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RESEARCH ARTICLE

Effects of selenomethionine on the growth, oxidative potential, digestive enzyme activity, and immune status of juvenile sterlet (*Acipenser ruthenus*)

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Abstract. In a 56-day experiment, the effects of selenium-enriched yeast (SeY) on the growth, enzymatic activity of the gastrointestinal tract, oxidative stress parameters, and selected immunological parameters of sterlet, *Acipenser ruthenus* (L.), were analyzed. The concentration of selenomethionine (SeMet) from SeY in the feed was 0 mg kg^{-1} (C), 0.7 mg kg^{-1} feed (S1), 1.4 mg kg^{-1} feed (S2), and 2.1 mg kg^{-1} feed (S3). Statistically significant differences were found in the final fish body weight between the control and groups S1 and S3. A significant increase in lipase activity was found in the initial section of the small intestine of fish from group S3. The highest activity of the antioxidant enzymes glutathione peroxidase and superoxide dismutase was recorded in the liver of fish from group S2. Analyses of non-specific humoral response parameters showed an increase in ceruloplasmin activity in group S1. The lowest active dose of SeMet derived from yeast tested in this study that stimulated humoral immunity and fish growth was 0.7 mg kg^{-1} feed (S1).

Keywords: antioxidant enzymes, growth parameters, gut enzymes, immunological assays, selenium-enriched yeast, selenomethionine, sturgeon

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Introduction

Selenium (Se) in fish nutrition is of interest because it is an essential mineral that plays a crucial role in how animal bodies function (Rotruck et al. 1973, Watanabe et al. 1997). It is a core component of glutathione peroxidase (GPx), the function of which is to protect cells from the damaging effects of peroxides produced by biochemical processes (Rotruck et

al. 1973, et al. 2008). GPx includes selenocysteine (SeCys), which enables the reduction of both hydrogen peroxide and organic peroxides (Watanabe et al. 1997). Thus, Se deficiency in fish feed can lead to an impaired ability in fishes to protect themselves against oxidative stress. Fishes reared in intense farming conditions that expose them to a range of additional stressors can require higher amounts of dietary Se (Rider et al. 2009). Unsurprisingly, the positive effects of Se supplementation, on both fish growth and immune parameters, are confirmed in many fish species (Jaramillo et al. 2009, Han et al. 2011, Wang et al. 2013, Naderi et al. 2017a).

Excessive Se, however, is associated with toxicity mainly manifested by inhibited growth, reduced feeding efficiency, and increased mortality (Hilton et al. 1980). Long-term exposure of fishes to excess Se can cause kidney calcification that can lead to degeneration in the organ (Hilton and Hodson 1983). Therefore, it is important to determine the precise requirements and intake for fish species, ages, and habitat conditions and the forms in which supplements are administered.

Se is found in numerous nutrients in various chemical forms. Its inorganic forms are selenides and selenates, while its organic forms are complexes containing the protein selenomethionine (SeMet), selenium-methyl-selenium-L-methionine, SeCys, and selenocystine (Watanabe et al. 1997). The bioavailability of Se supplements, and their effectiveness, depends largely on the chemical forms in which they are supplied to fishes. Organic forms of Se are more bioavailable to them than are inorganic forms (Lorentzen et al. 1994, Wang and Lovell 1997), and Bell and Cowey (1989) showed that SeMet was the most bioavailable. Le and Fotedar (2014) report similar results in a study on the bioavailability of different forms of Se in yellowtail amberjack (*Seriola lalandi* (Val.)). SeMet increases glutathione peroxidase activity most significantly and, consequently, increases the resistance of fishes to oxidative stress that can lead to immunosuppression.

A *Saccharomyces cerevisiae* strain is used to produce fodder selenium-enriched yeast (SeY), which is cultured on a suitable medium (molasses or gluten) with inorganic Se salts. During fermentation, the yeast absorbs Se and incorporates it in various forms into its structures predominantly in the form of selenomethionine SeMet, which accounts for about 90% of the element absorbed. SeCys is another form that is produced in much smaller amounts. The remaining Se, which is not absorbed and occurs in inorganic form, should not exceed 2% of the total content of the micronutrient in SeY supplements (Dobrzański et al. 2006).

Inorganic Se fish feed additives are garnering a lot of attention in aquaculture, while there are few reports about organic SeY being used as a dietary supplement. The organic Se in yeast has a similar level of bioavailability as pure SeMet; therefore, using it in fish nutrition permits almost halving the dose compared to the inorganic form. Consequently, the organic form reduces contamination in RAS (Wang and Lovell 1997).

In consideration of the important role of Se in fish nutrition, many studies have been conducted to determine the Se requirements of different fish species, e.g., rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Hilton et al. 1980); channel catfish, *Ictalurus punctatus* (Raf.) (Gatlin and Wilson 1984); malabar grouper, *Epinephelus malabaricus* (Bloch and Schneider) (Lin and Shiau 2005); yellowtail amberjack, *S. lalandi* (Le and Fotedar 2014); and common carp, *Cyprinus carpio* (L.) (Ashouri et al. 2015). Very few studies on the response of sturgeons to Se supplementation have been conducted to date. Tashijan et al. (2006) report that Se fed to white sturgeon, *Acipenser transmontanus* (Richardson), exhibits toxicity at levels between 10 and 20 g Se g^{-1} based on histopathological analyses of kidney tissues. Huang et al. (2012) present the issue of bioavailability and the accumulation and utilization of different forms of Se given to white sturgeon in single doses. In contrast, Arshad et al. (2011) showed positive effects of Se supplementation at doses of 11.56 and 20.26 g Se g^{-1} on the growth and survival of juvenile beluga, *Huso huso* (L.). However, the effect of Se supplementation has not been completely investigated in sterlet, *Acipenser ruthenus* (L.) (Hung 2017).

Sturgeons are biologically and economically important fishes, yet most species are rare in their

native range or critically threatened with extinction. Sterlet is a unique sturgeon that spends its entire life rivers, grows to a relatively small size (for sturgeons), and matures relatively quickly. Because of these characteristics, it is used in aquaculture to create hybrids that are economically important (bester, *Husso husso x Acipenser ruthenus* hybrid).

The present study aimed to determine the effect of long-term supplementation with different doses of SE in the form of SeY on sterlet immune parameters (humoral and cellular immunity), hematology, oxidative stress response, digestive enzymes, and growth.

Materials and Methods

Fish and rearing conditions

The sterlet was reared initially at the Wąsosze Fish Farm near Konin. When the fish reached an average body weight of approximately 2 g, they were transported in oxygenated bags to the Department of Ichthyology, Hydrobiology and Aquatic Ecology of the National Inland Fisheries Research Institute in Olsztyn. Before starting the experiment, the fish were acclimatized to the new conditions for two weeks. A total of 360 sterlet of an average initial body weight of approximately 2.6 g were selected for the experiment. The experiment consisted of four feeding variants (three experimental groups S1, S2, and S3 and control group C) in triplicate (n=3). Thirty individuals were placed in twelve 280 dm^3 tanks.

The physical and chemical parameters of the water were measured at the outlet of the rearing tanks during the experiment. The temperature was 18.0°C ± 0.1, oxygen content was 6.12 ± 0.44 mg O_2 dm^{-3}, concentrations of ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) and nitrite nitrogen (NO_2-N) were 0.152 ± 0.074 mg TAN dm^{-3}; 0.011 ± 0.012 NO_2-N dm^{-3}, respectively. The water pH ranged between 7.4 and 7.5. Temperature, oxygen content, and pH were measured daily with a YSI ProDSS probe. Every seven days nitrite and ammonium nitrogen were measured spectrophotometrically (Epoll Eco-20 and Shimadzu UV-1601). The light intensity above the tank water surface was 60 lx. The experiment lasted for eight weeks.

Experimental design and diets

Commercial feed Nutra T-2.0 (Skretting, France) with a basic composition of 54% protein and 18% fat was supplemented with SeY (SELEMAX, Poland). The concentration of the substance tested was 0.7 mg kg^{-1} feed (group S1); 1.4 mg kg^{-1} feed (group S2), and 2.1 mg kg^{-1} feed (group S3). SeY was mixed with distilled water (300 ml) and added to 1 kg of feed. The entire mixture was stirred until a homogeneous mass was obtained, and granules with a diameter of approximately 1 mm were formed mechanically. The

Table 1

Proximate composition (g kg^{-1} dry weight) of experimental diets containing 0.7 mg kg^{-1} (S1); 1.4 mg kg^{-1} (S2), and 2.1 mg kg^{-1} (S3) of selenomethionine from selenium yeast

	Experimental diets			
Proximate composition	C	S1	S2	S3
Crude protein	540.0	540.0	540.0	540.0
Crude lipid	180.0	180.0	180.0	180.0
Crude fiber	10.0	10.0	10.0	10.0
Crude ash	115.0	115.0	115.0	115.0
NFE[1]	155.0	155.0	155.0	155.0
Gross energy2 (MJ kg^{-1})[2]	22.6	22.6	22.6	22.6
Selenomethionine (Se-Met)[3]	0.0	0.7	1.4	2.1

[1] NFE (Nitrogen free extracts) = 100 – (crude protein + crude lipid + crude fiber + crude ash) (Shearer, 1994)

[2] Gross energy was calculated from the chemical composition using the following energy conversion factors: 24 kJ g^{-1} proteins, 39 kJ g^{-1} lipids, and 17 kJ g^{-1} NFE (Jobling 1994)

[3] SeMet value is given in mg kg^{-1} feed

prepared feed was then dried for 24 h at room temperature. The control group (group C) was fed only the base feed that was subjected to the same procedure without the addition of SeY. The chemical compositions of the feeds were similar (Table 1). The feed was then stored in a refrigerator at 4°C.

The fish were fed with belt feeders (4305 FIAP; Fish Technic Gmbh, Ursensollen, Germany) for 18 h per day (08:00-02:00). Daily feeding rates decreased with fish growth and were from 2.5 to 1.5% of the stocking biomass. Feeding was stopped 24 hours before the final sampling.

Experimental procedures

Rearing parameters

At the beginning and end of the experiment, fish body weights (W; ± 0.01 g) and total lengths (TL; ± 0.1 cm) were measured. Every seven days, mean weights only were measured to determine feed rations. The main measurements were taken at the end on the trials after the fish were euthanized. Rearing indices were calculated according to the following formulas: daily growth rate (DGR, g d^{-1}) = ($W_f - W_i$) × T^{-1}; specific growth rate (SGR, % d^{-1}) = 100 × [($\ln W_2 - \ln W_1$) × t^{-1}]; condition factor (CF) = (W × 100) × TL^{-3}; feed conversion ratio (FCR) = TFI × (FB – IB)$^{-1}$; protein efficiency ratio (PER) = (FB – IB) × TFP^{-1}; where: Wi – initial mean body weight (g), Wf – final mean body weight (g), T – rearing time (d), W – body weight (g), TL – total length (cm), FB – final stocking biomass (g), IB – initial stocking biomass (g), TFI – total weight of feed (g).

Activities of digestive and liver antioxidant enzymes

To determine the metabolic profile of the gastrointestinal tract, sections of the anterior and posterior intestines and liver samples were collected from 10 fish from each experimental group and fixed in liquid nitrogen. This material was stored at -80°C until analysis. Subsequently, the material was homogenized in distilled water and centrifuged at 14,000 × g for 10 min at 4°C. The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were examined using a SPINREACT kit. Lipase (LIP) and trypsin (TRYP) activities were analyzed with the methodology described in Kamaszewski et al. (2014). Liver enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPX), were determined using a RANDOX kit. To standardize enzyme activity, the total protein concentration of the samples was measured with the method described in Lowry et al. (1951).

Enzyme analysis of each sample was performed in triplicate at 37°C. Enzyme activity is expressed as the number of micromoles of reaction product per minute divided by one mg of protein (U mg^{-1} protein). Activity measurements were performed according to the manufacturer's instructions and the recommended methodologies. All enzyme analyses were performed with an Infinite 200 Pro (Tecan Austria, Grödig, Austria).

Immunological analysis

Non-specific cellular and humoral immunity parameters were assessed with the method in Wiszniewski et al. (2019). At the end of the experiment, blood was collected from the caudal vein, spleen, and kidneys of 10 individuals from each experimental variant to determine these parameters. Spectrophotometric methods were used to compare the following biochemical parameters and non-specific humoral immunity parameters: total protein and gamma-globulin levels and lysozyme and serum ceruloplasmin activities. Non-specific cellular immunity parameters were determined and compared–the proliferative activity of T-lymphocytes stimulated with concanavalin A (ConA, Sigma) with spectrophotometric methods, and B-lymphocytes stimulated with lipopolysaccharide (LPS) with the MTT assay. The method in Siwicki and Anderson (1993) was used to determine the metabolic activity of macrophages after cell stimulation with phorbol myristate acetate and

phagocyte potential killing activity (PKA) after stimulating cells with *Aeromonas hydrophila* (strain 35654 ATCC).

Statistical analysis

All data were tested for normal distribution and homogeneity of variance with the Shapiro-Wilk test. The data were subjected to a one-way ANOVA with Tukey's test as a post hoc or the Kruskal-Wallis test (for non-parametrical data) to determine the effects of different levels of SeY supplementation. Differences were considered statistically significant at $P < 0.05$. Values are presented as the mean ± standard deviation (SD). All analyses were performed in Statistica 13 (StatSoft).

Results

Growth analysis and feed utilization

The effects of SeY supplementation on fish growth are shown in Table 2. A statistically significantly higher final fish body weight was recorded in groups S1 (35.09 ± 0.78) and S3 (35.46 ± 1.65) compared to the control group C (32.78 ± 2.55) ($P < 0.05$; Table 2). However, greater fish body weight did not translate into higher values for daily (DGR) or specific (SGR) growth rates ($P > 0.05$; Table 2). The values of the final fish condition factor (CF), feed conversion ratio (FCR), and protein efficiency ratio (PER) did not differ among groups ($P > 0.05$; Table 2). During the eight weeks of the experiment, no mortality was observed in any feeding group.

Enzyme activity analysis

No significant effect on the activity of alkaline phosphatase (ALP) or acid phosphatase (ACP) was noted in the livers of fish fed feed with increasing doses of SeY compared to the control group ($P > 0.05$, Table 3). The activities of alkaline phosphatase (ALP), lipase (LIP), and trypsin (TRYP) in the anterior section of the intestine also did not differ significantly among the groups ($P > 0.05$, Table 3). LIP activity increased significantly in the posterior section of the intestines in fish from group S3 (7.41 ± 3.96) with the highest dose of SeY in the feed compared to the control group C (4.03 ± 2.80). However, the activities of ALP and TRYP did not differ significantly compared to the control group C ($P > 0.05$, Table 3).

The values of hepatic antioxidant enzymes in fish fed the experimental diet are presented in Table 3. The highest values of glutathione peroxidase (GPx) were recorded in group S2 (262.23 IU g^{-1} vs. 172.80 IU g^{-1} in control group C; $P < 0.05$; Table 3), while the lowest activity of this antioxidant enzyme in the liver was noted in group S1, although these

Table 2

Effect of dietary selenium supplementation at 0 mg kg^{-1} (C), 0.7 mg kg^{-1} feed (S1), 1.4 mg kg^{-1} feed (S2), and 2.1 mg kg^{-1} feed (S3) for 56 days on starlet sturgeon rearing parameters (mean ± SD). n – number of individuals. Means followed by different letters in the same row are significantly different ($P < 0.05$)

	Dietary treatment			
Rearing parameters	C	S1	S2	S3
Final total length TL (mm) (n = 90)	193.14 ± 2.86	196.55 ± 1.66	193.60 ± 1.91	196.00 ± 4.03
Final body weight BW (g) (n = 90)	32.78 ± 2.55^{a}	35.09 ± 0.78^{b}	34.44 ± 0.96^{ab}	35.46 ± 1.65^{b}
Daily growth rate DGR (g d^{-1}) (n = 3)	0.54 ± 0.05	0.57 ± 0.02	0.57 ± 0.02	0.59 ± 0.03
Specific growth rate SGR (% d^{-1}) (n = 3)	4.50 ± 0.32	4.40 ± 0.16	4.65 ± 0.14	4.67 ± 0.15
Initial condition factor (n = 90)	0.54 ± 0.02	0.53 ± 0.01	0.56 ± 0.02	0.54 ± 0.01
Final condition factor (n = 90)	0.45 ± 0.02	0.46 ± 0.01	0.47 ± 0.01	0.47 ± 0.01
Feed conversion ratio FCR (n = 3)	1.13 ± 0.08	1.15 ± 0.04	1.09 ± 0.04	1.08 ± 0.03
Protein efficiency ratio PER (n = 3)	1.59 ± 0.11	1.56 ± 0.56	1.65 ± 0.05	1.65 ± 0.05

Table 3
Effect of dietary selenium supplementation at 0 mg kg^{-1} (C), 0.7 mg kg^{-1} feed (S1), 1.4 mg kg^{-1} feed (S2), and 2.1 mg kg^{-1} feed (S3) for 56 days on sterlet digestive and antioxidant enzyme activity (mean ± SD, n=10). Means followed by different letters in the same row are significantly different (P < 0.05)

Enzyme activity	Dietary treatment			
	C	S1	S2	S3
Liver				
Alkaline phosphatase (ALP) (IU g^{-1})	105.69 ± 33.77	115.46 ± 36.74	117.78 ± 31.91	109.39 ± 33.88
Acid phosphatase (ACP) (IU g^{-1})	2.64 ± 0.64	2.64 ± 0.35	3.13 ± 0.89	2.95 ± 1.04
Anterior intestine				
Alkaline phosphatase (ALP) (IU g^{-1})	63.23 ± 34.09	61.44 ± 37.13	69.38 ± 56.12	67.46 ± 34.40
Lipase (LIP) (IU g^{-1})	18.42 ± 10.69	11.70 ± 9.39	18.57 ± 19.52	29.18 ± 15.10
Trypsin (TRYP) (IU g^{-1})	48.21 ± 34.55	37.23 ± 34.65	60.20 ± 48.37	60.01 ± 50.71
Posterior intestine				
Alkaline phosphatase (ALP) (IU g^{-1})	196.21 ± 162.90	228.15 ± 118.20	317.44 ± 229.62	115.80 ± 57.84
Lipase (IU g^{-1})	4.03 ± 2.80^{a}	2.42 ± 1.62^{a}	5.09 ± 2.96^{ab}	7.41 ± 3.96^{b}
Trypsin (IU g^{-1})	30.66 ± 36.53	69.06 ± 73.03	40.98 ± 36.79	23.46 ± 21.87
Antioxidant enzymes in the liver				
Glutathione peroxidase (GP) (IU g^{-1})	172.80 ± 74.18^{a}	93.00 ± 55.08^{a}	262.23 ± 81.30^{b}	166.97 ± 67.88^{a}
Superoxide dismutase (SOD) (IU g^{-1})	11.37 ± 2.50^{ab}	9.33 ± 1.37^{a}	14.39 ± 2.03^{b}	12.01 ± 4.83^{ab}

differences were not statistically significant (P > 0.05, Table 3). The highest activity of superoxide dismutase (SOD), at 14.39 IU g^{-1}, was recorded in group S2 compared to group S1 in which it was the lowest at 9.33 IU g^{-1} (P < 0.05; Table 3).

Immunological analysis

The effects of SeY supplementation on selected parameters of non-specific humoral and cellular immunity of fish blood at the end of the experiment are presented in Table 4. The humoral response, lysozyme activity, and total protein level increased with increasing doses of Se in the feed, but the differences were not statistically significant (P > 0.05; Table 4). In turn, the concentration of ceruloplasmin was significantly higher in group S1 compared to the control group C (51.29 IU vs. 36.57 IU; P < 0.05; Table 4). The non-specific cellular response parameters determined in fish spleen, phagocyte respiratory burst activity (RBA), and PKA remained unchanged (P > 0.05; Table 4). The proliferative activity of T and B lymphocytes of the pronephros in the groups analyzed did not differ significantly compared to the control group C (P > 0.05; Table 4).

Discussion

In studies examining the effects of SeY on fish growth, significant benefits have been observed when diets were supplemented with Se. According to Khalil et al. (2019), the growth of meagre, *Argyrosomus regius* (Asso), fed varying levels of SeY was significantly higher than that of the control group. Increases were especially evident at higher doses of Se (2.97 and 3.98 mg Se kg^{-1} diet) and significantly enhanced survival, food intake, nutrient utilization, and whole-body protein content were observed. Arshad et al. (2011) report similar observations on the benefits of supplementing diets with Se, and, in their study on beluga sturgeon (*H. huso*), groups fed diets containing more than 11 μg Se g^{-1} exhibited faster and higher growth rates than the other groups, and growth indices were particularly high for the fish fed a dose of 20.26 μg Se g^{-1}, which

Table 4

Effect of dietary selenium supplementation at 0 mg kg^{-1} (C), 0.7 mg kg^{-1} feed (S1), 1.4 mg kg^{-1} feed (S2), and 2.1 mg kg^{-1} feed (S3) for 56 days on non-specific humoral and cellular defense mechanisms in sterlet spleen and pronephros (*IS* ± SD, n = 10). Means followed by different letters in the same row are significantly different (P < 0.05)

	Dietary treatments			
Defense mechanisms	C	S1	S2	S3
Non-specific humoral immunity				
Lysozyme activity (mg L^{-1})	2.79 ± 0.83	3.20 ± 0.81	3.28 ± 0.96	3.33 ± 1.22
Ceruloplasmin (IU)	36.57 ± 10.52^{a}	51.29 ± 3.83^{b}	44.13 ± 9.04^{ab}	44.09 ± 10.03^{ab}
Total protein level (g L^{-1})	16.33 ± 4.85	20.93 ± 2.02	19.03 ± 3.23	19.45 ± 3.20
Total Immunoglobulin (Ig) level (g L^{-1})	6.20 ± 6.95	6.18 ± 1.59	5.00 ± 2.17	5.51 ± 2.61
Non-specific cellular immunity				
Spleen				
Metabolic activity of phagocytes (RBA) (OD)	1.06 ± 0.10	1.12 ± 0.04	1.10 ± 0.06	1.20 ± 0.40
Potential killing activity of phagocytes (PKA) (OD)	1.33 ± 0.31	1.35 ± 0.12	1.22 ± 0.28	1.29 ± 0.23
Pronephros				
Proliferative response of lymphocytes T stimulated by mitogen concanavaline Až(ConA) (OD)	1.10 ± 0.18	1.13 ± 0.25	0.96 ± 0.11	1.19 ± 0.35
Proliferative response of lymphocytes B stimulated by lipopolysaccharide (LPS) (OD)	1.02 ± 0.16	1.10 ± 0.22	0.83 ± 0.17	0.99 ± 0.34

differed significantly from the control and the other experimental groups. Additionally, indices such as the feed conversion ratio (FCR) and feed efficiency (FE) were dependent on Se dose, which underscores the potential benefits of adding Se to fish diets. However, it is worth noting that while Se is an essential nutrient for fish, it can become toxic at concentrations slightly above dietary requirements (Hilton et al. 1980). The recommended Se concentrations in fish diets range from 0.1 to 0.5 mg g^{-1} dry weight (Hilton and Hodson 1983, Gatlin and Wilson 1984). Thus, it is crucial to closely monitor and control Se doses in fish diets to ensure optimal benefits while avoiding the potential risks associated with toxicity.

The effects of Se supplementation on fish growth performance appear to vary across different studies, species, and conditions. Rider et al. (2009) and Pacitti et al. (2015, 2016) report that no significant changes in growth performance were observed in rainbow trout fed diets supplemented with 0.25–8 mg kg^{-1} Se from SeY. This contrasts with the present findings, in which diets supplemented with 2, 4, and 6 mg kg^{-1} Se (from SeY) led to a significant boost in fish growth performance. Such discrepancies can be attributed to differences in fish stages, diet composition, rearing conditions, feeding strategies, and other factors. Ozluer-Hunt et al. (2011) observed improved growth performance in rainbow trout fed diets supplemented with 3 or 4 mg kg^{-1} Se from SeY.

In another study on Nile tilapia, *Oreochromis niloticus* (L.), both SeY and enriched selenite (Se(IV)) showed a dual role, depending on the concentration (Chen et al. 2020). At 3 μg g^{-1} in the feed, both forms of Se significantly enhanced weight gain and specific growth rate up to 90 days. However, an increase in Se concentration to 12 μg g^{-1} led to significant decreases in these growth parameters at both 45 and 90 days. Interestingly, while the FCR remained largely unaffected until Se levels reached 12 μg g^{-1}, SeY appeared to have a slight advantage over Se(IV) in promoting better feed conversion. However, in our study, Se supplementation did not result in statistically significant differences in this parameter. Chen et al. (2020) also report the negative impact of higher

Se concentrations was further highlighted by the reduced total length of tilapia after 45 and 90 days of feeding diets with 12 μg^{-1} SeY and Se(IV). In general, parameters such as CF and hepatosomatic index (HSI) remained unchanged across different Se treatments, with the exception of a significant decrease in HSI for fish fed the 12 $\mu g\ g^{-1}$ SeY diet for 90 days compared to the control (Chen et al. 2020).

The dietary requirements of Se in fishes also differ based on the specific indicators of health or growth being monitored. Watanabe et al. (1997) suggest a broad requirement range for fishes at values between 0.05 and 1.0 mg kg^{-1} Se dry diet. However, specific species have more well-defined requirements. For instance, Gatlin and Wilson (1984) determined the Se requirement for channel catfish, *Ictalurus punctatus*, to be 0.25 mg kg^{-1} Se based on growth and liver glutathione peroxidase activity. Similarly, for rainbow trout, a dietary supplementation of 0.06 mg kg^{-1} Se prevented symptoms of Se deficiency (Bell and Cowey 1989). In Atlantic salmon, *Salmo salar* (L.), fry a mere 0.1 mg kg^{-1} Se dry diet was sufficient to prevent mortality caused by Se-deficient diets, with optimal growth observed at 0.15 mg kg^{-1} Se dry diet (Poston et al. 1976, Poston and Combs 1979). It is also noteworthy that Se, besides being a dietary requirement, has a threshold of toxicity. For white sturgeon, this threshold is between 10 and 20 $\mu g\ g^{-1}$ Se diet, which was determined based on histopathological changes in the kidneys (Tashjian et al. 2006). The form of Se is another crucial factor. SeY, an organic source of Se, mainly contains SeMet, which constitutes 70–90% of its selenocompounds (Ip et al. 2000, Block et al. 2004). This organic form is recommended as an excellent Se supplement, especially for rainbow trout, leading to significant Se accumulation in tissues (Pacitti et al. 2015). This assertion aligns with the results of several studies, in which a direct relationship between dietary Se levels and Se accumulation in various tissues was observed in rainbow trout (Küçükbay et al. 2009, Rider et al. 2009, Ozluer-Hunt et al. 2011, Pacitti et al. 2015, 2016). This pattern of Se accumulation has also been observed in other species, including common carp (Elia et al. 2011, Ashouri et al. 2015) and blunt snout bream, *Megalobrama amblycephala* (Yih) (Liu et al. 2017). When fishes are fed diets supplemented with Se yeast, Se primarily accumulates as SeMet and SeCys (Godin et al. 2015). While the advantages of Se supplementation are evident, it must be dosed precisely. The over-accumulation of selenoamino acids can trigger oxidative stress in the metabolic pathways of fishes, with rainbow trout being particularly susceptible (Palace et al. 2004, Misra et al. 2012). Therefore, careful dosing of dietary Se is essential to maximize its benefits while mitigating potential risks.

The results of enzymatic activity obtained in this experiment concur with the results in the literature on the supplementation of yeast or its ingredients and indicate positive effects on the growth and activity of digestive enzymes in animals reared in aquaculture. A study conducted on whiteleg shrimp, *Litopenaeus vannamei* (Boone), showed that feed supplementation with yeast hydrolysates increased the activity of digestive enzymes (amylase, trypsin, and lipase) and the expression of genes related to the use of nutrients that simultaneously improved the ability of the fish to process carbohydrates and lipids (Yang et al. 2020). An experiment conducted on larval European sea bass, *Dicentrarchus labrax* (L.), showed that a live yeast nutritional supplement had positive effects. Tovar-Ramírez et al. (2004) noted increased survival, earlier enterocyte maturation, and higher brush membrane enzyme activity compared to the control group. The second, more important, factor influencing the well-being of fish was the Se concentration in the fish diet. A dose of 2 mg kg^{-1} Se in fish feed stimulated growth in young tilapia (*O. niloticus*) and increased the activity of intestinal digestive enzymes (Iqbal et al. 2020). However, other researchers (Palace et al. 2004, Misra et al. 2012, Chen et al. 2020) report both beneficial and negative effects of various forms of Se depending on its concentration, where oxidative stress is considered to be the main factor regulating fish homeostasis. Oxidative stress occurs because of significant increases in concentrations of reactive oxygen species (ROS) and reactive nitrogen species. High levels of ROS have a toxic effect on DNA, proteins, and lipids (e.g.,

non-enzymatic lipid peroxidation occurs) that leads to the accumulation of oxidative damage in various parts of cells, dysregulation of sensitive metabolic and signaling pathways, and, ultimately, pathological conditions. To counteract these changes, cells have developed detoxification mechanisms in the form of enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Schrader and Fahimi 2006). In rainbow trout fed feed supplemented with organic Se (2, 4, or 6 mg of SeY kg^{-1} feed) for 10 weeks, an increase in body weight was observed without causing oxidative stress in their tissues (Wang et al. 2018).

In a study with channel catfish fed Se from organic sources showed better growth and higher glutathione peroxidase activity than individuals fed Se from inorganic sources (Wang and Lovell 1997). In general, the metabolism of inorganic Se is a multi-step process in which the valence of Se changes at each step, which can result in increased ROS, while the metabolism of organic Se does not involve a change in valence (Chen et al. 2020). When tested in vitro, SeMet was found to be toxic only at the highest dose (1,000 μM), causing a decrease in cell survival. At the same time, an increase in the activity of antioxidant enzymes and a decrease in the ratio of reduced to oxidized glutathione were observed, which indicated the development of an oxidative intracellular environment (Misra et al. 2012).

Se is known to be essential for the normal development of cellular and humoral immunity in fishes, although the mechanisms are not fully understood (Deilamy Pour et al. 2021). Cotter et al. (2008) studied the addition of organic and inorganic Se to the feed of hybrid striped bass and report no effects on lysozyme activity, similarly to our study. Instead, they demonstrated a peak in ceruloplasmin levels at the low doses tested, while they were reduced at the higher dose. This coincides with our results, which showed increased ceruloplasmin activity in group S1, which was fed the lowest Se dose. The administration of higher doses of Se to sterlet diets did not result in increased ceruloplasmin in blood plasma. Ceruloplasmin is an acute phase protein that is important in multiline immunity that maintains health and homeostasis in fishes. It inhibits the growth of bacteria by depriving them of essential nutrients including copper ions (Alexander 1985), is involved in blood clotting, fibrinolysis, and oxidative protection (Kushner 1993), and seems to play a potential role during parasitic infection in fishes (Das and Sahoo 2018). Other parameters of humoral immunity tested in sterlet, such as total protein level and lysozyme activity, increased with Se feed supplementation, but this was statistically insignificant. Perhaps the addition of higher doses of Se yeast would stimulate the activity of these parameters more. Other authors observed increases in total protein levels in fishes fed with the addition of Se: Nile tilapia (Dawood et al. 2020), rainbow trout (Naderi et al. 2017a, 2017b), European seabass (Abd El-Kader et al. 2021), and African catfish, *Clarias gariepinus* (Burchell), (Abdel-Tawwab et al. 2007). Plasma or serum total protein, which is mainly synthesized by liver parenchymal cells, has been used as a broad clinical indicator of health, immune competence, stress, and nutritional condition in fishes. The addition of Se to fish diets increased lysozyme activity in, among others, Siberian sturgeon, *Acipenser baerii* (Brandt), (Gholizadeh Zare Tavana et al. 2019); European seabass (Abd El-Kader et al. 2021); rainbow trout (Kohshahi et al. 2019); and pacu, *Piaractus mesopotamicus* (Holmberg), (Biller-Takahashi et al. 2015). Natural antibodies (immunoglobulins) have a wide range of functions in defense-related activities such as limiting the dispersal of infectious agents, killing microbes, repairing tissue damage, and restoring health. Our results showed stable immunoglobulin levels in all study groups, which could indicate a lack of harmful effects from the doses of SeMet doses on sterlet. Similarly, Se-Met and selenite supplementation did not affect serum immunoglobulin levels in common carp, but nanoparticles of Se caused an increase in this parameter (Saffari et al. 2018). The same results are reported in Nile tilapia (Ghaniem et al. 2022), but several other authors report the contrary that Se nanoparticles did not affect immunoglobulin levels in rainbow trout (Naderi et al. 2017a), Asian sea bass (Deilamy Pour et al. 2021), or Nile tilapia (Al-Deriny et al. 2020). These differences

might stem from the different requirements and tolerances of these fishes for this element and the form in which it was administered (Wang et al. 2022).

Cells such as monocytes, macrophages, and neutrophils are known as professional phagocytic cells. Phagocyte RBA is used frequently as an indicator of nonspecific immunity in fishes. Our results showed that spleen phagocyte RBA was no different compared to that in the control fish. According to Kumar et al. (2018), the respiratory burst in serum from striped catfish, *Pangasianodon hypophthalmus* (Sauvage), improved significantly with dietary Se nanoparticle supplementation at the lowest dose tested. Stimulating RBA is also demonstrated in rohu, *Labeo rohita* (Hamilton), (Swain et al. 2019) and Nile tilapia (Dawood et al. 2020).

Lymphocytes predominate in the peripheral blood of fishes, and relatively few studies have examined the effects of nutrients including supplemental Se on lymphocyte function. Our results showed that the proliferative response of pronephros lymphocytes stimulated with LPS as a B-cell mitogen did not increase. There were also no changes in pronephros lymphocytes stimulated with ConA as a T-cell mitogen. Xia et al. (2019) investigated chitosan--selenium nanoparticles for immune enhancement in zebrafish, *Danio rerio* (Hamilton), and showed that T cells were stimulated, but B cell proliferation did not occur. Additionally, using selenite without the addition of chitosan did not lead to splenocyte proliferation. Gholizadeh Zare Tavana et al. (2019) report a significant increase in lymphocyte percentage in Siberian sturgeon fed organic Se and suggest that higher lymphocyte proliferation could be attributed to it. The present study showed that the addition of SeY stimulated humoral immunity more than cellular immunity in sterlet.

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References

Abd El-Kader, M. F., Fath El-Bab, A. F., Abd-Elghany, M. F., Abdel-Warith, A. W. A., Younis, E. M., Dawood, M. A. (2021). Selenium nanoparticles act potentially on the growth performance, hemato-biochemical indices, antioxidative, and immune-related genes of European seabass (*Dicentrarchus labrax*). Biological Trace Element Research, 199, 3126–3134.

Abdel-Tawwab, M., Mousa, M. A., Abbass, F. E. (2007). Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. Aquaculture, 272(1-4), 335-345.

Al-Deriny, S. H., Dawood, M. A., Elbialy, Z. I., El-Tras, W. F., Mohamed, R. A. (2020). Selenium nanoparticles and

spirulina alleviate growth performance, hemato-biochemical, immune-related genes, and heat shock protein in Nile tilapia (*Oreochromis niloticus*). Biological Trace Element Research, 198, 661–668.

Alexander, J. B. (1985). Non-immunoglobulin humoral defence mechanisms in fish. In: Fish Immunology (Ed.) M. J. Manning, M. F. Tatner, Academic Press, London, 133-140.

Arshad, U., Takami, G. A., Sadeghi, M., Bai, S., Pourali, H. R., Lee, S. (2011). Influence of dietary L-selenomethionine exposure on growth and survival of juvenile *Huso huso*. Journal of Applied Ichthyology, 27(2), 761-765.

Ashouri, S., Keyvanshokooh, S., Salati, A. P., Johari, S. A., Pasha-Zanoosi, H. (2015). Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture, 446, 25-29.

Bell, J. G., Cowey, C. B. (1989). Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionine and selenocystine in Atlantic salmon (*Salmo salar*). Aquaculture, 81(1), 61-68.

Biller-Takahashi, J. D., Takahashi, L. S., Mingatto, F. E., Urbinati, E. C. (2015). The immune system is limited by oxidative stress: dietary selenium promotes optimal antioxidative status and greatest immune defense in pacu *Piaractus mesopotamicus*. Fish & Shellfish Immunology, 47(1), 360-367.

Block, E., Glass, R. S., Jacobsen, N. E., Johnson, S., Kahakachchi, C., Kamiński, R., Skowrońska, A., Boakye, H.T., Tyson, J.F., Uden, P. C. (2004). Identification and Synthesis of a Novel Selenium- Sulfur Amino Acid Found in Selenized Yeast: Rapid Indirect Detection NMR Methods for Characterizing Low-Level Organoselenium Compounds in Complex Matrices. Journal of Agricultural and Food Chemistry, 52(12), 3761-3771.

Chen, H., Li, J., Yan, L., Cao, J., Li, D., Huang, G. Y., Shi, W.J., Dong, W., Zha, J., Ying, G., Zhong, H., Wang, Z., Huang, Y., Luo, Y., Xie, L. (2020). Subchronic effects of dietary selenium yeast and selenite on growth performance and the immune and antioxidant systems in Nile tilapia *Oreochromis niloticus*. Fish & Shellfish Immunology, 97, 283-293.

Cotter, P. A., Craig, S. R., McLean, E. (2008). Hyperaccumulation of selenium in hybrid striped bass: a functional food for aquaculture? Aquaculture Nutrition, 14(3), 215-222.

Das, S., Sahoo, P. K. (2018). Ceruloplasmin, a moonlighting protein in fish. Fish & Shellfish Immunology, 82, 460-468.

Dawood, M. A., Zommara, M., Eweedah, N. M., Helal, A. I. (2020). Synergistic effects of selenium nanoparticles and vitamin E on growth, immune-related gene expression, and regulation of antioxidant status of Nile tilapia (*Oreochromis niloticus*). Biological Trace Element Research, 195, 624-635.

Deilamy Pour, H., Mousavi, S. M., Zakeri, M., Keyvanshokooh, S., Kochanian, P. (2021). Synergistic effects of selenium and magnesium nanoparticles on growth, digestive enzymes, some serum biochemical parameters and immunity of Asian sea bass (*Lates calcarifer*). Biological Trace Element Research, 199(8), 3102-3111.

Dobrzański, Z., Dolińska, B., Chojnacka, K., Opaliński, S., Ryszka, F. (2006). The use of yeasts in livestock feeding. Acta Scientiarum Polonorum-Medicina Veterinaria, 5(2), 49-66.

Elia, A. C., Prearo, M., Pacini, N., Dörr, A. J. M., Abete, M. C. (2011). Effects of selenium diets on growth, accumulation and antioxidant response in juvenile carp. Ecotoxicology and Environmental Safety, 74(2), 166-173.

Gatlin, D. M., Wilson, R. P. (1984). Dietary selenium requirement of fingerling channel catfish. The Journal of Nutrition, 114(3), 627-633.

Ghaniem, S., Nassef, E., Zaineldin, A. I., Bakr, A., Hegazi, S. (2022). A comparison of the beneficial effects of inorganic, organic, and elemental nano-selenium on Nile tilapia: growth, immunity, oxidative status, gut morphology, and immune gene expression. Biological Trace Element Research, 200(12), 5226-5241.

Gholizadeh Zare Tavana, B., Banaee, M., Yousefi Jourdehi, A., Nematdoost Haghi, B., Seyed Hassani, M. (2019). Effects of dietary Sel-Plex supplement on growth performance, hematological and immunological parameters in Siberian sturgeon (*Acipenser baerii* Brandt, 1869). Iranian Journal of Fisheries Sciences, 18(4) 830-846.

Godin, S., Fontagné-Dicharry, S., Bueno, M., Tacon, P., Jesu Prabhu, P. A., Kaushik, S., Medale, F., Bouyssiere, B. (2015). Influence of dietary selenium species on selenoamino acid levels in rainbow trout. Journal of Agricultural and Food Chemistry, 63(28), 6484-6492.

Han, D., Xie, S., Liu, M., Xiao, X., Liu, H., Zhu, X., Yang, Y. (2011). The effects of dietary selenium on growth performances, oxidative stress and tissue selenium concentration of gibel carp (*Carassius auratus gibelio*). Aquaculture Nutrition, 17(3), 741-749.

Hilton, J. W., Hodson, P. V. (1983). Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). The Journal of Nutrition, 113(6), 1241-1248.

Hilton, J. W., Hodson, P. V., Slinger, S. J. (1980). The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). The Journal of Nutrition, 110(12), 2527-2535.

Huang, S. S. Y., Strathe, A. B., Wang, W. F., Deng, D. F., Fadel, J. G., Hung, S. S. (2012). Selenocompounds in juvenile white sturgeon: evaluating blood, tissue, and urine selenium concentrations after a single oral dose. Aquatic Toxicology, 109, 158-165.

Hung, S. S. (2017). Recent advances in sturgeon nutrition. Animal Nutrition, 3(3), 191-204.

Ip, C., Thompson, H. J., Zhu, Z., Ganther, H. E. (2000). In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Research, 60(11), 2882-2886.

Iqbal, S., Atique, U., Mahboob, S., Haider, M. S., Iqbal, H. S., Al-Ghanim, K. A., Al-Misned, F., Ahmed, Z., Mughal, M. S. (2020). Effect of supplemental selenium in fish feed boosts growth and gut enzyme activity in juvenile tilapia (*Oreochromis niloticus*). Journal of King Saud University-Science, 32(5), 2610-2616.

Jaramillo Jr, F., Peng, L., Gatlin III, D. M. (2009). Selenium nutrition of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) bioavailability, toxicity and interaction with vitamin E. Aquaculture Nutrition, 15, 160-165.

Jobling, M. (1994). Fish bioenergetics. Fish & Fisheries Series (FIFI, vol. 13). Springer Dordrecht, 310 p.

Kamaszewski, M., Wójcik, M., Ostaszewska, T., Kasprzak, R., Kolman, R., Prusińska, M. (2014). The effect of essential fatty acid (EFA) enrichment of *Artemia* sp. nauplii on the enzymatic activity of Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill, 1815) larvae-preliminary study. Journal of Applied Ichthyology, 30, 1256-1258.

Khalil, H. S., Mansour, A. T., Goda, A. M. A., Omar, E. A. (2019). Effect of selenium yeast supplementation on growth performance, feed utilization, lipid profile, liver and intestine histological changes, and economic benefit in meagre, *Argyrosomus regius*, fingerlings. Aquaculture, 501, 135-143.

Kohshahi, A. J., Sourinejad, I., Sarkheil, M., Johari, S. A. (2019). Dietary cosupplementation with curcumin and different selenium sources (nanoparticulate, organic, and inorganic selenium): influence on growth performance, body composition, immune responses, and glutathione peroxidase activity of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 45, 793-804.

Küçükbay, F. Z., Yazlak, H., Karaca, I., Sahin, N., Tuzcu, M., Cakmak, M. N., Sahin, K. (2009). The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss*) under crowding conditions. Aquaculture Nutrition, 15(6), 569-576.

Kumar, N., Krishnani, K. K., Gupta, S. K., Sharma, R., Baitha, R., Singh, D. K., Singh, N. P. (2018). Immuno-protective role of biologically synthesized dietary selenium nanoparticles against multiple stressors in *Pangasinodon hypophthalmus*. Fish & Shellfish Immunology, 78, 289-298.

Kushner, I. (1993). The acute phase response: an overview. Acute-Phase Proteins Molecular Biology, Biochemistry and Clinical Applications, 3-19.

Le, K. T., Fotedar, R. (2014). Bioavailability of selenium from different dietary sources in yellowtail kingfish (*Seriola lalandi*). Aquaculture, 420, 57-62.

Lin, Y. H., Shiau, S. Y. (2005). Dietary selenium requirements of juvenile grouper, *Epinephelus malabaricus*. Aquaculture, 250(1-2), 356-363.

Liu, G. X., Jiang, G. Z., Lu, K. L., Li, X. F., Zhou, M., Zhang, D. D., Liu, W. B. (2017). Effects of dietary selenium on the growth, selenium status, antioxidant activities, muscle composition and meat quality of blunt snout bream, *Megalobrama amblycephala*. Aquaculture Nutrition, 23(4), 777-787.

Lorentzen, M., Maage, A., Julshamn, K. (1994). Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (Salmo salar). Aquaculture, 121(4), 359-367.

Lowry, O., Rosebrough, N., Farr, A. L., Randall, R. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1), 265-275.

Margis, R., Dunand, C., Teixeira, F. K., Margis-Pinheiro, M. (2008). Glutathione peroxidase family-an evolutionary overview. The FEBS Journal, 275(15), 3959-3970.

Misra, S., Hamilton, C., Niyogi, S. (2012). Induction of oxidative stress by selenomethionine in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). Toxicology In Vitro, 26(4), 621-629.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1-2), 55-63.

Naderi, M., Keyvanshokooh, S., Salati, A. P., Ghaedi, A. (2017a). Combined or individual effects of dietary vitamin E and selenium nanoparticles on humoral immune status and serum parameters of rainbow trout (*Oncorhynchus mykiss*) under high stocking density. Aquaculture, 474, 40-47.

Naderi, M., Keyvanshokooh, S., Salati, A. P., Ghaedi, A. (2017b). Proteomic analysis of liver tissue from rainbow trout (*Oncorhynchus mykiss*) under high rearing density after administration of dietary vitamin E and selenium nanoparticles. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 22, 10-19.

Ozluer-Hunt, A., Berkoz, M., Ozkan, F., Yalin, S., Ercen, Z., Erdogan, E., Gunduz, S. G. (2011). Effects of organic selenium on growth, muscle composition, and antioxidant system in rainbow trout. The Israeli Journal of Aquaculture-Bemidgeh, IIC: 63.2011.562, 10 p.

Pacitti, D., Lawan, M. M., Feldmann, J., Sweetman, J., Wang, T., Martin, S. A. M., Secombes, C. J. (2016). Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®. BMC Genomics, 17, 1-26.

Pacitti, D., Lawan, M. M., Sweetman, J., Martin, S. A., Feldmann, J., Secombes, C. J. (2015). Selenium supplementation in fish: A combined chemical and biomolecular study to understand Sel-Plex assimilation and

impact on selenoproteome expression in rainbow trout (*Oncorhynchus mykiss*). PloS One, 10(5), e0127041.

Palace, V. P., Spallholz, J. E., Holm, J., Wautier, K., Evans, R. E., Baron, C. L. (2004). Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety, 58(1), 17-21.

Poston, H. A., Combs Jr, G. F. (1979). Interrelationships between requirements for dietary selenium, vitamin E, and L-ascorbic acid by Atlantic salmon (*Salmo salar*) fed a semipurified diet. Fish Health News, 8(4), 6-7.

Poston, H. A., Combs Jr, G. F., Leibovitz, L. (1976). Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical deficiency signs. The Journal of Nutrition, 106(7), 892-904.

Rider, S. A., Davies, S. J., Jha, A. N., Fisher, A. A., Knight, J., Sweetman, J. W. (2009). Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): implications on selenium status and health responses. Aquaculture, 295(3-4), 282-291.

Rook, G. A. W., Steele, J., Umar, S., Dockrell, H. M. (1985). A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ-interferon. Journal of Immunological Methods, 82(1), 161-167.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science, 179(4073), 588-590.

Saffari, S., Keyvanshokooh, S., Zakeri, M., Johari, S. A., Pasha-Zanoosi, H., Mozanzadeh, M. T. (2018). Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). Fish Physiology and Biochemistry, 44, 1087-1097.

Schrader, M., Fahimi, H. D. (2006). Peroxisomes and oxidative stress. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1763(12), 1755-1766.

Siwicki, A. K., Cossarini-Dunier, M. (1990). Effect of levamisole on the lymphocyte and macrophage activity in carp (*Cyprinus carpio*). Annales de Recherches Veterinaires, 21(2), 95-100.

Siwicki, A. K., Anderson, D. P. (1993). Immunostimulation in fish: measuring the effects of stimulants by serological and immunological methods. Symposium on Fish Immunology. Lysekil, Sweden.

Siwicki, A. K., Fuller, J. C., Nissen, S., Morand, M., Pozet, F., Vincent, F., Kazun, B. (2004). Effect of HMB (β-hydroxy-β-methylbutyrate) on in vitro proliferative responses of sheatfish (*Silurus glanis*) and catfish (*Ictalurus melas*) lymphocytes stimulated by mitogens. Acta Veterinaria Brno, 73(1), 119-122.

Swain, P., Das, R., Das, A., Padhi, S. K., Das, K. C., Mishra, S. S. (2019). Effects of dietary zinc oxide and selenium nanoparticles on growth performance, immune responses and enzyme activity in rohu, *Labeo rohita* (Hamilton). Aquaculture Nutrition, 25(2), 486-494.

Tashjian, D. H., Teh, S. J., Sogomonyan, A., Hung, S. S. (2006). Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquatic Toxicology, 79(4), 401-409.

Tovar-Ramırez, D., Infante, J. Z., Cahu, C., Gatesoupe, F. J., Vázquez-Juárez, R. (2004). Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture, 234(1-4), 415-427.

Wang, C., Lovell, R. T. (1997). Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (*Ictalurus punctatus*). Aquaculture, 152(1-4), 223-234.

Wang, K. Y., Peng, C. Z., Huang, J. L., Huang, Y. D., Jin, M. C., Geng, Y. (2013). The pathology of selenium deficiency in *Cyprinus carpio* L. Journal of Fish Diseases, 36(7), 609-615.

Wang, L., Sagada, G., Wang, R., Li, P., Xu, B., Zhang, C., Qiao, J., Yan, Y. (2022). Different forms of selenium supplementation in fish feed: The bioavailability, nutritional functions, and potential toxicity. Aquaculture, 549, 737819.

Wang, L., Zhang, X., Wu, L., Liu, Q., Zhang, D., Yin, J. (2018). Expression of selenoprotein genes in muscle is crucial for the growth of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with selenium yeast. Aquaculture, 492, 82-90.

Watanabe, T., Kiron, V., Satoh, S. (1997). Trace minerals in fish nutrition. Aquaculture, 151(1-4), 185-207.

Wiszniewski, G., Jarmołowicz, S., Hassaan, M. S., Mohammady, E. Y., Soaudy, M. R., Łuczyńska, J., Tońska, E., Terech-Majewska, E., Ostaszewska, T., Kamaszewski, M., Skrobisz, M., Adamski, A., Schulz, P., Kaczorek, E., Siwicki, A. (2019). The use of bromelain as a feed additive in fish diets: Growth performance, intestinal morphology, digestive enzyme and immune response of juvenile Sterlet (*Acipenser ruthenus*). Aquaculture Nutrition, 25, 1289-1299.

Xia, I. F., Cheung, J. S., Wu, M., Wong, K. S., Kong, H. K., Zheng, X. T., Wong, K.H., Kwok, K. W. (2019). Dietary chitosan-selenium nanoparticle (CTS-SeNP) enhance immunity and disease resistance in zebrafish. Fish & Shellfish Immunology, 87, 449-459.

Yang, X., Chi, S., Tan, B., Nie, Q., Hu, J., Dong, X., Yang, Q., Liu, H., Zhang, S. (2020). Yeast hydrolysate helping the complex plant proteins to improve the growth performance and feed utilization of *Litopenaeus vannamei*. Aquaculture Reports, 17, 100375.