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Selenium Nanoparticles Act Potentially on the Growth Performance, Hemato-Biochemical Indices, Antioxidative, and Immune-Related Genes of European Seabass (*Dicentrarchus labrax*)

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Abstract

The current study investigated the role of selenium (Se) nanoparticles on the growth performance, hemato-biochemical indices, antioxidative, and immune-related genes of European seabass (*Dicentrarchus labrax*). Therefore, fish with initial weight of 20.53 ± 0.10 g/fish were fed diets with 0, 0.25, 0.5, and 1 mg Se nanoparticles/kg diet for 90 days. The final body weight, weight gain, and specific growth rate of fish fed dietary nano-Se varying levels were significantly higher than the control with the highest performances and lowest FCR in the group of fish fed nano-Se at 0.5 mg/kg. The values of Hb, PCV, RBCs, and WBCs were significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets. The values of total serum protein and globulin were significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets. Additionally, globulin had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg ($P < 0.05$). No significant alterations were observed on albumin, ALT, and AST variables ($P > 0.05$). Phagocytic index, phagocytic, lysozyme activities were significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets in a dose dependent manner ($P < 0.05$). Further, SOD activity had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg, whereas CAT was increased in the group of fish fed dietary 0.5 mg nano-Se/kg diet ($P < 0.05$). The level of MDA was significantly lowered by dietary nano-Se where the group of fish fed 0.25 mg/kg had the lowest level followed by those fed 0.5 and 1 mg/kg. The expression of *GH*, *IGF-1*, *IL-8*, and *IL-1β* genes had the highest mRNA levels in the group of fish fed 0.25 and 0.5 mg/kg followed by those fed 1 mg/kg, whereas *HSP70* was downregulated. Based on the overall results, Se nanoparticles are recommended at the rate of 0.5–1 mg/kg diet to maintain the optimal growth performance, hemato-biochemical indices, antioxidative status, and immune-related genes in European seabass.

Keywords Nano minerals · Seabass · Growth rate · Antioxidative status · Immunity

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Introduction

The aquaculture sector is one of the primary sources for the animal proteins required for human nutrition [1]. In this regard, feeding aquatic animals with nutritionally balanced diets is one of the key factors for successful and sustainable aquaculture [2, 3]. Nutritious aquafeed should contain optimal requirements from minerals and vitamins besides protein, lipids, and carbohydrates [4, 5]. Additionally, trace minerals which associated with several physiological, metabolic, and functional properties in organism body [6].

Selenium (Se) is a vital micromineral required to sustain the growth and metabolic activity of fish and assists as an essential element of the active core of glutathione peroxidase (GPx) enzymes [7]. Se is needed in trace amounts only and deserves particular attention among microminerals due to its role in the organism's growth performance and physiological function [8, 9]. Using nanoparticles in aquafeed have gained considerable attention due to novel functionalities, including higher chemical consistency, bioactivity, safety, and the ability to rapidly trigger Se after digestion compared to the other types of Se [10, 11]. Dietary supplementation of Se nanoparticles enhanced the growth performance, feed efficiency, and well-being of several aquatic species, including common carp (*Cyprinus carpio*) [12, 13], catfish (*Clarias gariepinus*) [14], and meager (*Argyrosomus regius*) [15]. Additionally, dietary Se poses antioxidative, immunostimulant, and antibacterial influences in Asian seabass (*Lates calcarifer*) [16], red sea bream (*Pagrus major*) [17], Nile tilapia (*Oreochromis niloticus*) [18], rainbow trout (*Oncorhynchus mykiss*) [19], rohu (*Labeo rohita*) [20], and *Pangasinodon hypophthalmus* [21].

European seabass (*Dicentrarchus labrax*) is widespread in the Mediterranean area and well-known for its fast growth and high survival rate in high stocking density, and it also can tolerate a wide range of environmental fluctuations [22, 23]. However, malnutrition and lack of essential trace minerals are among the crucial factor to hamper aquaculture growth. Therefore, the present study was undertaken to examine the efficacy of dietary nano-Se to strengthen nutrition physiology, immune response, and antioxidant system in European seabass.

Materials and Methods

Fish and Experimental Facilities

Three hundred and sixty European seabass (*D. labrax*) fingerlings with an average initial body weight of 20.53 ± 0.10 g/fish were obtained from private farm, Damietta, Egypt. Prior to the trial, the fish were acclimated to the experimental conditions for 1 week in two indoors circular fiberglass tanks (1 m^3). During this period, fish were fed control diet (45% crude protein). After acclimatization, the fish were randomly distributed into 12 hapa measuring (1 × 2 × 1.25 m each), representing four experimental treatments (in triplicate) at a stocking density of 10 fish per hapa.

Experimental Design and Diets

Four experimental diets are formulated to contain 4 different levels of Se nanoparticles (60 ± 20 nm) at 0, 0.25, 0.5, and 1 mg/kg (Supplementary File). Se nanoparticles were thoroughly mixed with the basal diet (45% crude protein, AQUA International for Food Industries, Cairo, Egypt) in the presence of fish oil and water. Then, the experimental diets were prepared by thoroughly mixing the dry ingredients of each diet; then, 200 ml of water was added per kg diet thereafter, the mixture (ingredients and water) was blended to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through laboratory pellet machine with a 1 mm diameter die; the resulting wet pellets were dried at room temperature until the full drying. The diets were stored in plastic bags in refrigerator (4 °C) until use. The chemical composition of diet samples was assessed according to procedures of AOAC [24]. Se content in the test diets was checked by using atomic absorption spectrophotometer and averaged 0.07 (control), 0.31, 0.58, and 1.09 mg/kg diet. The daily ration was offered three times a day (09.00, 12.00, and 15.00 h) 6 days a week until satiation level for 90 days.

Water temperature, dissolved oxygen, pH, and ammonia were monitored weekly during the trial, to maintain water quality at optimum range for European seabass fingerlings. Water temperature ranged from 18.0 to 19.0 °C, dissolved oxygen (DO) from 4.8 to 5.32 mg/L, pH from 7.0 to 7.5, ammonia (NH_3) from 0.03 to 0.038 mg/L, total salinity (23.41 ppt), and a photoperiod regime (12:12 h light:dark).

Growth and Feed Utilization Indices

At the end of the trial, all fish weighed to calculate the weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR) using the following equations:

$$\mathrm{WG} = \text{final body weight (g)} - \text{initial body weight (g)}$$
$$\mathrm{FCR} = \text{feed intake (g)} / \text{weight gain (g)}$$
$$\mathrm{SGR} = 100 \times ((\ln \text{final body weight (g)} - \ln \text{initial body weight (g)}) / \text{duration of feeding (day)})$$

where W is the fish weight (wet weight in g).

Blood Sampling

Blood samples were collected at the end of the experiment. Five fish per net enclosure were sampled for blood collection. The fish were anesthetized with MS-222 at 25 mg/L, and the blood samples were taken by puncturing the caudal vessels.

The collected blood was divided into two tubes, one containing heparin (1600 UI/ml) as anticoagulant agent for hematological assessment, and the other was anticoagulant free for biochemical estimation. Serum was collected by centrifugation at 3000 rpm/ 15 min at 4 °C and stored at − 20 °C. The hematological parameters are expressed in international units (SI).

The red blood cell counts (RBCs) were determined by using a Bürker counting chamber and Hayem solution. The findings and instructions published by Blaxhall and Daisley [25], and Hrubec and Smith [26] were followed when the RBCs were determined. Hematocrit (PCV) was determined by using microhematocrit-heparinized capillary tubes and a microhematocrite centrifuge (10,000*g* for 5 min). The values of PCV were determined within 30 min alter bleeding. Hemoglobin concentrations (Hb) were determined by the cyanhemoglobin method, at 540 nm. The total WBCs count was determined according to the method of Stoskopf [27]. WBCs and Hb values were determined within 6 h after blood sampling.

Total serum protein (g/dL) was determined using biuret method according to Doumas and Bayse [28]. Albumin (g/dL) was determined by the bromocresol green method according to Reinhold [29], and globulin (g/dL) was calculated as the difference between total protein and albumin. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using the method of Gella and Olivella [30]. The absorbance is read at a wavelength of 505 nm. The absorbance was then interpolated in the calibration curve.

Immunological and Oxidation Assays

Analysis of serum lysozyme activity was performed using turbidimetric assay according to Ellis and Stolen [31] based on the lysis of Gram-positive bacterium *Micrococcus lysodeikticus* (Sigma, USA). Leukocyte phagocytic function followed the method of Cai and Li [32]. The number of leukocytes that engulfed bacteria was counted as percentages in relation to the total leukocyte number in the smear from the phagocytosis assay. By following Kawahara and Ueda [33], the phagocytic activity and phagocytic index were determined.

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malonaldehyde (MDA) levels in serum were measured using diagnostic reagent kits following the manufacturer's (Cusabio Biotech Co., Ltd., China) instructions.

Gene Expression

The expression of hepatic genes was determined using RT-PCR. Briefly, TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) was utilized to extract total RNA from approximately 100 mg of hepatic tissue. RNA samples of 1,8 or more A260/A280 were used for DNA synthesis using a cDNA synthesis package (Fermentas, Waltham, MA, USA) using Nanodrop quantitative. The SYBR Green Master Mix and Table 1 primers with (GAPDH) as a standard gene have been added to amplify cDNA. Obtained results on amplification were assessed using $2^{-\Delta\Delta}$ methods [34].

Statistical Analyses

The obtained data were subjected to one-way ANOVA using SPSS version 22 (SPSS Inc., IL, USA). Differences between the means were tested at the 5% probability level using Duncan's test as a post hoc test.

Results

Growth Performance

The final body weight and weight gain of fish fed dietary nano-Se varying levels are significantly higher than the control (Table 2). The specific growth rate recorded higher value in the group of fish fed nano-Se at 0.5 mg/kg than the other groups (Table 2). However, the FCR was significantly reduced in fish fed dietary nano-Se with the lowest value in fish 0.5 mg nano-Se/kg ($P < 0.05$).

Hemato-Biochemical Indices

The hematological indices of European seabass show regular values except for the Hb, PCV, RBCs, and WBCs (Table 3). The values of Hb, PCV, RBCs, and WBCs were significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets. Additionally, PCV had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg ($P < 0.05$).

The values of total serum protein and globulin are significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets (Table 4). Additionally, globulin had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg ($P < 0.05$). No significant alterations were observed on albumin, ALT, and AST variables ($P > 0.05$).

Immune and Antioxidative Response

Phagocytic index, phagocytic, lysozyme activities were significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets (Table 5). Further, the phagocytic activity had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg ($P < 0.05$).

Table 1 Primers of RT-qPCR used in this study

Gene	Sequence	Accession number
IL-8	Forward: GTCTGAGAAGCCTGGGAGTG Reverse: GCAATGGGAGTTAGCAGGAA	AM490063.1
IL-1β	Forward: ATCTGGAGGTGGTGGACAAA Reverse: AGGGTGCTGATGTTCAAACC	AJ269472.1
HSP70	Forward: GCTCCACTCGTATCCCCAAG Reverse: ACATCCAGAAGCAGCAGGTC	AY423555.2
GH	Forward: TGAGGAAGAGGAGGAGGTGA Reverse: GGAGGTGGAGCTACAGAACA	GQ918491
IGF-1	Forward: CGCTGCAGTTTGTGTGTGG Reverse: CTCTTGGCATGTCTGTGTGG	AY800248
GAPDH	Forward: GAAGGTTATCAAGGCCGCTG Reverse: CACACACGGTTGCTGTATCC	AJ567450

SOD, CAT, and GPx activities are significantly higher in fish fed varying levels of Se nanoparticles than fish fed the basal diets (Table 6). Further, SOD activity had higher value in the group of fish fed 0.25 and 0.5 mg nano-Se/kg than fish fed 1 mg nano-Se/kg, whereas CAT was increased in the group of fish fed dietary 0.5 mg nano-Se/kg diet ($P < 0.05$). The level of MDA is significantly lowered by dietary nano-Se where the group of fish fed 0.25 mg/kg had the lowest level followed by those fed 0.5 and 1 mg/kg (Table 6).

Gene Expression

The relative expression of liver *GH*, *IGF-1*, *HSP70*, *IL-8*, and *IL-1β* genes are significantly varied among the group of fish fed nano-Se ($P < 0.05$) (Fig. 1). The expression of *GH*, *IGF-1*, *IL-8*, and *IL-1β* genes had the highest mRNA levels in the group of fish fed 0.25 and 0.5 mg/kg followed by those fed 1 mg/kg. However, the expression of *HSP70* was significantly downregulated in fish fed dietary nano-Se with regard to the control group.

Discussion

The new trend in the aquaculture sector is to increase fishery intensification through the management of the aquatic environment and feeding strategies. The well-balanced nutritional formulations should contain the essential nutrients (e.g., proteins, lipids, carbohydrates, vitamins, and minerals) required for the maintenance of body performance, metabolism, and physiological behavior [2]. Selenium (Se) is an essential element that has been indicated to improve the quality of aquafeeds. The Se-depended proteins' activity in the body can be malformed due to the lack of Se, resulting in the signs and symptoms of deficiency [8]. The lack of Se in aquafeeds induces diverse effects on the performances and feed efficiency of fish. Nano mineral form is ranged between 1 and 100 nm, which makes it easy for absorption and available within the body tissues and fluids [35]. Further, nanoparticles are characterized by the small size and large surface, which potentiate their biological and physiological ability. The results showed increased final body weight, weight gain, SGR, and FCR in fish fed dietary nano-Se at 0.5–1 mg/kg. Similarly, the growth rate was enhanced by dietary Se in Nile tilapia [36], crucian carp [37], and rainbow trout [38]. Wang and Yan [39] illuminated that a certain concentration of nano-Se increased the protein contents of intestinal epithelial cells of the crucian carp. The increase in intracellular protein of intestinal epithelial cells may cause better metabolism of feed ingredients resulting in higher growth enhancement. Another possible reason is the role of Se, which acts as co-enzymes for digestive enzymes synthesis and hence increases

Table 2 Growth performance of European seabass fed with diets supplemented with different levels Se nanoparticles for 90 days

Item	0	0.25	0.5	1
IBW (g)	20.17 ± 0.06	20.83 ± 0.32	20.44 ± 0.05	20.67 ± 0.04
FBW (g)	82.78 ± 0.08^{a}	84.77 ± 0.12^{b}	86.44 ± 0.27^{b}	84.29 ± 0.05^{b}
WG (%)	62.61 ± 0.08^{a}	63.94 ± 0.44^{b}	66.00 ± 0.29^{b}	63.63 ± 0.07^{b}
SGR (%/day)	1.68 ± 0.00^{a}	1.67 ± 0.02^{a}	1.72 ± 0.01^{b}	1.67 ± 0.00^{a}
FCR	1.84 ± 0.09^{c}	1.68 ± 0.00^{b}	1.59 ± 0.05^{a}	1.69 ± 0.00^{b}

Data are presented as mean ± S.E. Data in the same row with different superscript are significantly different ($P < 0.05$)

Table 3 Hematological indices of European seabass fed with diets supplemented with different levels Se nanoparticles for 90 days

Item	0	0.25	0.5	1
Hb (g/100 ml)	10.53 ± 0.01[a]	11.66 ± 0.01[b]	11.71 ± 0.01[b]	11.23 ± 0.01[b]
RBCs (10/mm^6)	3.44 ± 0.01[a]	3.83 ± 0.01[b]	3.91 ± 0.01[b]	3.72 ± 0.01[b]
PCV (%)	34.00 ± 0.00[a]	38.00 ± 0.00[c]	38.50 ± 0.29[c]	36.50 ± 0.29[b]
MCV (mm^3)	98.84 ± 0.17	99.09 ± 0.22	98.59 ± 0.52	98.11 ± 0.47
MCH (Pg)	30.61 ± 0.07	30.39 ± 0.05	29.99 ± 0.10	30.18 ± 0.12
MCHC (%)	30.97 ± 0.02	30.67 ± 0.02	30.42 ± 0.26	30.76 ± 0.27
WBCs (10/mm^3)	12.84 ± 0.01[a]	14.67 ± 0.01[b]	14.77 ± 0.01[b]	14.30 ± 0.01[b]
Heterophils (%)	15.50 ± 0.29	10.50 ± 0.29	10.50 ± 0.29	11.50 ± 0.29
Lymphocytes (%)	76.00 ± 0.00	80.50 ± 0.29	81.00 ± 0.58	79.00 ± 0.58
Monocytes (%)	7.00 ± 0.00	8.00 ± 0.58	7.50 ± 0.29	7.00 ± 0.58
Eosinophils (%)	0.50 ± 0.29	0. 50 ± 0.25	0.50 ± 0.29	1.00 ± 0.00
Basophils (%)	1.00 ± 0.58	0.50 ± 0.29	0.50 ± 0.29	1.50 ± 0.29

Data are presented as mean ± S.E. Data in the same row with different superscript are significantly different ($P < 0.05$)

its activity [40]. The activation of digestive enzymes increases nutrient digestibility and release more nutrients for absorption by the intestinal epithelial cells. In this context, the level of FCR is expected to enhance, and the growth performance increases. Interestingly, the current study results displayed upregulated expressions of GH and IGF-1 gene expressions in European seabass, which explains the enhanced growth performance by dietary Se nanoparticles.

The values of RBCs, WBCs, PCV, and Hb were increased in fish fed 0.5–1 mg dietary nano-Se. These results probably are associated with the regulation of the metabolic rate and well-being of seabass induced by nano-Se feeding. Increasing of RBCs and Hb causes oxygen availability in the body tissues owing to the absence of anemic features. The balanced levels of RBCs, Hb, and PCV also attributed to the role of dietary nano-Se in affording the nutritional requirements for seabass, which induced enhanced health status. The antioxidant influence of the Se results in the balance of the protection of RBCs membranes and increases their life span by defending them against harmful impacts of oxygen-free radicals (ROS) and alleviating anemia and membrane disruption as well as the reducing cell hemolysis and degeneration [12, 41]. In this regard, Le and Fotedar [42] reported that yellowtail fed dietary Se at 0 to 2 mg/kg had enhanced the GPx activity of RBCs. Additionally, the results showed similar enhancements in the Hb, RBCs, and PCV indices with dietary Se in common carp [43].

The high count of WBC refers to enhanced adaptive immunity to counteract with pathogens and invaders, which occurs under stressful conditions [44]. WBC functions as the main component of the front lines of body defense, and its number increases rapidly when infections occur [45, 46]. In the present study, increased WBCs and platelet counts in European seabass strongly supported cell-mediated immunity by dietary nano-Se [47]. In the parallel to the RBCs results, hemoglobin values increased with increasing nano-Se levels as hemoglobin acts as a protein carried by RBCs and having a role in respiration [48].

The protein content is required for several functions, including the cell function, metabolism, secretion of enzymes and hormones, and regulating physiological processes in the fish body [49]. Therefore, the detection of the total protein in fish blood is vital to evaluate the general health condition and immunity of fish. Besides, the high level of albumin can protect the blood vessels from leaking out during stress, while globulins include several immunological related contents

Table 4 Biochemical parameters of European seabass fed with diets supplemented with different levels Se nanoparticles for 90 days

Item	0	0.25	0.5	1
ALT (U/I)	32.50 ± 0.87	32.50 ± 2.02	29.50 ± 0.29	31.50 ± 0.29
AST (U/I)	25.50 ± 0.29	20.50 ± 0.29	23.50 ± 1.44	25.50 ± 0.29
Blood total protein (mg/dl)	4.08 ± 0.01[a]	4.59 ± 0.05[b]	4.65 ± 0.02[b]	4.41 ± 0.03[b]
Albumin (g/dl)	1.52 ± 0.00	1.56 ± 0.01	1.52 ± 0.02	1.53 ± 0.02
Globulin (g/dl)	2.55 ± 0.01[a]	3.03 ± 0.06[c]	3.13 ± 0.05[c]	2.88 ± 0.00[b]

Data are presented as mean ± S.E. Data in the same row with different superscript are significantly different ($P < 0.05$)

Table 5 Immune parameters in sera of European seabass fed with diets supplemented with different levels Se nanoparticles for 90 days

Item	0	0.25	0.5	1
Lysozyme activity (unit/ml)	9.19 ± 0.02[a]	10.49 ± 0.02[b]	10.60 ± 0.03[b]	10.28 ± 0.01[b]
Phagocytic activity (%)	11.99 ± 0.02[a]	14.59 ± 0.01[c]	14.68 ± 0.01[c]	13.61 ± 0.02[b]
Phagocytic index	0.94 ± 0.02[a]	1.15 ± 0.00[b]	1.19 ± 0.00[b]	1.10 ± 0.00[b]

Data are presented as mean ± S.E. Values in each row with different superscripts shows significant difference ($P < 0.05$)

[50]. The globulins are the major humoral components among serum proteins, which perform a significant role in the immune action. The results indicated that the blood total protein and globulin were increased by dietary nano-Se. The results assured the positive impact of dietary protein on the health condition of seabass reared under the current trial conditions. The increased total proteins and globulin levels are similar to the study of Ashouri and Keyvanshokooh [12] in common carp, Abdel-Tawwab and Mousa [51] in African catfish, and Dawood and Zommara [36] in Nile tilapia. The possible importance of increased total protein may be related to the high protein content induced by the role of nano-Se in increasing the levels of selenoprotein in the intracellular intestines of seabass.

Phagocytes generate ROS during infection to combat pathogenic invades [52]. Hence, the oxygen demand increased after stimulation of the phagocytic cell membrane. The lysozyme activity is a vital response in fish attributed to the ability of leukocytes (neutrophils and macrophages) to release lysozyme as a humoral constituent of the non-specific defense mechanism [53]. The current study results illustrated that European seabass fed nano-Se had enhanced phagocytic index, phagocytic, and lysozyme activities. In a similar sense, dietary Se nanoparticles increased the phagocytosis and lysozyme activities in Nile tilapia [36], *Piaractus mesopotamicus* [47], and meager [15].

The oxidative stress occurs during stressful environmental conditions, and pathogenic invades that liberates ROS due to the imbalance of ROS production and removal [54]. The overproduced ROS impair the lipid contents in the cell membranes and damage RNA. Excessive ROS also induce lipid peroxidation, which can be measured by the level of malondialdehyde (MDA) [55]. As a functional component of the glutathione peroxidase (GPx), Se performs a highly significant function in activating the antioxidant defense system [56]. The antioxidant activity is the most significant biological function of Se as it makes selenocysteine, an integral part of the active core of the GPx [57]. The antioxidant enzymes (CAT, SOD, and GPx) are served as the first line of the antioxidant defense system in fish [58]. The results displayed enhanced CAT, SOD, and GPx activities in fish fed Se nanoparticles. Similar results were reported for Nile tilapia [36], common carp [12], gilthead seabream (*Sparus aurata*) [59], and meager (*Argyrosomus regius*) [15]. The MDA level has decreased in fish fed dietary Se nanoparticles, which confirms the role of Se in protecting European seabass against lipid peroxidation. Similarly, Ashouri and Keyvanshokooh [12] stated that the MDA level was lowered in fish fed dietary Se nanoparticles.

The heat shock protein (HSP70) is considered one of the most important genes that maintain the cellular protein and thus protect cells from apoptosis due to its role in raising cellular immunity under stressful conditions [60]. The current study demonstrated that Se nanoparticles downregulated the HSP70 which indicates that nano-Se has no inflammatory or stressful impacts on European seabass.

Interleukin 12 (*IL-12*) is responsible about the expression of pro-inflammatory cytokine (*IL-8*), which is helping to resist pathogenic invasions and regulate the immune response in the host [61, 62]. The current results revealed upregulated intestinal *IL-8* cytokine in European seabass by feeding nano-Se. The *IL-1β* cytokine is also among the genes which regulate the immune response through the readiness against infection through cell proliferation, differentiation, and apoptosis [63, 64]. The results displayed upregulated *IL-1β* gene by nano-Se feeding. Although in the present study no disease challenge

Table 6 Antioxidative capacity in sera of European seabass fed with diets supplemented with different levels Se nanoparticles for 90 days

Item	0	0.25	0.5	1
SOD (IU/I)	10.09 ± 0.01[a]	11.11 ± 0.26[c]	11.05 ± 0.09[c]	10.50 ± 0.08[b]
CAT (IU/I)	11.09 ± 0.03[a]	12.65 ± 0.46[b]	13.82 ± 0.10[c]	11.98 ± 0.34[b]
GPx (IU/I)	16.67 ± 0.13[a]	17.45 ± 0.06[b]	17.56 ± 0.06[b]	17.08 ± 0.01[b]
MDA (IU/I)	19.95 ± 0.05[c]	17.85 ± 0.10[a]	18.84 ± 0.42[b]	18.70 ± 0.02[b]

Data presented as mean ± S.E. of individual fish. Values in a row with different superscripts show significant difference ($P < 0.05$)

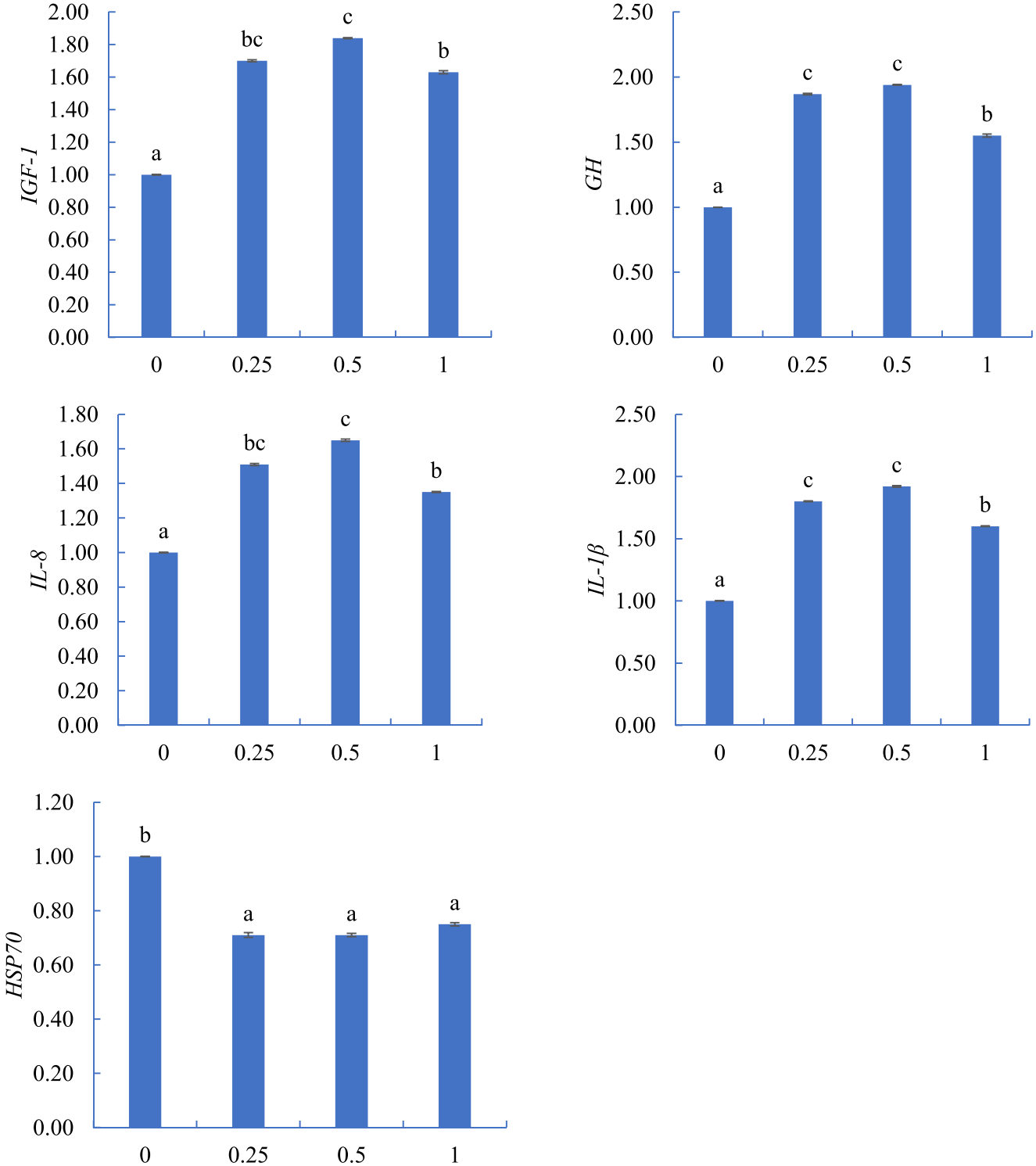

Fig. 1 Relative expression of *GH*, *IGF-1*, *HSP70*, *IL-8*, and *IL-1β* genes in European seabass fed diets enriched with different levels of Se nanoparticles for 90 days. Data are presented as mean ± S.E. ($n = 3$). Bars with different superscript are significantly different ($P < 0.05$)

has been conducted, however, the obtained results are supporting the role of nano-Se as an immunostimulant which can be applied in the European seabass diets to increase the resistance against the possible infection under farm conditions. The upregulation of *HSP70*, *IL-1β*, and *IL-8* genes in the liver of European seabass means that nano-Se induced enhanced antistressor and pro-inflammatory responses.

Conclusion

Based on the overall results, Se nanoparticles is recommended at the rate of 0.5–1 mg/kg diet to maintain the optimal growth performance, hemato-biochemical indices, antioxidative status, and immune-related genes in European seabass.

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