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Evaluating the possible feeding strategies of selenium nanoparticles on the growth rate and wellbeing of European seabass (*Dicentrarchus labrax*)

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ABSTRACT

The study aimed at evaluating the possible feeding strategies of selenium (Se) nanoparticles on the performances of European seabass (*Dicentrarchus labrax*). The first group fed the basal diet continuously (control) while the second group offered Se (1 mg/kg diet) every day (R1). The third group fed the basal diet one day then Se diet every other day (R2), and the fourth group basal diet for two days then fed Se diet every two days (R3). The FBW, WG, and SGR were significantly improved in the R1 group than fish in the other groups ($P < 0.05$). The FCR was significantly reduced by feeding Se nanoparticles to fish in R1, R2, and R3 groups than the control with the lowest being in R1 group ($P < 0.05$). The R1, R2, and R3 groups had enhanced values of Hb, RBCs, and WBCs ($P < 0.05$). Additionally, the blood total protein and globulin of fish fed in R1, R2, and R3 groups was significantly higher than the control ($P < 0.05$). Phagocytic index, phagocytic, lysozyme activities were significantly higher in fish fed Se nanoparticles (R1, R2, and R3 groups) than fish fed the basal diets ($P < 0.05$). The antioxidative related indices (SOD, CAT, and GPx) were enhanced in fish of R2 group while the concentration of MDA was reduced ($P < 0.05$). Further, the level of MDA was decreased in fish of R1 group that fed dietary Se nanoparticles every day. The relative expression of liver GH, IGF-1, IL-8, and IL-1β genes were significantly upregulated in fish of R1, R2, and R3 groups while HSP70 gene was reduced ($P < 0.05$). In conclusion, fish fed dietary Se nanoparticles at 1 mg/kg either daily, every other day, or every other two days had enhanced growth rate, feed efficiency, haemato-biochemical indices, immunity, antioxidative responses, and anti-inflammatory impacts on European seabass.

1. Introduction

The sustainability of the aquaculture sector is one of the feasible activities that guarantee food security for a growing population worldwide (Dawood, 2020). The successful aquaculture practices are also recommended to avoid the possible loss of production under unstable environmental conditions (Yukgehnaish et al., 2020). The balanced aquafeed should contain rich protein, lipid, and carbohydrate sources with the optimum requirements throughout the rearing season (Vali et al., 2020). Not only that but also the micronutrients (e.g., vitamins

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and minerals) that are required for several physiological and biological functions in the aquatic animals' body have to be included in the aquafeed at adequate supplemental levels and durations (Dawood et al., 2018).

A particular focus has been given to trace elements that have a potential role in aquatic animals' performances (Dawood et al., 2020a). Of them, selenium (Se) which associated with several functions in the body of aquatic organisms (Khan et al., 2016). The mineral of Se is required to activate animals' antioxidative capacity as they build the core of glutathione peroxidase (GPx) enzymes (Khan et al., 2017). Besides, Se contributes to many physiological tasks, including building selenoproteins attributed to the activity of digestive enzymes and nutrient digestion (Skalickova et al., 2017). The functionality of Se well evaluated in most of the aquatic animals and most of these studies recommended Se supplementation in aquafeed for improving the growth performance, feed efficiency, and antioxidative status (Ashouri et al., 2015; Dawood et al., 2020c; Longbaf Dezfouli et al., 2019; Saffari et al., 2017). The efficacy of Se on aquatic animals' performances depends on several factors, including the form of inclusion and feeding duration. The nanoform of trace minerals characterized by its efficient influence due to the small particle size and the immense surface area, which increases Se permeability and functionality (Dawit Moges et al., 2020). Concurrently, the duration and regimens of Se feeding also determine the efficacy of Se on aquatic animal performances.

European seabass (*Dicentrarchus labrax*) is an important cultured fish species in the Mediterranean area with a realizable commercial value (Magalhães et al., 2017; Torrecillas et al., 2007; Yılmaz and Ergün, 2012). It is proposed that the impact of Se nanoparticles' continuous feeding can be equal to day/day and day/two days regimens. Hence, the present study aimed at evaluating the possible feeding strategies of Se nanoparticles on the growth rate, haemato-biochemical indices, antioxidative status, and immune-related genes of European seabass.

2. Materials and methods

2.1. Trial conditions, design, and diet preparation

The basal commercial diet with a protein content of 45 % is obtained from AQUA International for Food Industries, Cairo, Egypt, and considered the control diet. The basal diet fined well and mixed with Se nanoparticles in the presence of fish oil and water and pelleted with a meat chopper to produce dough (2 mm die) and left to air dry. Selenium nanoparticles (60 ± 20 nm) (Nano Gate, Cairo, Egypt) were included in the basal diet at the rate of 1 mg/kg (Abd El-Kader et al., 2020). The diets were stored in plastic bags in the refrigerator (4 °C) until use. The chemical composition of diet samples was assessed according to the procedures of AOAC (2007). Se content in the test diets was checked using the Atomic absorption spectrophotometer and averaged 0.07 (control) and 1.09 mg/kg diet. The daily ration was offered three times a day (09.00, 12.00, and 15.00 h) six days a week till satiation level for 90 days.

Fingerlings of European sea bass were gently collected from Damietta city and kept for adaptation for one week in 1000 L fiberglass tanks and fed the basal diet (45 % crude protein). Then fish with equal initial weight (20.27 ± 0.12 g) were distributed into four groups (triplicates). Fish were cultured in 12 enclosures (hapa) (1 × 2 × 1.25 m each) at 10 fish/hapa. The first group fed the basal diet continuously (control) while the second group offered Se supplemented diet every day (R1). The third group fed the basal diet one day then Se supplemented diet every other day (R2), and the fourth group basal diet for two days then fed Se supplemented diet every two days (R3) (Table 1).

Water temperature (18.0–19.0 °C), dissolved oxygen (4.8–5.32 mg/L), pH (7.0–7.5), ammonia (0.03 to 0.038 mg/L), and total salinity (23.41 ppt) were monitored weekly during the trial, and a photoperiod regime (12:12 h light: dark).

Table 1
Groups of the trial feeding regimens.

Item	Control	R1	R2	R3
First day	Basal diet	Se supplemented diet	Basal diet	Basal diet
Second day	Basal diet	Se supplemented diet	Se supplemented diet	Se supplemented diet
Third day	Basal diet	Se supplemented diet	Basal diet	Se supplemented diet
Fourth day	Basal diet	Se supplemented diet	Se supplemented diet	Basal diet

*The table shows the regime of the basal diet or Se supplemented diet feeding for four continuous days then can be repeated during the whole trial (90 days).

2.2. Blood sampling

Five fish per hapa were randomly collected for blood sampling. 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 haematological 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 haematological 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 (1973) and Hrubec and Smith (1999) 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 (1993). 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 et al. (1981). Albumin (g/dL) was determined by the bromocresol green method according to Reinhold (1953) 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 et al. (1985). The absorbance is read at a wavelength of 505 nm. The absorbance was then interpolated in the calibration curve.

2.3. Immunological and oxidation assays

Analysis of serum lysozyme activity was performed using turbidimetric assay according to Ellis et al. (1990) based on the lysis of Gram-positive bacterium *Micrococcus lysodeikticus* (Sigma, USA). Leukocyte phagocytic function followed the method of Cai et al. (2004). The number of leukocytes that engulfed bacteria was counted as

Table 2
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

percentages in relation to the total leukocyte number in the smear from the phagocytosis assay. By following Kawahara et al. (1991), 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.

2.4. 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, United States) using Nanodrop quantitative. The SYBR Green Master Mix and Table 2 primers with (GAPDH) as a standard gene have been added to amplify cDNA. Obtained results on amplification were assessed using $2^{-\Delta\Delta}$ methods (Pfaffl, 2001).

2.5. Calculations and statistical analyses

Based on the initial body weight (IBW) and final body weight (FBW), the weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR) were calculated where the WG = FBW (g) - IBW (g); FCR = feed intake (g)/WG (g); SGR = 100× ((ln FBW (g) - ln IBW (g))/ duration of feeding (day)).

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.

3. Results

3.1. Growth performance

The FBW, WG, and SGR were significantly improved in the group of fish fed dietary Se nanoparticle everyday (R1) than fish in the other groups ($P < 0.05$) without significant differences with fish fed Se nanoparticles every other day (R2) ($P > 0.05$) in terms of FBW and WG (Table 3). The FCR was significantly reduced by feeding Se nanoparticles to fish in R1, R2, and R3 groups than the control with the lowest being in R1 group ($P < 0.05$) (Table 3).

3.2. Haemato-biochemical indices

No abnormal features were observed on the hematological indices of European seabass. The groups of fish fed dietary Se nanoparticles every day, every other day, or every other two days had enhanced values of Hb, RBCs, and WBCs ($P < 0.05$) (Table 4). Additionally, the blood total protein and globulin of fish fed in R1, R2, and R3 groups was significantly higher than the control ($P < 0.05$) (Table 5). The globulin content in fish of R1 group showed the highest value among the groups.

3.3. Immune and antioxidative response

Phagocytic index, phagocytic, lysozyme activities were significantly higher in fish fed Se nanoparticles (R1, R2, and R3 groups) than fish fed the basal diets ($P < 0.05$) (Table 6). The antioxidative related indices (SOD, CAT, and GPx) were enhanced in fish of R2 group while the concentration of MDA was reduced ($P < 0.05$) (Table 7). Further, the level of MDA was decreased in fish of R1 group that fed dietary Se nanoparticles every day.

Table 5
Biochemical parameters of European seabass fed with diets Se nanoparticles at varying feeding regimens.

Item	Control	R1	R2	R3
ALT (U/I)	32.50 ± 0.87	35.00 ± 0.58	31.50 ± 0.29	28.50 ± 0.29
AST (U/I)	25.50 ± 0.29	26.00 ± 1.16	25.50 ± 0.29	23.50 ± 0.29
Blood total protein (mg/dl)	4.08 ± 0.01a	4.78 ± 0.03b	4.41 ± 0.03b	4.31 ± 0.01b
Albumin (g/dl)	1.52 ± 0.00	1.56 ± 0.03	1.53 ± 0.02	1.55 ± 0.01
Globulin (g/dl)	2.55 ± 0.01a	3.22 ± 0.01c	2.88 ± 0.00b	2.76 ± 0.01b

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

Table 3
Growth performance of European seabass fed with diets Se nanoparticles at varying feeding regimens.

Item	Control	R1	R2	R3
IBW (g)	20.17 ± 0.06	20.27 ± 0.02	20.67 ± 0.04	19.97 ± 0.08
FBW (g)	82.78 ± 0.08a	89.34 ± 0.08b	84.29 ± 0.05ab	81.80 ± 0.09a
WG (%)	62.61 ± 0.08a	69.07 ± 0.08b	63.63 ± 0.07ab	61.83 ± 0.06a
SGR (%/day)	1.68 ± 0.00a	1.77 ± 0.00b	1.67 ± 0.00a	1.68 ± 0.00a
FCR	1.84 ± 0.09c	1.52 ± 0.04a	1.69 ± 0.00b	1.73 ± 0.00b

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

Table 4
Hematological indices of European seabass fed with diets Se nanoparticles at varying feeding regimens.

Item	Control	R1	R2	R3
Hb (g/100 mL)	10.53 ± 0.01a	11.23 ± 0.01b	11.81 ± 0.01b	11.07 ± 0.01b
RBCs ($10/mm^6$)	3.44 ± 0.01a	3.72 ± 0.01b	3.92 ± 0.01b	3.67 ± 0.00b
PCV (%)	34.00 ± 0.00	36.50 ± 0.29	38.50 ± 0.29	36.00 ± 0.00
MCV (mm^3)	98.84 ± 0.17	98.11 ± 0.47	98.21 ± 0.45	97.96 ± 0.08
MCH (Pg)	30.61 ± 0.07	30.18 ± 0.12	30.13 ± 0.10	30.12 ± 0.06
MCHC (%)	30.97 ± 0.02	30.76 ± 0.27	30.68 ± 0.25	30.75 ± 0.03
WBCs ($10/mm^3$)	12.84 ± 0.01a	14.30 ± 0.01b	14.81 ± 0.01b	14.08 ± 0.01b
Heterophils (%)	15.50 ± 0.29	11.50 ± 0.29	9.50 ± 0.29	13.00 ± 0.00
Lymphocytes (%)	76.00 ± 0.00	79.00 ± 0.58	82.50 ± 0.87	79.50 ± 0.29
Monocytes (%)	7.00 ± 0.00	7.00 ± 0.58	7.00 ± 0.58	7.00 ± 0.00
Eosinophils (%)	0.50 ± 0.29	1.00 ± 0.00	0.50 ± 0.29	0.00 ± 0.00
Basophils (%)	1.00 ± 0.58	1.50 ± 0.29	0.50 ± 0.29	0.50 ± 0.29

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

Table 6
Immune parameters in sera of European seabass fed with diets Se nanoparticles at varying feeding regimens.

Item	Control	R1	R2	R3
Lysozyme activity (unit/mL)	9.19 ± 0.02a	10.28 ± 0.01b	10.82 ± 0.04b	10.02 ± 0.02b
Phagocytic activity (%)	11.99 ± 0.02a	13.61 ± 0.02b	14.84 ± 0.01b	12.88 ± 0.01b
Phagocytic index	0.94 ± 0.02a	1.10 ± 0.00b	1.19 ± 0.00b	1.02 ± 0.01b

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

3.4. Gene expression

The relative expression of liver GH, IGF-1, IL-8, and IL-1β genes were significantly upregulated in fish of R1, R2, and R3 groups while HSP70 gene was reduced ($P < 0.05$) (Fig. 1). Markedly, the expression of liver GH, IGF-1, IL-8, and IL-1β genes were significantly upregulated in fish of R2 group while HSP70 gene was reduced when compared to the other groups ($P < 0.05$).

4. Discussion

Selenium nanoparticles have a small size and large surface, which causes the high permeability and availability in the body of fish (Dawit Moges et al., 2020). The present study showed that fish fed Se nanoparticles every day (R1) had higher FBW, WG, and SGR with lower FCR than the control and R3 groups, while fish in the R2 group, which provided Se nanoparticles every other day, showed no difference with the R1 group. In a similar sense, the growth performance was enhanced by the inclusion of dietary Se nanoparticles in Asian seabass (*Lates calcarifer*) (Longbaf Dezfouli et al., 2019), red sea bream (*Pagrus major*) (Dawood et al., 2019), Nile tilapia (*Oreochromis niloticus*) (Dawood et al., 2020c), and rainbow trout (*Oncorhynchus mykiss*) (Naderi et al., 2017). The influence of Se nanoparticles on the growth of fish is probably attributed to the role of Se in building the selenoproteins in the intestinal epithelial cells, which followed by the efficient utilization and digestion of nutrients (Wang et al., 2013). Intestinal epithelial cells' intracellular protein can lead to improved feed ingredients' metabolism, resulting in increased growth. Further, Se acts as co-enzymes for the synthesis of digestive enzymes and thus increases its activity (Shenkin, 2006). The activation of digestive enzymes increases nutrient digestibility and release more nutrients for absorption by the intestinal epithelial cells. The decreased FCR also declares that dietary Se nanoparticles enhanced the feed utilization and the growth rate, which is supported by the upregulation of GH and IGF-1 genes.

The health condition of fish can be evaluated by the measurement of hematological and blood biochemical variables (Dawood et al., 2020b). The results revealed that fish fed Se nanoparticles had no abnormal values, which indicate the regular health condition of European seabass. Additionally, the Hb, RBCs, and WBCs indices were significantly improved by dietary Se nanoparticles in fish in the R1, R2, and R3 groups. 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 (Ashouri et al., 2015; Khan et al., 2016). Similarly, Saffari et al. (2018) reported that common carp fed diets with Se had improved Hb and RBCs. The present study also indicated improved WBCs in European seabass which strongly supported cell-mediated immunity by dietary nano-Se (Takahashi et al., 2017). 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 (Awal et al., 2012; de Azevedo et al., 2015d).

Table 7
Antioxidative capacity in sera of European seabass fed with diets Se nanoparticles at varying feeding regimens.

Item	Control	R1	R2	R3
SOD (IU/l)	10.09 ± 0.01a	10.50 ± 0.08a	11.31 ± 0.07b	10.15 ± 0.04a
CAT (IU/l)	11.09 ± 0.03a	11.98 ± 0.34a	13.93 ± 0.04b	11.67 ± 0.22a
GPx (IU/l)	16.67 ± 0.13a	17.08 ± 0.01a	18.06 ± 0.01b	17.08 ± 0.02a
MDA (IU/l)	19.95 ± 0.05c	18.70 ± 0.02b	17.96 ± 0.29a	19.70 ± 0.03c

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

The blood total proteins and globulins are humoral immune components associated with the fish body's defense system and can be affected by the nutritional value of aquafeed (Uribe et al., 2011). The enhanced levels of blood total protein and globulin in the present study in the groups of fish fed dietary Se nanoparticles are in line with Ashouri et al. (2015); Abdel-Tawwab et al. (2007), and Dawood et al. (2020d) who reported that dietary Se nanoparticles enhanced the blood proteins in common carp, African catfish, and Nile tilapia, respectively.

Among the main immune activities, the lysozyme and phagocytosis can protect the fish body during invades by breaking down pathogenic bacterial cells' walls and activating the phagocytic capacity (Itou et al., 1996). Interestingly, fish fed dietary Se nanoparticles had enhanced lysozyme and phagocytic activities in all feeding regimes regarding fish-fed Se-free diets. In this context, dietary Se nanoforms enhanced the lysozyme and phagocytic activities in meagre (Mansour et al., 2017), *Piaractus mesopotamicus* (Takahashi et al., 2017), and Nile tilapia (Dawood et al., 2020d).

Antioxidant activity is the most important biological function of Se, as it builds selenocysteine, an essential part of glutathione peroxidase (GPx) (Köhrle et al., 2000) which stimulate the antioxidant defense mechanism in fish body (Gamble et al., 1997). Besides, organisms' secrets further antioxidative enzymes (e.g., SOD and CAT) to cope with the oxidative stress occurred under stressful conditions (Martínez-Álvarez et al., 2005). The oxidative stress induces the lipid peroxidation of cell membranes (e.g., immune cells) and DNA damage that is measured by malondialdehyde (MDA) level (Ashouri et al., 2015). The results markedly showed enhanced SOD, CAT, and GPx activities and reduced MDA levels in fish fed dietary Se nanoparticles. The effect of dietary Se was observed in the R2 group, which fed Se nanoparticles every other day. In a similar sense, common carp (Ashouri et al., 2015), Nile tilapia (Dawood et al., 2020d), meagre (Mansour et al., 2017), and gilthead seabream (Saleh et al., 2015) fed dietary Se nanoparticles had enhanced antioxidative capacity.

The results showed that IL-1β and IL-8 genes were upregulated, and HSP70 was downregulated in the liver of European seabass fed dietary Se nanoparticles. These results attributed to the anti-stressor role of Se, which induced a reduced mRNA level of HSP70 gene expression (Ming et al., 2010). Additionally, the upregulated IL-1β and IL-8 genes revealed the anti-inflammatory role of Se nanoparticles on European seabass (Ethuin et al., 2001; Liu et al., 2005). However, the present study lacks stress challenges (e.g., disease challenge and environmental-related tests) required to understand Se nanoparticles' role to relieve stress-induced inflammation.

5. Conclusion

The study results efficiently dealt with an essential strategy that can be applied to guarantee the balanced aquafeed for better European seabass performances with the possible lowest cost. Fish fed dietary Se nanoparticles at 1 mg/kg either daily, every other day, or every other two days had enhanced growth rate, feed efficiency, and haemato-

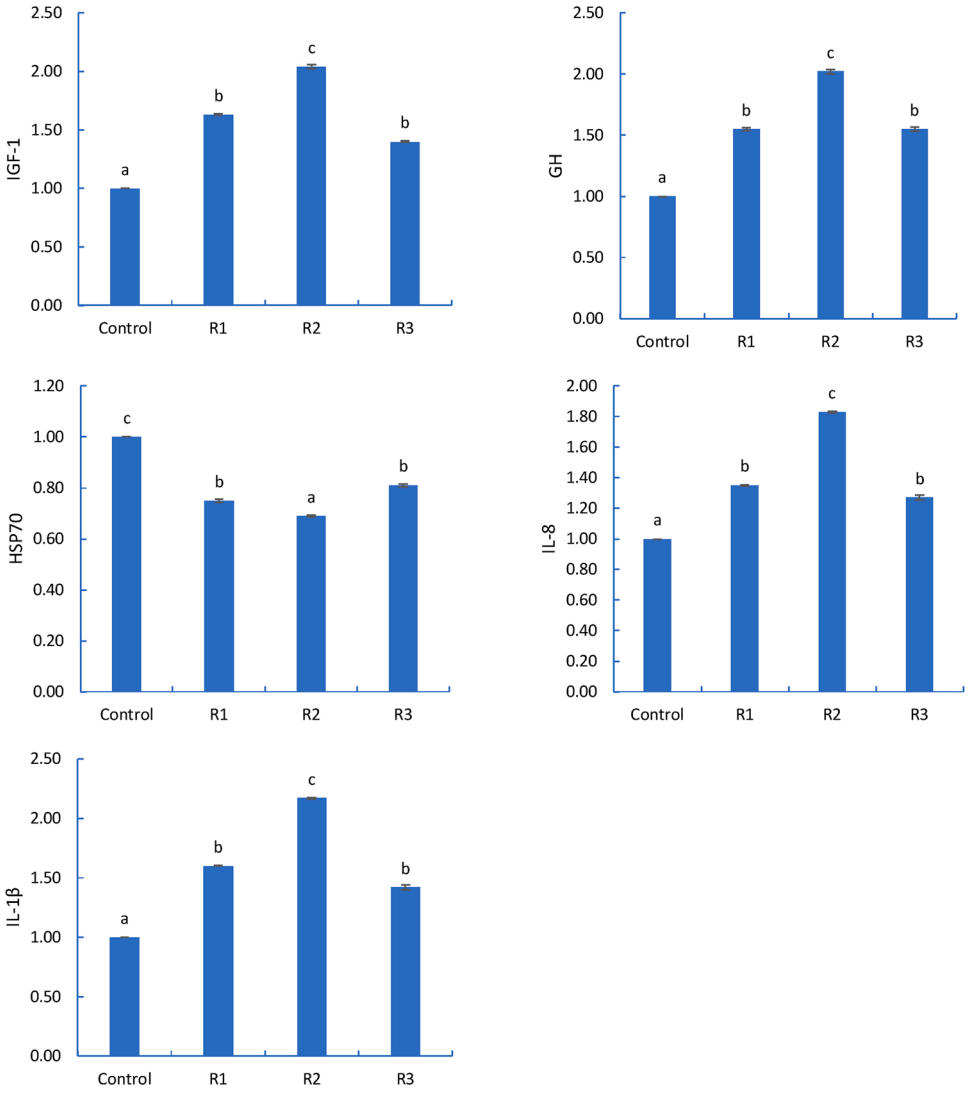

Fig. 1. Relative expression of GH, IGF−1, HSP70, IL−8, and IL−1β genes in European seabass fed diets enriched with different levels of Se nanoparticles for 90 days. Data are presented as mean ± S.E. (n=3). Bars with different superscript are significantly different (P<0.05).

biochemical indices. Further, the immune and antioxidative responses and anti-inflammatory impacts were enhanced by dietary Se nanoparticles in the same manner.

CRediT authorship contribution statement

Marwa F. Abd El-Kader: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision. **Ahmed. F. Fath El-Bab:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision. **Moustafa Shoukry:** Formal analysis, Investigation. **Abdel-Wahab A. Abdel-Warith:** Conceptualization, Funding acquisition, Investigation. **Elsayed M. Younis:** Conceptualization, Funding acquisition, Investigation. **Eman M. Moustafa:** Investigation. **Hanan B. El-Sawy:** Investigation. **Hamada A. Ahmed:** Investigation, Writing - original draft. **Hien Van Doan:** Investigation, Writing - original draft. **Mahmoud A.O. Dawood:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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