 340 nm over a period of 3 minutes (Moss and Henderson, 1999).

Lactate Dehydrogenase (LDH) activity was measured by converting pyruvate to lactate. Optical absorption intensity was measured at 340 nm over a period of 3 minutes (Moss and Henderson, 1999).

Cholesterol and triglyceride concentrations were determined by CHOD-PAP/endpoint and GPO-PAP/endpoint, respectively. Optical absorption of the samples was read at 500 and 520 nm respectively (Thomas, 1998).

Creatinine Phosphokinase (CPK) was assayed by enzymatic calometric method. In this method, creatinine is colored by alkaline picrate. Optical absorption rates were measured at 500 nm (Johnson *et al*., 1999).

Plasma creatinine was measured according to the JAFFE method (Newman and Price, 1999). Optical absorption at 492 nm was measured in one minute.

STATISTICAL ANALYSIS

A completely randomized design was used in the experiment. Normality of data was tested using the Anderson–Darling test (MINITAB 13.31 software). Statistical comparison of treatments was performed using one-way analysis of variance (ANOVA) at 95% level. All data means were compared using Duncan's multiple range test (SPSS software). A significance level of $P< 0.05$ was used for all tests. Data are reported as means±standard errors.

RESULTS

Growth and Nutrition Indicators

The highest growth rate was observed in fish fed 0.5 mg kg^{-1} SeN supplemented diet, which was significantly different from the control group ($P< 0.05$) but was not significantly different from the other treatments ($P> 0.05$) (Figure2).

There was no significant difference ($P> 0.05$) in either SGR or FCR between the control and the treatments that received SeN. (Figure3&4).

No mortality was observed in all treatments and therefor no significant differences were observed between treatments (Figure 5).

Biochemical Indices

Administration of the SeN at 0.5 mg kg^{-1} significantly decreased ALP and ALT compared to the control, but AST and LDH activites in yellow-tail seabream were not significantly different among SeN treatments and the control (Table 3).

The results showed that CPK enzyme significantly decreased in 0.5 mg kg^{-1} treatment compared to 1 and 2 mg kg^{-1} ($P< 0.05$). However, no significant difference was observed between treatments under selenium nanoparticles and control ($P< 0.05$).

Results showed no significant difference in triglyceride levels between the control and the other groups ($P< 0.05$). In terms of cholesterol, no significant difference was recorded between control and 1 and 2 mg kg^{-1}, but a

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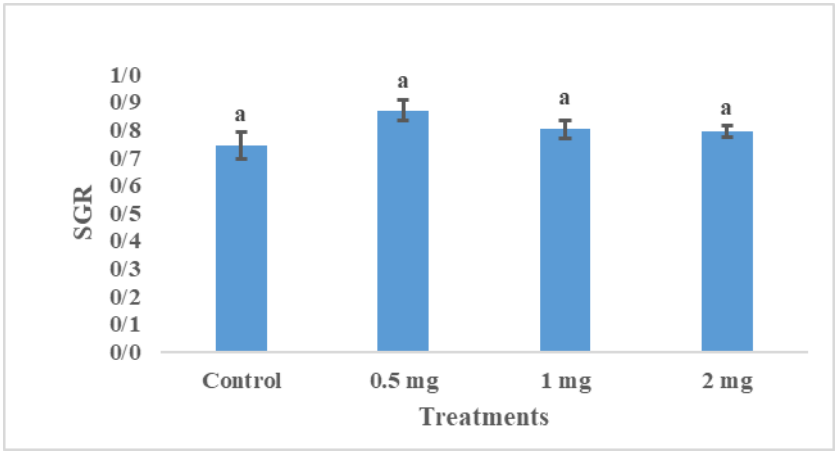

Figure 3. Influence of different levels of SeN dietary supplementation on specific growth coefficient of yellow-tail seabream (*Acanthopagrus latus*). Means with the same superscript are not significantly different (P> 0.05).

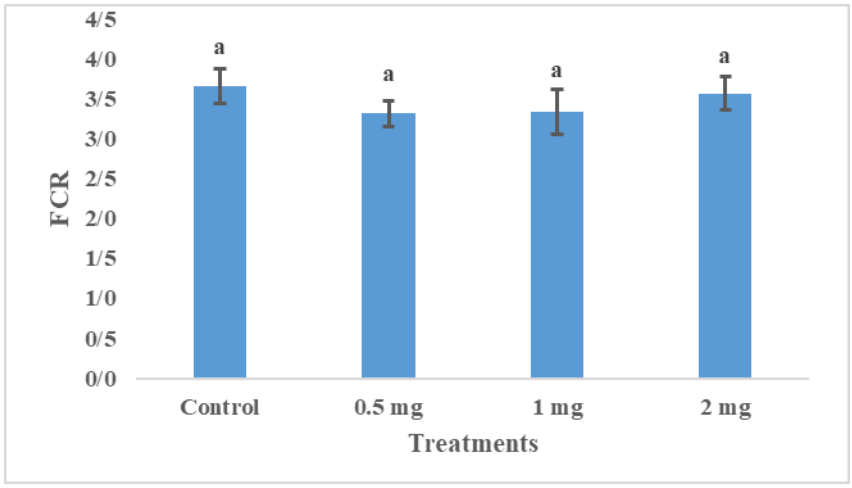

Figure 4. Influence of different levels of SeN dietary supplementation on FCR of yellow-tail seabream (*Acanthopagrus latus*). Means with the same superscript are not significantly different (P> 0.05).

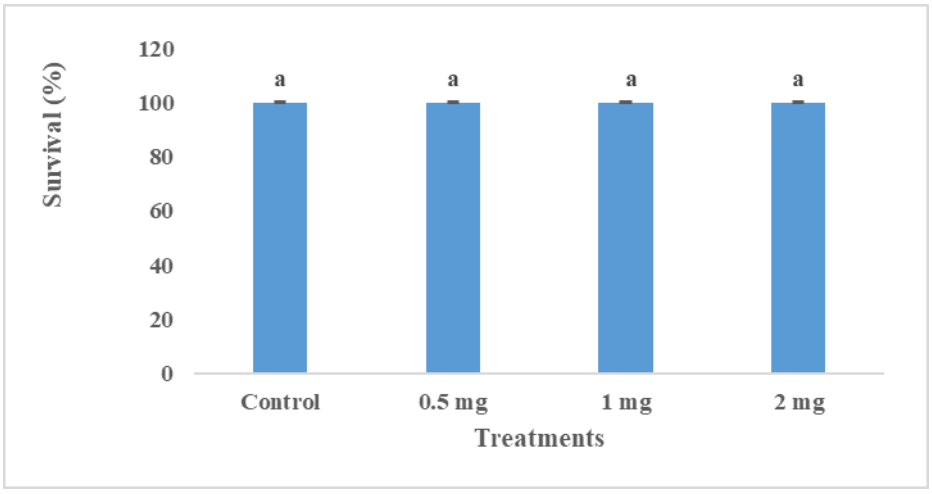

Figure 5. Influence of different levels of SeN dietary supplementation on survival of yellow-tail seabream (*Acanthopagrus latus*). Means with the same superscript are not significantly different (P> 0.05).

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Table 3. Influence of different levels of selenium nanoparticles dietary supplementation on blood biochemical parameters of yellow-tail seabream (*Acanthopagrus latus*) (n= 3).

Biochemical parameters	Treatments [a]			
	0 mg (Control)	0.5 mg	1 mg	2 mg
ALP	912.75±15.10[b]	838.31±26.30[a]	891.43±17.90[ab]	873.96±24.39[ab]
ALT	12.73±1.81[b]	6.88±2.67[a]	14.52±1.33[b]	23.01±1.12[c]
AST	118.22±24.62[a]	105.70±26.19[a]	155.16±12.86[a]	166.60±10.20[a]
LDH	3532.90±148.80[a]	3149.35±304.21[a]	3477.12±148.06[a]	3740.36±216.22[a]
CPK	1474.71±79.01[b]	1011.93±198.94[a]	1690.46±224.05[b]	2005.03±194.19[b]
Triglyceride	174.08±8.43[a]	164.23±12.45[a]	149.41±5.09[a]	153.25±6.22[a]
Cholesterol	155.01±6.75[b]	132.31±4.54[a]	135.68±2.18[ab]	146.35±9.98[ab]
Creatinine	0.087±0.019[a]	0.059±0.004[a]	0.055±0.004[a]	0.07±0.015[a]
Protein	2.07±0.14[a]	2.24±0.08[ab]	2.62±0.10[b]	2.69±0.10[b]
Albumin	1.44±0.23[a]	1.64±0.12[ab]	1.81±0.13[b]	1.90±0.19[b]
Globulin	0.51±0.17 [a]	0.61±0.10 [a]	0.85±0.15 [a]	0.90±0.10 [a]

[a] Means with the same superscript are not significantly different (P> 0.05). Control: No Selenium Nanoparticles (SeN) provided; 0.5 mg: 0.5 mg Se N added per kg of diet; 1 mg: 1 mg SeN kg^{-1} of diet; 2 mg: 2 mg SeN kg^{-1} of diet.

significant decrease was observed in 0.5 mg kg^{-1} treatment compared to the control (P< 0.05).

Creatinine content in yellow-tail seabream was not affected by SeN content in the diet and no significant differences were observed between treatments (P< 0.05).

Total protein content was lower in the control than other treatments, such that the treatment with 1 and 2 mg kg^{-1} of SeN showed a significant increase compared to the control group (P< 0.05). According to the results, the level of albumin in the 1 and 2 mg kg^{-1} treatments showed a significant difference with the control group (P< 0.05). Globulin assay results showed that there was a significant increase in the treatments under different concentrations of SeN compared with the control (P< 0.05).

DISCUSSION

In this study, yellow-tail seabream was fed diets containing 0 (control), 0.5, 1 and 2 mg nano selenium kg^{-1} diet. Increasing the amount of SeN to 0.5 mg kg^{-1} diet increased fish Weight (WG) compared to the control group but decreased at levels above 0.5 mg kg^{-1} diet. Failure to grow at higher concentrations of selenium may be due to the long-term energy expenditure of the body to maintain and improve tissues (Deng *et al*., 2007). Zhu *et al*. (2012) reported that the addition of selenium to the diet of bass fish (*Micropterus salmoide*) increased fish weight. Ashouri *et al*. (2015) also reported a significant increase in weight gain in common carp with increasing selenium content in the diet, but at levels above 1 mg kg^{-1}, there was a marked decrease in growth, that was dependent on the high concentration of selenium in the diet. Also, similar to the present study, SGR and survival rate were not significantly improved by inclusion of SeN levels in the diet.

The optimum level of selenium for hybrid striped bass was reported to be 1.42 mg kg^{-1} (Cotter *et al*., 2008), which is not consistent with the present study. This may be due to the greater efficacy of the nano-sized material and, as a result, they will have their beneficial effects in smaller quantities. However, 0.5 mg kg^{-1} of SeN obtained for yellow-tail seabream is approximately equal to the results for loach (0.50- 0.48 mg selenium in diet) (Hao *et al*., 2014). However, as Hao *et al*. (2014) have pointed out, the differences in optimum selenium content in different studies can be related to fish species, fish age, selenium chemical

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form, dietary factors, selenium response to other elements, water conditions and management.

According to the results of this study, the use of SeN (at concentrations of 0.5 to 2 mg kg^{-1} of diet) did not affect the survival of yellow-tail seabream .Various studies have shown that high concentrations of selenium can decrease survival in fish. For example, survival of chinook salmon (*Oncoryhnchus tshawytscha*) decreased by 38% when fed a diet containing 4.35 mg kg^{-1} selenium (Hamilton *et al*., 1990). On the other hand, survival of Beluga (*Huso huso*) fed with concentrations of 20.26-2.26 μg selenium g^{-1} of diet decreased by 75% (Arshad *et al*., 2011). These studies show that the use of selenium at high concentrations can be toxic and lethal to aquatic life, so, chronic and acute toxicity should be considered in its use for aquatic life.

Blood can provide important information about the internal environment of organisms, and the study of blood parameters in fish is an important tool in understanding the effects of pathological and natural processes (Borges *et al*., 2007).

Alkanine phosphatase plays an important role in phosphate hydrolysis and membrane translocation and acts as a biological indicator of stress in biological systems (Banaee *et al*., 2011). Low doses of SeN (0.5 mg kg^{-1}) significantly reduced the ALP activity in yellow-tail seabream compared to the control group, indicating a decrease in the oxidative effects of liver tissue and, consequently, a decrease in stress.

Asparate aminotransferase and alanine transferase play an important role in protein breakdown to produce ATP (Banaee *et al*., 2011). The activities of ALT and AST enzymes are important in the cellular metabolism of nitrogen, amino acid oxidation and hepatic gluconeogenesis (Banaee *et al*., 2011). The activity of transaminases (ALT and AST) and LDH in serum indicate a stress state in fishes and the increase in the concentration of these enzymes can be considered as a response to stressful conditions in animals (Bitiren *et al*., 2004). In the present study, although there was no significant difference between the levels of AST and LDH enzymes in the treatments, the amount of these enzymes decreased in the 0.5 mg dose, but this decrease was not statistically significant. However, the activity of ALT at 0.5 mg dose showed a significant decrease in 2 mg kg^{-1} treatment. Elevated levels of these enzymes at high doses of SeN may be indicative of the effects of selenium accumulation in the liver and its toxicity (Bitiren *et al*., 2004). In fact, the increase in LDH, AST and ALT in fish serum can be due to their release from damaged tissues, especially the liver (Abdel-Tawwab and Wafeek, 2010). Significant reduction of ALT in 0.5 mg kg^{-1} treatment may indicate a favorable and beneficial effect of low levels of SeN on liver enzyme activity. The results of this study were consistent with studies by Abdel-Tawwab and Wafeek (2010), who reported that feeding fish with organic selenium decreased serum ALT.

Increased Creatine Phosphokinase (CPK) in the blood indicates tissue damage, heart failure, muscular dystrophy, renal failure, seizures, and inflammatory conditions (Haagensen *et al*., 2008). In other words, increasing the activity of this enzyme can be a clinical indicator in the diagnosis of damage to muscle fibers or other tissues (Ozawa *et al*., 1999).

Elevated plasma cholesterol also indicates liver disorders. Biliary obstruction, hepatotoxicity, pancreatic dysfunction, and even elevated blood glucose, damage to biological membrane structures, including nerve cell membranes, can increase plasma cholesterol. Based on the results of the present study, serum cholesterol decreased with the addition of SeN to the diet, but this decrease was significant at a dose of 0.5 mg nano selenium. Bunglavan *et al*. (2014) and Ashouri *et al*. (2015) also showed that selenium can significantly lower serum cholesterol levels in vistas rat and common carp, respectively. Al-Dawairi *et al*. (2014) stated that selenium supplementation reduced cytosolic malic enzyme activity. It is an enzyme that produces NADPH (nicotinamide

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adenine dinucleotide phosphate) used in fatty acid and cholesterol metabolism. Thus, increased dietary selenium probably reduced the activity of malic cytosolic enzyme, resulting in the NADPH required for the production of cholesterol and fatty acid synthesis and decreased cholesterol and lipid levels. Based on the results, no significant increase in triglyceride content was observed with increasing selenium nanoparticles up to 2 mg kg^{-1} diet, which is consistent with the results of Ashouri *et al*. (2015).

Measured nonspecific immune response parameters such as albumin and globulin concentrations are used to assess the effects of nutrients on fish immunity (Abdel-Tawwab *et al*., 2007). Measurement of globulin in laboratory animal studies is an important factor as it depends on general nutrition status, vascular system integrity, and liver function (Abdel-Tawwab *et al*., 2007). Low albumin can be a result of problems with its production, its loss through the stool, and its increased catabolism (Nguyen, 1999). An increase in albumin and globulin is associated with an increase in organic selenium levels in fish diets that increase their production in the liver (Abdel-Tawwab *et al*., 2007). By increasing the amount of SeN up to 2 mg kg^{-1} diet, total protein and globulin were significantly increased, which was consistent with the results of Abdel-Tawwab *et al*. (2007) and Ashouri *et al*. (2015) on African catfish and common carp, respectively.

Creatinine is the latest product of creatine metabolism in skeletal muscle cells that is excreted through the kidneys (Grzyb and Skorkowski, 2005). Therefore, elevated creatinine levels in fish blood may be due to damage to skeletal muscles or kidney dysfunction due to excess creatinine excretion in the blood.

CONCLUSIONS

Comparison of reported amounts of selenium in previous studies and the present study showed that yellow-tail seabream required lower concentrations of selenium nanoparticles, and a dietary supplement containing 0.5 mg of selenium per kg of diet can improve the weight gain. The increase of CPK, ALT, and ALP at levels above 0.5 mg is indicative of the onset of toxic effects of selenium at doses above 0.5 mg. Therefore, based on the results of this experiment, it is suggested that adding 0.5 mg SeN per kg diet can improve growth and biochemical indices in yellow-tail seabream with minimal negative effects of selenium.

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اثرات غذای مکمل با نانو ذرات سلنیوم بر شاخص های بیوشیمیایی ، رشد و بازماندگی ماهی شانک زرد باله(*Acanthopagrus latus*)

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چکیده

هدف از این مطالعه بررسی اثرات غذای مکمل با مقادیر مختلف نانو ذرات سلنیوم بر رشد ، بازماندگی و شاخص های بیوشیمیایی *Acanthopagrus latus* بود. ماهی شانک زرد باله با میانگین

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وزن ۳ ± ۵۲ گرم با چهار جیره غذایی حاوی ۰ ، ۰.۵ ، ۱ و ۲ میلی گرم در کیلوگرم نانوذرات سلنیوم به مدت ۸ هفته تغذیه شدند. در پایان آزمایش ، ماهیانی که با نانوذرات سلنیوم ۰.۵ میلی گرم در کیلوگرم تغذیه کردند ، افزایش قابل توجهی در وزن نسبت به شاهد نشان دادند ($P<0/05$). نرخ رشد ویژه، ضریب تبدیل غذایی و بازماندگی تفاوت معنی داری بین تیمارها نداشت ($P>0/05$). کمترین فعالیت آلکالین فسفاتاز ، آلانین آمینو ترانسفراز ، کراتینین فسفوکیناز و کلسترول در تیمار نانوذرات سلنیوم ۰/۵ میلی گرم بر کیلوگرم مشاهده شد که به طور معنی داری کمتر از شاهد بود ($P<0/05$). اگرچه آسپارتات آمینو ترانسفراز ، لاکتات دهیدروژناز ، تری گلیسیرید و کراتینین تفاوت معنی داری نداشتند ($P>0/05$). سطح کل پروتئین و آلبومین در تیمارهای نانوذرات سلنیوم ۱ و ۲ میلی گرم در کیلوگرم در مقایسه با گروه کنترل به طور قابل توجهی افزایش یافت. بالاترین سطح گلوبولین در تیمار نانوذرات سلنیوم ۲ میلی گرم بر کیلوگرم مشاهده شد. طبق نتایج ، افزودن نانو ذرات سلنیوم به میزان ۰.۵ میلی گرم در کیلوگرم به خوراک ماهی می تواند رشد و پارامترهای بیوشیمیایی را بهبود بخشد.

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