



Synergistic Effects of Selenium and Magnesium Nanoparticles on Growth, Digestive Enzymes, Some Serum Biochemical Parameters and Immunity of Asian Sea Bass (*Lates calcarifer*)

Hamed Deilamy Pour¹ · Seyed Mohammad Mousavi^{1,2} · Mohammad Zakeri¹ · Saeed Keyvanshokoh¹ · Preeta Kochanian¹

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Abstract

This study aimed to investigate the effect of selenium and magnesium nanoparticles and their combination on growth, serum biochemical and immune parameters, serum hepatic enzymes, and digestive enzymes in Asian Sea bass (*Lates calcarifer*), with an average weight of 32.78 ± 1.16 g for 42 days. After 4 weeks of adaptation to experimental conditions, 96 fish were randomly distributed equally in 12 cylindrical 300-l fiberglass tanks. Four treatment groups included control, 4 mg nano-selenium (Nano-Se), 500 mg nano-magnesium (Nano-Mg), and a combined treatment of 4 mg Nano-SE + 500 mg Nano-Mg treatments examined in this study. The fish fed twice a day as satiation. The results showed fish fed with combined treatment showed the highest rate of body weight gain and the specific growth rate. Digestive enzymes were significantly different between experimental treatments ($p < 0.05$), whereas amylase did not show a significant difference between experimental treatments ($p > 0.05$). The results of this study showed that serum levels of glucose, cholesterol, triglyceride, and protein indices did not show any significant difference ($p > 0.05$). The immunoglobulin, IgM, C3, and ACH50 indexes did not differ significantly between the experimental treatments ($p > 0.05$). But cortisol and lysozyme were significantly different between the experimental treatments ($p < 0.05$). Serum levels of SGPT, SGOT, ALP, and LDH decreased in comparison with the control group ($p < 0.05$). The results of the present study showed that adding Nano-Se and Nano-Mg to the diet of *L. calcarifer* had a positive effect on the growth and non-specific immune system with no disturbance in serum biochemical and hepatic enzymes parameters.

Keywords Nano-selenium · Nano-magnesium · *Lates calcarifer* · Digestive enzymes · Hepatic enzymes · Growth

Introduction

In an aquaculture farm, more than 50% of current expenses are related to food and nutrition. Diet quality and quantity are among the topics that can have a significant impact on growth rate and productivity. Also combining the right amounts of nutrients in a balanced diet improves this process [1, 2].

Selenium is a rare and essential micronutrient for humans and animals, and plays a significant role in the antioxidant

system, regulating thyroid hormone metabolism, and cellular growth [3]. Selenium also plays a key role in the growth mechanism, fertility, and immune system of farmed organisms. Selenium can be found naturally in foods and organic matter. Fish meal and seafood are also the best sources of selenium. However, some species, such as tuna, have poor biological access to selenium. Magnesium is an important cofactor for several enzymatic reactions. This trace element involves in activity of phosphokinase and phosphate hydrolysis to transfer phosphate groups. Also magnesium involves in activity of phosphokinase and pyrophosphatase for transfer pre-phosphate groups. It also activates the saturated fatty acids acetyl coenzyme A (thiokinase) and activates the synthesis of amino acids. The enzymatic function of magnesium is well established. Magnesium is also vital for skeletal tissue metabolism, osmotic regulation, and neuromuscular transmission [4]. Although smaller nanoparticles can absorb more and more easily from the intestinal wall, so, Nano-Mg can exacerbate the positive effects of magnesium and reduce the negative

✉ Seyed Mohammad Mousavi
seied1356@yahoo.com

¹ Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

² Excellence Center of Warm Water Fish Health and Diseases, Ahvaz, Iran

effects of magnesium [5]. With the advancement of nanotechnology, Nano-Se is widely considered for animal and fish nutrition because nanometer particles exhibit new properties such as high surface activity, high catalytic coefficient, strong adsorption capacity, and low toxicity [6, 7]. Nano-Se has been reported to have comparable efficacy with selenium and selenium-methylselenocysteine in regulating the mechanism of selenium enzymes but has been significantly reduced [7].

The Asian Sea bass (*Lates calcarifer*), also is known as Barramundi in Australia. *L. calcarifer* is a member of the Centropomidae family. This fish has a euryhaline and reported to spread from the west of the Indian Ocean to the Pacific Ocean from the Persian Gulf to China, Taiwan, Papua New Guinea, and northern Australia. Barramundi divided into the category of catadromous, which during the breeding season, leaves its habitat in freshwater and brackish water and migrates to seawater [8–11]. In latest years, Barramundi farming in low salinity or freshwater is expanding in southwestern area in Iran. Given the different physiological role of selenium and magnesium in farmed organisms, in the present study, the possibility of using Nano-Se and Nano-Mg in Asian Sea bass was investigated. This study aimed to investigate the effects of Nano-Se and Nano-Mg, individually and in combination together on growth, digestive enzymes, immune system, serum hepatic enzyme, and serum biochemical parameters of *L. calcarifer*.

Material and Methods

Experimental Trials

A commercial diet (Faradaneh Co.) for Asian Sea bass prepared as the basic diet. This diet contained 0.65 mg/kg selenium, and 207.3 mg/kg magnesium. The biochemical composition of the basic diet (Table 1) was analyzed according to the standard methods [12]. The basic diet grinded and mixed with

the Nano-Se (purity: 99.95%, particle size: 30–45 nm; prepared from Iranian Nanomaterials Pioneers Co.) and/or Nano-Mg (purity: 98%, particle size: 20 nm; prepared from Iranian Nanomaterials Pioneers Co.). Then, water was gradually added to the mixture to produce firm dough. The resulted firm dough mixed completely using a mixer for 10 min. The experimental diets were as follows: (1) control treatment group: without any nanoparticle supplements, (2) Nano-Se treatment group: 4 mg Nano-Se/kg food, (3) Nano-Mg treatment group: 500 mg Nano-Mg/kg food, and (4) combination treatment group: 4 mg Nano-Se/kg food + 500 mg Nano-Mg/kg food [5, 13]. Then, the mixture pelleted and dried at 25 °C. The dried pellets garbled into suitable pellet size and stored at refrigerator before use.

The Nano-Mg and Nano-Se mean particle sizes confirmed by a scanning electron microscopy [14].

The practical and rearing section of this experiment was conducted in the wet laboratory. The Juveniles of *L. calcarifer* were transferred from a local farm located in Khorramshahr, Iran to the wet laboratory. The fish adapted to freshwater (2 ppt) by gradually reducing the salinity level (initial salinity, 25 ppt) over 1-week period. Then, the fish acclimatize to the experimental condition and fed with the basal diet for 14 days. At the start of the trials, 10 fish (mean weight: 32.78 ± 0.16 g) distributed in each of 12 fiberglass 300-L tanks. The fish fed with experimental diets two times per day at 10:00 and 17:00 as satiation for 6 weeks. During the trials, the water quality parameters checked and recorded features maintained as daily (temperature 28.6 ± 0.8 °C, pH 7.2 ± 0.2 , and dissolved oxygen 7.8 ± 0.2 mg/L). Natural photoperiod was considered during the experiment. The rate of water exchange was 20% every 2 days.

Growth Parameter Analyses

At the end of the experiment, all animals in feeding trials, weighed for growth measurement. Growth performance was

Table 1 The dietary analysis used during the experimental period for Asian Sea bass juveniles and the amounts of nano-magnesium and nano-selenium used in different nutritional treatments. All of these values based

on milligrams per kilogram of diet, based on biochemical analysis and measurement of mineral values in the diet

Parameter	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
Nano-magnesium	207.3	235.7	685.3	740.8
Nano-selenium	0.65	4.44	0.73	4.36
Protein	508.9	509.7	511.3	506.2
Lipid	122.3	124.1	119.8	125.4
Ash	113.8	109.9	114.3	107.7
Moisture	71.6	68.4	70.1	67.8

*All data recorded on average from 3 replications

measured based on the standard formulae: Weight gain (WG) = $100 \times (\text{final weight} - \text{initial weight}) / (\text{initial weight})$; Specific growth rate (SGR) = $100 \times \ln(\text{final weight}) - \ln(\text{initial weight}) / \text{days}$; Survival rate = $100 \times (\text{final fish number} / \text{initial fish number})$.

Sampling

At the end of the experimental period and 1 day before sampling, the fish did not feed. Initially, the fish captured and transferred to the sampling site and anesthetized with 200 ppm Eugenol [15]. Blood sampling was performed with 2.5-ml syringe from caudal vein, and blood samples were collected to 1.5-ml microtubes and centrifuged at 3000 rpm for 10 min and sera collected. Serum samples stored at $-80\text{ }^{\circ}\text{C}$ until serum analyses.

Serum Biochemical Parameter Analyses

Serum levels of total protein, glucose, albumin, triglyceride, cholesterol, LDL, HDL, calcium, phosphorus, magnesium, urea, and creatinine were measured by the photometric method using biochemical auto analyzer (Mindray BS-200, China) with Diagnostic Kits (Pars Azmoon, Iran) [16, 17]. Serum levels of globulin calculated by deducting total serum albumin from total serum protein [18].

Immunological Parameter Assay

Serum Lysozyme Activity Assay

This measurement quantified using turbidity assay and based on the method recommended by Ellis (1990) [19]. To perform this analyses, 200 μL of *Micrococcus lysodeikticus* suspension (0.2 mg/ml sodium phosphate buffer 0.6 M, pH: 6.2) with 10 μL of serum samples in 96-well ELISA plates, mixed, and the optical absorption of the samples read after 1, and 6 min using an ELISA plate reader at 530 nm. PBS used as blank. Each unit of enzyme activity calculated as the amount of enzyme that reduces the absorption by 0.001 per min/ml of serum, in comparison with standard curves of egg white lysozyme concentrations (prepared by Sigma).

Complement Activity Assay

Serum C3 and C4 complement activities measured by a quantitative diagnostic kit (Pars Azmoon, Iran). In this experiment, the concentration of C3 and C4 determined by photometric measurement of the reaction between the antibodies sensitive to C3 and C4 in the kit and the serum C3 and C4 antigen, and assayed on the wavelength of 340 nm. C3 and C4 calculated in U/L [16].

ACH50 (Alternative complement activity) measured by the method described by Sunyer and Tort (1995) [20]. Serum complement volume determined based on 50% hemolysis, and the ACH50 units per ml of the sample, calculated. ACH50 activity measured by hemolysis of rabbit erythrocytes. Rabbit erythrocytes were washed using veronal buffer three times. Their cells adjusted by the Neubauer slides per ml of buffer to 2×10^8 cells/ml. First, the optical absorption of 100% rabbit lysate determined by adding 100 μL of the above suspension to 3.4 ml of distilled water. Then the serum samples diluted 100 times with the above buffer, and the volumes were prepared in a sterile test tube, and volume of all tubes adjusted to 250 μL by the buffer. Finally, 100 μL of rabbit erythrocyte was added to all tubes, and the mixture was placed at $20\text{ }^{\circ}\text{C}$ for 90 min. As a final point, 3.15 ml of 0.85% sodium chloride solution was added to each tube. The tubes were centrifuged at 1600g for 10 min at $4\text{ }^{\circ}\text{C}$ (Model 5417R, Eppendorf Co., Germany), and optical absorption of supernatant read at 414 nm. A volume of serum that causes 50% hemolysis (k) determined and used to calculate the ACH50 activity of the samples: $\text{ACH50, U/ml} = 0.5 \times k$.

Serum Total Immunoglobulin Level Assay

Total serum immunoglobulin level determined by Amar et al. method [21]. For this purpose, 100 μL of serum sample (diluted 1: 100 in phosphate buffer solution) incubated in 12% (V/V) polyethylene glycol solution for 2 h at room temperature. Immunoglobulin molecules precipitated by centrifugation (5000 rpm at $4\text{ }^{\circ}\text{C}$ for 10 min). Then, serum protein levels measured by the Biuret method. Total immunoglobulin level calculated based on subtraction of serum protein after immunoglobulin participation, from serum protein level before immunoglobulin participation. The results presented as mg/ml.

Measurement of Serum Cortisol

To measure cortisol, a colorimetric method [22] and a special cortisol measuring kit of Pars Azmoon company were used using Eliza Reader device (Mindary MR-69A, China) with a wavelength of 450 nm.

Serum Hepatic Enzyme Analyses

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) measured by quantitative diagnostic kits (Pars Azmoon, Iran) [23].

Digestive Enzyme Activity Analyses

The intestine of the fish homogenized into buffer solution (KCl 50 mM, Tris-HCl 50 mM, and CaCl 20 mM, pH: 7.5)

at 9:1 (v/w) ratio using a homogenizer (IKA, Ultra-turrax®, USA). The homogenates centrifuged at 12,000 rpm for 10 min at 4 °C. Then, the supernatant separated and poured into 1.5-ml microtubes and administered for enzyme analyses. The protein content of the extract assayed by the Bradford method (1976) [24]. Activity of trypsin, chymotrypsin, lipase, α -amylase, and alkaline phosphatase (ALP) measured in intestine tissue extract. Trypsin activity evaluated with N α -Benzoyl-L-arginine ethyl ester (BAEE). For this purpose, the intestine tissue extract incubated for 2 min at 25 °C in 2 mL of the Tris buffer (pH: 8.1). Chymotrypsin activity evaluated using N-Benzoyl-L-tyrosine ethyl ester (BTEE) as substrate. For chymotrypsin activity assay, the tissue extract incubated for 2 min in 2 mL of Tris buffer (pH: 7.8). The activity of trypsin and chymotrypsin enzyme evaluated at wavelength of 253, and 256 nm, respectively [25]. Lipase, amylase, and ALP activities evaluated using photometric diagnostic kits (Pars Azmoon, Tehran, Iran), by using a biochemical auto-analyzer (Mindray BS-200, China) at a wavelength of 580, 405, and 405 nm, respectively [26].

Data Analyses

Each tank considered as a test unit, and the results reported as mean \pm standard error. In this study, all statistical calculations performed using SPSS software version 20. One-way ANOVA used to compare the means and the Duncan test to examine the presence or absence of a significant difference between the treatments. The significance level in this study considered to be less than 0.05.

Results

Growth Parameters

The effect of different levels of Nano-SE and Nano-Mg on growth performance was presented in Table 2. At the beginning of the experimental period, there was not any significant

difference among the four experimental treatments in terms of initial mean weight ($p > 0.05$). At the end of the experimental period, fish fed with combined selenium nanoparticles and magnesium nanoparticles showed the highest rate of body weight gain ($131.33 \pm 26.58\%$) and the specific growth rate ($2.08 \pm 0.28\%/day$) ($p < 0.05$). Also, the survival rate did not significantly differ between experimental treatments. Although, the results showed that Se and Mg nanoparticles (individually or in combination together) improve survival rate.

Serum Biochemical Parameters

The effect of supplementation of selenium and magnesium nanoparticles on serum biochemical composition was presented in Table 3. According to the results of measuring these parameters, there was no significant difference in experimental treatments and control groups for glucose, cholesterol, triglycerides, and protein levels ($p > 0.05$). The lowest amount of calcium level was observed in the combined treatment and showed a significant difference with other treatments. The highest amount of phosphorus was observed in the control treatment and there was a significant difference with other treatments ($p < 0.05$). The highest amount of albumin was in the Nano-Se treatment, and the lowest amount was seen in the combined treatment, which showed a significant difference with other treatments ($p < 0.05$).

Serum Hepatic Enzyme Analyses

According to the results obtained at the end of the experiment, the highest levels of SGOT, ALP, and LDH were observed in the control treatment ($p < 0.05$). Also, there was no significant difference between SGPT levels in different treatments ($p < 0.05$) (Table 4).

Serum Immune Parameters

The results of this study showed that there was no significant difference between globulin, IgM, C3, and ACH50 and

Table 2 Growth parameters of Asian Sea bass juveniles fed with diets supplemented with selenium and magnesium nanoparticles (mean \pm SE, $n = 3$)

Growth parameter	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
Initial weight (g)	33.02 \pm 2.35	34.33 \pm 1.21	31.94 \pm 3.56	31.83 \pm 0.41
Final weight (g)	54.22 \pm 4.41 ^b	75.02 \pm 2.40 ^a	64.36 \pm 3.92 ^{ab}	73.69 \pm 9.32 ^a
Weight gain (%)	65.01 \pm 21.26 ^b	118.77 \pm 13.01 ^a	103.91 \pm 32.91 ^{ab}	131.33 \pm 26.58 ^a
SGR (%/day)	1.23 \pm 0.32 ^b	1.95 \pm 0.14 ^a	1.75 \pm 0.42 ^{ab}	2.08 \pm 0.28 ^a
Survival rate (%)	74.07 \pm 27.96	88.88 \pm 19.24	77.77 \pm 19.24	85.18 \pm 6.41

*Different letters in each row indicate a significant difference between the experimental groups (mean \pm S.E) ($p < 0.05$)

Table 3 Serum biochemical parameters of Asian Sea bass juveniles fed with diets supplemented with selenium and magnesium nanoparticles (mean \pm SE, $n = 3$)

Serum biochemical parameter	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
Glucose (mg/dl)	72.33 \pm 20.30	82.00 \pm 14.18	79.67 \pm 12.91	54.33 \pm 7.31
Urea (mg/dl)	12.43 \pm 1.50 ^{ab}	9.23 \pm 1.39 ^b	16.16 \pm 0.98 ^a	14.96 \pm 0.97 ^a
Cholesterol (mg/dl)	130.33 \pm 19.09	132.33 \pm 14.31	124.00 \pm 7.02	100.33 \pm 6.64
Triglyceride (mg/dl)	242.00 \pm 78.25	225.00 \pm 14.74	172.00 \pm 43.24	149.00 \pm 25.48
Calcium (mg/dl)	11.66 \pm 0.88 ^a	11.60 \pm 0.20 ^a	11.56 \pm 0.28 ^a	10.03 \pm 0.31 ^b
Phosphorous (mg/dl)	10.86 \pm 0.46 ^a	7.80 \pm 0.36 ^b	8.83 \pm 1.13 ^{ab}	7.56 \pm 0.40 ^b
Protein (g/dl)	4.47 \pm 0.18	4.53 \pm 0.08	4.16 \pm 0.02	4.00 \pm 0.45
Albumin (g/dl)	1.97 \pm 0.16 ^a	2.11 \pm 0.13 ^a	1.97 \pm 0.28 ^a	1.25 \pm 0.01 ^b

*Different letters in each row indicate a significant difference between the experimental groups (mean \pm S.E) ($p < 0.05$)

different treatments ($p > 0.05$). However, in the case of lysozyme, the highest amount was observed in the combined treatment and the lowest in the control treatment ($p < 0.05$). Cortisol has been shown to be the most abundant in control treatments and Nano-Mg, respectively, and the lowest was in Nano-Se treatment and combined treatment ($p < 0.05$) (Table 5).

Digestive Enzyme Activity

In this study, the lowest amount of ALP was observed in the combined treatment and was significantly different from other treatments ($p < 0.05$). The highest amount of lipase was observed in Nano-Mg treatment, which showed a significant difference with other treatments ($p < 0.05$). The maximum amount of trypsin enzyme was observed in the treatment of Nano-Mg and the minimum in the Nano-SE treatment ($p < 0.05$). The highest levels of the chymotrypsin were observed in the Nano-Mg treatment, and the lowest levels were observed in the control treatment ($p < 0.05$). Also, the amount of amylase did not show a significant difference between the four experimental treatments ($p > 0.05$) (Table 6).

Table 4 Serum hepatic enzymes levels of Asian Sea bass juveniles fed with diets supplemented with selenium and magnesium nanoparticles (mean \pm SE, $n = 3$)

Serum hepatic enzymes	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
SGOT (U/L)	130.33 \pm 8.51 ^a	97.67 \pm 10.83 ^{ab}	66.67 \pm 14.17 ^b	98.67 \pm 19.85 ^{ab}
SGPT (U/L)	6.67 \pm 1.76	8.67 \pm 0.88	6.33 \pm 2.02	9.00 \pm 0.57
ALP (U/L)	147.33 \pm 20.49 ^a	93.33 \pm 2.40 ^b	121.67 \pm 6.33 ^{ab}	104.67 \pm 18.27 ^{ab}
LDH (U/L)	3181.00 \pm 144.58 ^a	2518.00 \pm 621.50 ^{ab}	1214.67 \pm 68.33 ^b	3041.00 \pm 752.55 ^a

*Different letters in each row indicate a significant difference between the experimental groups (mean \pm S.E) ($p < 0.05$)

Discussion

Growth Parameters

In the growth parameters, the highest values were observed in the combined treatment of Nano-Se and Nano-Mg ($p > 0.05$). BW% and SGR indicating the nutritional status of fish [1]. Given these high characteristics in the Se nanoparticle group and the combined group of Mg and Se nanoparticles, it can be said that these treatments had better growth conditions than the control treatment. The results coincided with the results of some other studies on hybrid striped bass: *Morone chrysops* x *Morone saxatilis* [27], *Rachycentron canadum* [28], *Carassius gibelio* [29], *Cyprinus carpio* [30], *Lates calcarifer* [31], *Carassius auratus* [32], and *Lates calcarifer* [33].

Due to the properties of Se nanoparticles (high surface activity, high catalytic coefficient, strong adsorption capacity, and low toxicity) [6, 7] and comparable better efficiency to Se and Se-methyl selenocysteine in regulating the mechanism of Se-enzymes [7], the increase in growth in treatments supplemented with Nano-Se and Nano-Mg is quite justified. Mg is an important cofactor for many enzymatic reactions and involves the transfer of phosphate and pre-phosphate groups. It

Table 5 Serum immune parameters of Asian Sea bass juveniles fed with diets supplemented with selenium and magnesium nanoparticles (mean \pm SE, $n = 3$)

Serum immune parameters	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
Globulin (g/dl)	2.50 \pm 0.17	2.42 \pm 0.17	2.19 \pm 0.29	2.75 \pm 0.45
Ig (μ g/ml)	35.80 \pm 1.82	37.18 \pm 1.82	35.79 \pm 0.78	34.82 \pm 2.82
Lysozyme (μ g/ml)	0.04 \pm 0.00 ^c	0.07 \pm 0.00 ^{ab}	0.06 \pm 0.01 ^{bc}	0.09 \pm 0.01 ^a
C3 (mg/dl)	28.66 \pm 0.88	29.66 \pm 0.33	25.33 \pm 4.63	30.33 \pm 1.20
ACH50 (μ g/ml)	31.70 \pm 0.70	31.33 \pm 1.38	30.97 \pm 1.06	32.44 \pm 0.48
Cortisol (ng/ml)	60.00 \pm 3.60 ^a	43.33 \pm 1.20 ^b	58.30 \pm 2.40 ^a	47.67 \pm 0.88 ^b

*Different letters in each row indicate a significant difference between the experimental groups (mean \pm S.E) ($p < 0.05$)

also activates the saturated fatty acids acetyl coenzyme A (thiokinase) and activates the synthesis of amino acids. The enzymatic function of Mg is well established. Mg is also vital for skeletal tissue metabolism, osmotic regulation, and neuromuscular transmission [4]. A closer look reveals that the level of Nano-Mg used in this study is much lower than the Mg levels used in other researches. Nanoparticles, despite their smaller particles, can absorb more and more easily from the intestinal wall, intensifying the positive effects of Mg and reducing the negative effects of Mg [5]. Also, although *Barramundi* is a marine catadromous species, this fish adapted and reared in freshwater in the current study. The basic diet used for this experiment contained an appropriate Mg concentration (207.3 mg Mg/kg food), which is similar to the previous report for dietary levels of Mg requirement in some freshwater fish species (200–700 mg Mg/kg food) [33]. This specifies that the Mg content of the basic diet was adequate for the Mg requirement of Asian Sea bass when reared in freshwater.

The possible reason for such a beneficial role is the increase in activity of growth hormones by Mg nanoparticles [34]. Reigh et al. showed the critical effects of magnesium on appetite and growth performance [35]. Mg plays a critical role in the normal metabolism of lipids, proteins, and carbohydrates as a cofactor in a large number of enzymatic and metabolic reactions [36]. Mg influences cell division and plays a critical role as a cofactor in pathways of many enzymes involved in cell metabolism and in energy production [37]. In acclimation to low salinity media, more protein would be required to the hemolymph as a source of amino acids to maintain the osmotic pressure, which reduced growth because of the loss of amino acids from muscle [38]. Therefore, in this study, supplementation of Mg to diets improved growth performance and feed efficiency of fish, perhaps owing to the sparing action of Mg on nutrient consumption in osmotic regulation. Also, the higher growth of tilapia fed the selenium-supplemented diet can be explained by the fact that selenium is an important micronutrient that is needed for the better growth performance and feeding parameters of fish. Its supplementation enhances

erythrocytes production and prevents high quantity fat accumulation in the liver tissue [39]. Also, it is an important constituent of the deiodinase enzyme, which is necessary for the proper functioning of the thyroid hormones [40], and the thyroid hormones are required for growth hormone secretion from the pituitary gland [41]; thus, selenium is indirectly involved in the secretion of the growth hormone [42].

Gastric Enzyme Activity

At the end of the trial period, the highest and lowest levels of the activity of the ALP were observed in the treatment of Nano-Se and the Nano-Se and Nano-Mg combination. The highest activity rates of trypsin, chymotrypsin, and lipase were found in the treatment of Nano-Mg. Also, the activity of amylase did not show a significant difference between the four experimental treatments. The level of digestive enzyme activity depends on several aspects such as the intrinsic properties of the enzyme, its production, and secretion [43]. Adkins and Ewan (1984) examined the effect of selenium on pancreatic pig digestive enzymes [44]. The results showed that supplementing selenium did not have any effect on trypsin, chymotrypsin, amylase, and lipase activity in the pancreas.

Serum Biochemical Parameters

Serum biochemical parameters in fish may change under the influence of physiological factors such as age, size, sex, reproductive stage, and health status [45]. These factors also changed under the influence of external factors such as season, water temperature, environmental conditions, food, stress, types of pollution, and diseases [46]. Any changes in albumin, globulin, and total plasma protein levels can use as a clinical indicator in monitoring the health of the immune system, liver, and kidneys [47]. In the present study, among the measured biochemical parameters, glucose, cholesterol, triglycerides, and total protein at the end of the experiment did not have a significant difference between the experimental treatments ($p > 0.05$). The highest serum

urea levels were observed in the treatment of 500 mg Nano-Mg, and the lowest urea was observed in 4 mg Nano-Se. Uric acid, which produced in fish from endogenous and exogenous purine nucleotides as well as catabolism of proteins by purines, converted to urea in the liver and a lesser extent in the kidneys and excreted through gills. Due to the positive effects of Se on the cell membrane of body tissues and the inhibition of the activity of enzymes such as glutathione peroxidase [48], especially in liver tissue, low urea levels can be related to Se's ability to reduce liver damage and subsequently, decreased urea levels. Also, due to the effect of Mg as a cofactor in enzymes complicated in protein metabolism [49], high urea levels in Nano-Mg-treated treatments are justifiable. Serum calcium levels have shown a significant decrease in combined treatment in comparison with other treatments ($p > 0.05$). The balance between salts and minerals in aquatic food can indirectly affect the serum levels of mineral elements [50]. Also, serum phosphorus levels in all nutritional treatments of Nano-Se and Nano-Mg had a significant decrease compared to the control treatment, which can be found in the imbalance of minerals in the diet, and competition between Ca and Mg absorption from intestinal mucosa and the effect on phosphorus absorption from intestinal mucosa is stated [50]. The lowest serum albumin levels were observed in the combined treatment. The optimal amount of Se is a protective agent against heavy metals through the synthesis of glutathione peroxidase. However, high levels of Se can have negative effects on physiological indicators [51]. Changes in plasma protein concentrations, especially albumin, are proportional to changes in total plasma Ca concentrations. In the opposite direction of increasing albumin in plasma, it is linked with an increase in plasma Ca concentration [49]. Butler et al. also showed a significant positive correlation between Ca and albumin [52], which coincided with the results obtained in the current study. Total protein, albumin, triglycerides, glucose, and cholesterol in the present study were not affected by the nanoparticles in the diet, which could be due to possible causes such as test duration, nanoparticle size, the dose used and type of nanoparticles, which coincided with the results reported by Longbaf Dezfooli et al. [33].

Serum Hepatic Enzyme Activity

At the end of the trial period, the highest levels of SGOT, ALP, and LDH were observed in the control treatment group. Also, there was not any significant difference between SGPT levels and different treatments. Serum SGPT and SGOT belong to plasma inactive enzymes that are naturally present in the hepatic cells, heart, kidneys, and other organs [53]. Wells et al. reported that increased SGPT and plasma SGOT activity probably provided information about liver damage or liver dysfunction [54]. In the current study, similar to Zhang et al. study, serum SGOT enzyme activity did not increase with the use of Mg and Se nanoparticles in the diet [5], which indicates a better process of liver activity by adding these nanoparticles, and this may be

related to the increased antioxidant performance of these nanoparticles, membrane stabilization, and cell damage prevention [55]. Increased SGPT and creatine kinase activity indicate the induction of transamination processes that occur as amino acid input to the tri-carboxylic acid cycle. Kouba et al., similar to the current study, observed the highest SGOT activity rate in the control group [56] and, contrary to the present study, the highest SGPT and LDH activity in 1 g Nano-Se-consuming treatments with microalgae and 0.3 g of Se. Ashouri et al., in contrast to the current study, obtained the highest levels of SGOT, SGPT, and ALP activity in *Cyprinus carpio* serum fed 2 mg of Nano-Se per kg of food [30]. A significant increase in serum ALP, SGOT, and SGPT activity is thought to be the body's response to stressors [57]. Therefore, it can be stated that the health status of the liver in fish that used nanoparticles was better than the control group.

Serum Immune Responses

The results of the current study showed that at the end of the experimental period, the amount of globulin, IgM, C3, and ACH50 did not differ significantly between different treatments. The results of this study are inconsistent with the results of Longbaf Dezfooli et al. in *Lates calcarifer* [33]. Lovell and Wang reported a significant increase in antibody production and survival rate in catfish infected with the *Edwardsiella ictaluri* upon feeding with dietary selenium levels [58]. Ashouri et al. measured the biochemical characteristics of blood by adding different levels of Se nanoparticles to a common carp diet [30]. At the end of the experimental period, the highest biochemical index of globulin obtained in the treatment of 2 g of Se per kg of diet. Impairment of globulin, IgM, C3, and ACH50 parameters between different researches can be due to possible reasons such as the length of the test period, the size of the nanoparticles, the dose used, and the type of nanoparticles. The highest amount of lysozyme was observed in the combined treatment and showed a significant difference with other treatments. Dietary Se is essential for optimal immune response, although mechanisms are not fully understood, but Se affects innate and acquired immune systems [59, 60]. The highest levels of cortisol were observed in the control treatment and showed a significant difference. Sathya et al. stated in their study that adding Se to the buffalo diet reduced oxidative stress and plasma cortisol levels [61]. Increasing the lipid peroxidation under stress conditions stimulates the stress axis and leads to an increase in cortisol concentrations [62]. Decreases in lipid peroxidation following the use of vitamin E and Se, as well as a further reduction in MDA, can also reduce cortisol concentrations [61].

Se plays an important role in the formation of several important types of selenoproteins such as glutathione peroxidase and thioredoxin reductase [63] and protects the body against oxidative stress [64]. Wang et al. found that dietary Mg supplementation could improve the antioxidant capacity of juvenile grass carp by increasing antioxidase activities [65]. Likewise, in this study,

Table 6 Gastric enzymes activity levels of Asian Sea bass juveniles fed with diets supplemented with selenium and magnesium nanoparticles (mean \pm SE, $n = 3$)

Growth parameter	Experimental treatments			
	Control	Nano-Se	Nano-Mg	Nano-Se + Nano-Mg
ALP (U/mg protein)	507.45 \pm 112.16 ^a	611.06 \pm 82.00 ^a	498.52 \pm 79.59 ^a	145.24 \pm 17.70 ^b
Amylase (U/mg protein)	4.20 \pm 1.57	1.91 \pm 0.43	2.35 \pm 0.04	2.48 \pm 0.04
Lipase (U/mg protein)	0.53 \pm 0.01 ^b	0.53 \pm 0.04 ^b	0.86 \pm 0.03 ^a	0.47 \pm 0.11 ^b
Trypsin (U/mg protein)	0.050 \pm 0.002 ^{ab}	0.044 \pm 0.002 ^b	0.054 \pm 0.001 ^a	0.047 \pm 0.002 ^{ab}
Chymotrypsin(U/mg protein)	0.168 \pm 0.024 ^b	0.197 \pm 0.004 ^{ab}	0.214 \pm 0.004 ^a	0.181 \pm 0.008 ^{ab}

*Different letters in each row indicate a significant difference between the experimental groups (mean \pm S.E) ($p < 0.05$)

supplementation of dietary Mg might promote growth performance by reducing oxidative stress of Japanese seabass fed oxidized oil diets. Also, numerous researches reported that Mg could reduce oxidative stress and free radicals in organisms [66]. An increase in supplemented Se led to an increase in stress tolerance, as shown by the decreased cortisol levels. Se plays an important role to prevent oxidative stress and, in chinook salmon (*Oncorhynchus tshawytscha*) has been found to induce a loss of whole-body Se. Se supplementation has been reported to allow salmonids to better cope with stressful conditions caused by handling [67].

Conclusion

In general, the administration of combination of Se and Mg nanoparticles improve growth indices. However, the growth parameters indicated that there is not any significant difference between nano-selenium treatment and combined treatment. Also, Mg nanoparticles improve growth indices, but there is not any significant difference with control group. The results of this study specified that the Mg content of the basic diet was adequate for the Mg requirement of Asian sea bass when reared in freshwater. Thus, the results confirmed that extra levels of magnesium in diet could not significantly affect growth and other analyses parameters during the period of the study. Also, using Se and Mg nanoparticles (individually or in combination together) improves survival rate. The supplementation of Se and Mg nanoparticles had no negative effect on glucose, cholesterol, triglycerides, and protein. The results showed that the use of Se and Mg nanoparticles in the diet had positive effects on the non-specific immune system, serum biochemical composition, hepatic enzymes, and digestive enzymes of *L. calcarifer*. Finally, this research presented that combination of Se and Mg nanoparticles may be proposed as a nutrient additive to *L. calcarifer* farms.

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Compliance with Ethical Standards

Conflicts of Interest/Competing Interests The authors declare that they have no conflict of interest.

Ethics Approval The authors of the manuscript declared that the experimental conditions were standard and ethical statements about experimental animals were observed during the experimental. The minimum samples were collected and the animals were anesthetized before any sampling.

Consent to Participate All authors of the manuscript are aware from submission of this manuscript.

Consent for Publication I hereby accept liability for the scientific integrity of the manuscript contents. I hereby declared that this manuscript is an original research article which has not been submitted or published in other journals. Also, my study does not involve human subjects. This manuscript is resulted from Research Project under financial support of Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.

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