Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Effects of selenomethionine and synbiotic Diproplus (Dip$^+$) on growth, hematology, carcass analysis, some immune parameters and IL-1 and TNF-α relative genes expression in *Salmo caspius*

Maziyar Akbarabadi[1], Hossein Khara[1]*, Habib Vahabzadeh Roudsari [1], Mohaddeseh Ahmadnezhad[2], Roghieh Safari[3]

1. Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran
2. Inland Waters Aquaculture Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Bandar Anzali, Iran
3. Department of Aquaculture, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

** Corresponding author's Email: h.khara1974@yahoo.com*

ABSTRACT

Limited research has focused on the nutritional requirements of *Salmo caspius*. This study investigates the effects of Selenomethionine (SelMet) and synbiotic Diproplus (Dip+) oral supplements on growth, hematology, carcass analysis, immune responses, and the IL-1 and TNF-α relative gene expression in *S. caspius*. By employing a randomized design, the dietary control group and various treatments (per kg of diet) were established, including T_1 (2 g Dip$^+$ + 0 mg SelMet), T_2 (3 g Dip$^+$ + 0 mg SelMet), T_3 (0 g Dip$^+$ + 2 mg SelMet), T_4 (0 g Dip$^+$ + 4 mg SelMet), T_5 (2 g Dip$^+$ + 2 mg SelMet), T_6 (2 g Dip$^+$ + 4 mg SelMet), T_7 (3 g Dip$^+$ + 2 mg SelMet) and T_8 (3 g Dip$^+$ + 4 mg SelMet). Significant increases in growth performance and the highest lysozyme activity were observed in T_6 ($p < 0.05$). T_3 and T_7 showed the highest total Ig and IgM levels, respectively ($p < 0.05$). Additionally, T_8 exhibited significantly elevated WBCs, RBCs, Hb, and Hct ($p < 0.05$). Carcass analysis indicated that the control group had the highest fat content and lowest amounts of ash, protein, and moisture ($p < 0.05$). Furthermore, the IL-1 and TNF-α relative gene expression levels increased approximately 16 and 22 times in T_8, respectively ($p < 0.05$). The findings suggest that the combined use of Dip$^+$ and SelMet yields superior results compared to their individual use. We recommend combining 4 mg SelMet and 3 g Dip$^+$ in fish farms.

Keywords: Growth, IgM, Lysozyme, Probiotic, Prebiotic.
Article type: Research Article.

INTRODUCTION

Salmonidae are among the most significant family that can be cultivated worldwide, with breeding practices being employed in various countries (Lee & Donaldso 2001). Given the escalating global population and the increasing demand of societies for healthy and high-quality protein sources, consuming aquatic animals is a crucial means of meeting protein needs. Consequently, it is anticipated that aquaculture will play a pivotal role in protein provision in the next two decades and contribute to alleviating global poverty. Other studies have been conducted on the utilization of food additives aimed at enhancing the health of fish, encompassing probiotics, prebiotics, and synbiotics (Ai *et al.* 2011). The concurrent administration of probiotics and prebiotics has been proposed to regulate intestinal microbial flora in aquatic animals (Li & Gatlin 2003). The utilization of synbiotics enhances the host's overall condition by augmenting the digestive system's efficiency, exerting either a direct or indirect influence on the microbial flora within the host's intestine (Merrifield *et al.* 2010). Several studies have explored the positive impact of commercial synbiotics on the growth, reproduction, and safety of aquatic animals. In

summary, the effects of utilizing the synbiotic BiominImbo® (comprising probiotic *Enterococcus faecium* and prebiotic fructo-oligosaccharide with cell wall fragments of beneficial bacteria and phycophytic compounds) have been investigated in rainbow trout (Ghafari farsani *et al.* 2021), *Acipenser gueldenstaedtii* (Vaezi *et al.* 2016), *Danio rerio* (Nekoubin *et al.* 2012a) and *Pterophyllum scalare* (Nekoubin *et al.* 2012b). The inclination to utilize mineral food supplements, particularly micro-elements, has garnered significant attention from farm animals and aquaculture. This attention stems from the necessity of providing essential nutrients through the diet for living organisms and the recognized impact of deficiencies on health and growth (Dato-Cajegas *et al.* 1996; Oliva-Teles 2012). Beyond enhancing growth indicators, these supplements bolstered fish resistance against environmental stress, various infectious diseases, and the stimulation of the non-specific immune system in aquatic animals (Roubach *et al.* 2019). Utilizing microelements bonded with amino acids represents a nutritional strategy aimed at mitigating the stress induced by free radicals, owing to their substantial impact on enhancing the body's antioxidant status (Rizzo *et al.* 2007). Selenium (Se) is a rare yet essential element for all living organisms (Hamilton 2004). It serves as a crucial micronutrient, acting as the primary structural component of the enzyme glutathione peroxidase. Research indicates that a deficiency of Se in fish can lead to a reduction or cessation of growth, and in some instances, it may result in muscle wasting (Ilham & Fotedar 2016). Se deficiency has been associated with oxidative stress, diminished appetite, and compromised health in fish (Poston & Combs 1979). However, excessive Se levels can manifest symptoms such as decreased hematological indices, reduced food consumption efficiency, appearance abnormalities, toxicity, and even mortality (Hilton *et al.* 1980 and Pacini *et al.* 2012). In a study by Naiel *et al.* (2021), it was reported that the inclusion of Selenomethionine (SelMet) in the diet of Nile tilapia, *Oreochromis niloticus* improved growth performance, serum biochemical indices, the immune system, and antioxidant defense. Tseng *et al.* (2021) assessed the impact of SelMet on total body fatty acids and the relative expression of the Mx gene in *Sparus aurata* under viral infection conditions. Their results revealed that while fish growth remained unaffected by Se consumption, an elevation in dietary SelMet led to increased Se accumulation in tissues, heightened lipid content in the entire body, and modulation of the synthesis of n-3 unsaturated fatty acids. Ghaniem *et al.* (2022) compared the varied effects of Se, SelMet, and nano-selenium (NanSe) in the Nile tilapia, *O. niloticus*. They examined growth performance, immunity, antioxidant defense, intestinal morphology, and the relative expression of specific genes involved in immunity. The findings indicated that NanSe proved more effective than Se and SelMet supplements in enhancing Nile tilapia's growth performance and its overall health. Ajdari *et al.* (2022) reported the effects of food supplements Primalac (probiotic), Inulin (prebiotic), and BiominImbo® (synbiotic) on growth performance, the innate immune system and antioxidant defense in common carp, *Cyprinus carpio*. Their study demonstrated improvements in growth indicators, increased biochemical indicators in blood serum, immune system modulation, and antioxidant defense enhancement. Additionally, a significant decrease in the feed conversion ratio was observed. The researchers reported that the impact of synbiotic supplementation surpassed that of prebiotic and probiotic supplementation.

The Caspian brown trout, *Salmo caspius* is a Salmonid indigenous to Iranian Waters, and its population in the Caspian Sea is decreasing strikingly (Rahbar *et al.* 2011; Khara 2016). In recent years, there has been a limited reports of the nutritional requirements specific to *S. caspius*, often relying on research findings from rainbow trout (*Oncorhynchus mykiss*) to fill the gaps. Understanding the impact of various dietary supplements is crucial for successfully cultivating a fish species, as it allows for adjusting growth indicators and improving both the immune and digestive systems. This holds for Se and synbiotics. The effects of utilizing SelMet and the synbiotic Diproplus (Dip^+), either alone or in combination, on *S. caspius* remain unexplored. Therefore, this study investigates the effects of administering SelMet and Dip+ orally on the growth, blood indices, carcass composition, certain innate immunity indicators, and the relative expression of IL-1 and TNF-α genes in *S. caspius*.

MATERIALS AND METHODS

Experiment design and feed preparation

The experiment was conducted employing a completely randomized design comprising eight experimental treatments (T_1: Diet containing 2 g Dip^+ + 0 mg SelMet; T_2: 3 g Dip^+ + 0 mg SelMet; T_3: 0 g Dip^+ + 2 mg SelMet; T_4: 0 g Dip^+ + 4 mg SelMet; T_5: 2 g Dip^+ + 2 mg SelMet; T_6: 2 g Dip^+ + 4 mg SelMet; T_7: 3 g Dip^+ + 2 mg SelMet; T_8: 3 g Dip^+ + 4 mg SelMet per kg of diet), along with a control group each with three replications (Vaezi *et al.* 2016 and Safabakhsh *et al.* 2020). *Salmo caspius* (n = 500), with an average weight of 66.34 ± 1.45 g, were purchased for the study. Following an adaptation period to the experimental conditions, the fish were stocked and

evenly distributed among 27 tanks. Extruded diet (EX-TG2) from 21 Beyza Feed Mill, Shiraz, Iran, was utilized in this study. The physical characteristics and nutritional analysis of the consumed feed are presented in Table 1.

Table 1. Physical characteristics and proximate analysis of basic commercial feed. 21 Beyza Feed Mill Co., Shiraz, Iran.

Feed diameter (mm)	Minimum crude protein (%)	Minimum crude Fat (%)	Digestible energy (kcal/kg)	Maximum crude fiber (%)	Minimum absorbable phosphorus (%)	Maximum moisture (%)
2.4-3.3	44	14.5	4000	2.2	0.8	10

The research lasted 60 days and was carried out at the Shahid Bahonar Fish Reproduction and Restoration Center in Kelardasht, Mazandaran, Iran. Dip^+ was sourced from Takgene Company (Tehran, Iran), while SelMet was obtained from Ariana Company (Mashhad, Iran). The constituents of Dip^+ and SelMet are presented in Table 2.

Table 2. Compositions of Dip^+ (Takgene Co.) and SelMet (Ariana Co.).

Probiotic content	*Bacillus subtilis*, *Bacillus licheniformis*, *Pediococcus acidilactici* and 4 strains of lactic acid bacteria
Prebiotic content	Mannan-oligosaccharides (MOS)
Organic selenium	It consists of selenium ion bonded with methionine

Growth parameters

Fish biometry was conducted in three phases: initial, middle (every two weeks), and final. A ruler with an accuracy of 1 mm was employed to measure the length, while a digital scale with an accuracy of 0.01 g to measure the weight of the fish. Specific growth rate (SGR), body weight increase (BWI), percent of body weight increase (PBWI), feed conversion rate (FCR), condition factor (CF), protein efficiency (PER), and fish survival percentage (SR) were calculated using following equations for different experimental treatments and the control group (Khara *et al.* 2016).

BWI (g) = Final weight (g) - Initial weight (g)

PBWI (%) = [Final weight (g)-Initial weight (g)] / [Initial weight (g)] ×100

SGR = [Ln final Weight-Ln initial weight] / [Duration (day)] ×100

CF = [Weight (g)] / [Length (cm) ^{3}] ×100

FCR = [Feed intake (g)] / [BWI (g)]

PER = Final weight (g) / Consumed protein (g)

SR = [Final fish count / Initial fish count] ×100

Blood indices

After the experimental period, three fish from each replication were anesthetized using a 100 ppm Eugenol solution (Mohammadi & Khara 2015). Subsequently, blood was collected from the caudal vein using a sterile syringe. The collected samples were then transported to the Gorgan University of Agricultural Sciences and Natural Resources Laboratory, Gorgan, Iran, where white blood cells (WBCs) and red blood cells (RBCs) were quantified following the methods outlined by Gao *et al.* (2007) and Khara *et al.* (2016). Differential diagnosis of white blood cells was conducted using the method described by Gao *et al.* (2007), hematocrit (Hct) percentage according to the Ellis method (1990), and the amount of hemoglobin (Hb) using the Pars Azmoon kit (Tehran, Iran), based on the instructions provided within the kit. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indexes were measured using standard relationships based on following equations, as provided by Khara *et al.* (2016).

MCV (fL)= [Hct (%) ×10] / [RBC (10^6 mm^{-3})]

MCH (pg)= [Hb (g dL^{-1}) ×10] / [RBC (10^6 10^6 mm^{-3})]

MCHC (g dL^{-1}) = [Hb (gr/dL) ×100] / [Hct (%)]

Innate immune system indices

The quantification of total serum immunoglobulin (Total Ig) was conducted in g dL^{-1} using a biochemistry autoanalyzer (Hitachi, Japan) and the Pars Azmoon detection kit (Tehran, Iran) through the photometric method.

Serum immunoglobulin M (IgM) levels were measured in mg dL^{-1} utilizing the Minineph nephelometry device (Binding Site, England) and the corresponding Binding Site laboratory kit. Lysozyme activity in serum samples was determined according to the method outlined by Ellis (1990).

IL-1 and TNF-α relative gene expression

Following the methodology outlined by Mansourghanaei *et al.* (2022) and Zargari *et al.* (2023), to assess the relative expression of IL-1 and TNF-α genes after the experimental period, samples were obtained from the liver tissue of fish under sterile conditions, and the containers containing the samples were promptly submerged in liquid nitrogen. RNA extraction was performed using the RNx-Plus kit (SinaClon Company, Tehran, Iran), following the manufacturer's instructions. Qualitative and quantitative evaluations of the extracted RNA were confirmed through electrophoresis with a 1% agarose gel and measurement of the 260/280 absorbance using a Nanodrop device (USA). cDNA synthesis was performed using Genet Bio's cDNA synthesis master mix (Korea), following the manufacturer's instructions. Normal PCR was conducted to validate the primer sequences and the synthesized cDNA. In the final step, the relative expressions of TNF-α and IL-1 genes were measured through real-time PCR. The specific primer sequences for TNF-α, IL-1, and the reference gene β-actin are provided in Table 3.

Table 3. Specific primers for TNF-α, IL-1 and β-actin genes (Jami *et al.* 2019).

Primer name	Primer sequence	Accession number
TNF-α	F: CAAGAGTTTGAACCTTGTTCAA R: GCTGCTGCCGCACATAGAC	AJ277604.2
IL-1	F: ACATTGCCAACCTCATCATCG R: TTGAGCAGGTCCTTGTCCTTG	AJ223954
β-actin	F: CAAGGATATCCGTCGTGGCA R: ACAGCGAAACGACCAAGAGG	XM_029735646.1

Feed and carcass analysis

The prepared diet's analysis, including measuring the carcass's moisture, fat, protein, ash, and fiber content and determining the Se levels in the food and water, was conducted in accordance with the standard methods outlined by AOAC in 1990.

Statistical analysis

This experiment was executed utilizing a completely randomized design in the structure of a factorial test, encompassing two factors, the first factor being Dip^+ at three levels and the second factor being SelMet at three levels, with each factor having three replications. The normality of the data was assessed through the Kolmogorov-Smirnov test. Subsequently, a two-way analysis of variance was applied, and Duncan's test, with a confidence level of 95%, was employed in SPSS version 22 software to compare the means. Graphs and tables were generated using Excel 2010. The relative gene expression was evaluated using the $2^{-\Delta\Delta CT}$ method, as described by Zargari *et al.* (2024).

RESULTS

Growth parameters

The results obtained from the investigation on the growth performance of *S. caspius* fed different levels of SelMet and Dip^+ over 60 days are presented in Table 4. According to the results, the most favorable growth performance was observed in T_6, significantly different from the control group ($p < 0.05$). T_6 exhibited the highest final weight, final length, PBWI, SGR, and the lowest FCR ($p < 0.05$). However, the CF and PER did not differ significantly among treatments ($p > 0.05$). The data in Table 4 suggests a significant interaction between Dip^+ and SelMet in the treatments ($p < 0.05$).

Measurement of Se in water and feed

The Se contents in the food ration, incoming water source, and wastewater discharged from the tanks were measured (Table 5). The analyses revealed that the Se content in the diet was 4370 μg kg^{-1}. The Se concentration in the incoming water remained constant at 2 μg L^{-1} for all diets before adding SelMet supplementation. In the

wastewater discharged from the treatments where SelMet was added, the Se content increased to 6740 µg kg^{-1} (in T_3, T_5, and T_7) and 10960 µg kg^{-1} (in T_4, T_6, and T_8; $p < 0.05$). However, no significant difference was observed in the Se concentration measured in wastewater discharged from treatments that received the same Se level in the diet ($p > 0.05$).

Innate immune system indices

The results obtained from the evaluation of safety indicators are presented in Table 6. The activity levels of lysozyme, total Ig and IgM in the control group exhibited the lowest significant levels ($p < 0.05$). The most significant difference in lysozyme activity was recorded in T_6 ($p < 0.05$). T_3 demonstrated the highest significant amount of Total Ig ($p < 0.05$), while T_7 exhibited the highest significant level of IgM ($p < 0.05$). The findings in Table 6 suggest the potential for interaction between total Ig and IgM concerning to the two oral supplements, Dip^+ and SelMet ($p < 0.05$).

Table 4. Average growth indices of *S. caspius* fed different levels of SelMet and Dip^+ for 60 days.

	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Initial weight (g)	67.28 ± 1.54^a	67.11 ± 0.77^a	64.95 ± 0.88^a	67.21 ± 0.33^a	66.13 ± 1.17^a	66.53 ± 1.64^a	55.2 ± 64.53^a	68.63 ± 1.61^a	64.67 ± 2.55^a
Final weight (g)	119.19 ± 1.12^{ab}	114.28 ± 4.96^b	116.8 ± 4.43^b	116.21 ± 1.28^b	116.87 ± 4.99^b	121.81 ± 1.28^{ab}	130.1 ± 5.64^a	120.7 ± 2.77^{ab}	118.77 ± 1.69^{ab}
Initial length (cm)	18.13 ± 0.07^a	18.05 ± 0.04^a	18.12 ± 0.06^a	18.13 ± 0.05^a	18.11 ± 0.05^a	18.17 ± 0.01^a	18.13 ± 0.03^a	18.16 ± 0^a	18.12 ± 0.03^a
Final length (cm)	21.49 ± 0.12^{ab}	21.17 ± 0.24^b	21.52 ± 0.24^{ab}	21.31 ± 0.03^b	21.52 ± 0.36^{ab}	21.78 ± 0.1^{ab}	22.13 ± 0.24^a	21.62 ± 0.14^{ab}	21.49 ± 0.08^{ab}
BWI (%)	77.41 ± 5.58^{ab}	70.41 ± 8.3^b	79.96 ± 7.87^{ab}	72.9 ± 1.15^b	76.77 ± 7.48^{ab}	83.39 ± 6.18^{ab}	102.84 ± 1.62^a	76.25 ± 8.07^{ab}	84.37 ± 9.01^{ab}
CF (g/cm^3)	1.2 ± 0.01^a	1.2 ± 0.01^a	1.17 ± 0.01^a	1.2 ± 0.01^a	1.17 ± 0.01^a	1.18 ± 0^a	1.2 ± 0.01^a	1.19 ± 0.01^a	1.2 ± 0^a
Food intake rate (g)	3.07 ± 0.03^{ab}	2.93 ± 0.13^b	3 ± 0.113^b	2.97 ± 0.03^b	2.97 ± 0.15^b	0.06 ± 3.1^{ab}	3.33 ± 0.15^a	3.1 ± 0.06^{ab}	3.03 ± 0.03^{ab}
FCR	1.028 ± 0^b	1.029 ± 0^a	1.028 ± 0^{ab}	1.028 ± 0^{ab}	1.028 ± 0^{ab}	1.028 ± 0^{ab}	1.027 ± 0^b	1.028 ± 0^{ab}	1.028 ± 0^{ab}
SGR (%/day)	0.95 ± 0.05^{ab}	0.88 ± 0.08^b	0.98 ± 0.07^{ab}	0.91 ± 0.01^{ab}	0.94 ± 0.07^{ab}	1.01 ± 0.06^{ab}	1.17 ± 0.13^a	0.94 ± 0.07^{ab}	1.01 ± 0.08^{ab}
PER (%)	0.62 ± 0^a	0.63 ± 0^a	0.61 ± 0^a	0.62 ± 0^a	0.61 ± 0^a	0.63 ± 0^a	0.62 ± 0^a	0.63 ± 0^a	0.62 ± 0^a

Different letters indicate significant differences in each group ($p < 0.05$).

Table 5. Se contents in feed and wastewater of *S. caspius* tanks fed different levels of SelMet and Dip^+ for 60 days.

	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Se in feed (µg kg^{-1})	4370	4370	4370	6740	10960	6740	10960	6740	10960
Se in wastewater (µg kg^{-1})	3	3	3	1	2	1	2	1	2

Blood indicators

The results obtained from the analyses of blood indices are depicted in Table 7. Notably, T_8 exhibited the highest WBCs, RBCs, Hb, and Hct levels compared to the control group ($p < 0.05$). Conversely, the control group demonstrated the lowest significant levels of RBCs, WBCs, and Hb ($p < 0.05$). The hematocrit levels in T_1, T_4, and T_5 did not differ significantly from the control group ($p < 0.05$). Table 7 suggests an interaction between the two oral supplements, Dip^+ and SelMet, across all blood parameters ($p < 0.05$).

Blood indices

The results derived from the analyses of blood indices are outlined in Table 8. The control group exhibited the highest significant level of MCV compared to the experimental treatments ($p < 0.05$). T_3 displayed the highest

significant level of MCH ($p < 0.05$). T_1, T_2, and the control group displayed lower MCH levels than other treatments ($p < 0.05$). The control group and T_5 demonstrated the lowest and highest MCHC levels respectively, significantly different from other treatments ($p < 0.05$). Based on the results obtained from examining blood indices in Table 8, it can be inferred that there is a mutualistic relationship between Dip^+ and SelMet ($p < 0.05$).

Table 6. Average levels of lysozyme activity, total Ig and IgM in *S. caspius* fed different levels of SelMet and Dip^+ for 60 days.

	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
lysozyme activity (U mL^{-1} min^{-1})	26±2.52^{c}	29.67±2.33^{bc}	26±0.58^{c}	26±1.15^{c}	25.33±1.33^{c}	32.67±2.67^{abc}	37±1^{a}	35±4.04^{ab}	28±1^{bc}
IgM (mg dL^{-1})	69±0.58^{e}	79.33±0.67^{b}	67.68±0.67^{e}	79.67±0.67^{b}	73±0^{d}	70.33±0.67^{e}	75±0.58^{c}	82.33±0.67^{a}	78.67±0.67^{b}
Total Ig (mg mL^{-1})	24.83±0.07^{h}	27.43±0.07^{d}	28.6±0.1^{b}	29.83±0.03^{a}	25.5±0^{g}	26.43±0.07^{e}	28.23±0.09^{c}	24.77±0.09^{h}	25.77±0.07^{f}

Different letters indicate significant differences in each group ($p < 0.05$).

Table 7. Average levels of WBCs, RBCs, Hb and Hct in *S. caspius* fed different levels of SelMet and Dip^+ for 60 days.

	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
WBC (10^3 mm^3)	7410 ± 20.82^{h}	9683.3 ± 44.1^{c}	9583.3 ± 44.1^{d}	8333.3 ± 33.33^{f}	7883.3 ± 16.67^{g}	8316.7 ± 16.67^{f}	8433.3 ± 33.33^{e}	9916.7 ± 16.67^{b}	11533 ± 33.33^{a}
RBC (mm^3)	1.680000 ± 5773.5^{f}	1756700 ± 333.33^{d}	1831700 ± 1666.6^{a}	1770000 ± 2886.7^{c}	1703300 ± 1666.6^{e}	1763300 ± 1666.6^{cd}	1755000 ± 5000^{d}	1785000 ± 2886.^{7b}	1825000 ± 2886.7^{a}
Hb (g dL^{-1})	8.24 ± 0.01^{g}	8.35 ± 0.01^{f}	8.72 ± 0.02^{bc}	8.77 ± 0.03^{b}	8.3 ± 0.01^{f}	8.68 ± 0.01^{c}	8.48 ± 0.02e	8.62 ± 0d	8.82 ± 0.03a
Hct (%)	48.33 ± 0.88^{c}	48.33 ± 0.33^{c}	50.67 ± 0.33^{a}	50.67 ± 0.67^{a}	48.67 ± 0.33^{bc}	49.67 ± 0.33^{abc}	50.33 ± 0.33^{a}	50.33 ± 0^{ab}	50.67 ± 0.33^{a}

Different letters indicate significant differences in each group ($p < 0.05$).

Table 8. Average level of MCV, MCH and MCHC in *S. caspius* fed different levels of SelMet and Dip^+ for 60 days.

	Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
MCV (fL)	297 ± 1.15^{a}	282 ± 1.15^{cd}	278.67 ± 0.67^{d}	288 ± 2.08^{b}	287.33 ± 0.33^{b}	284 ± 1^{c}	290 ± 1^{b}	281.67 ± 0.88^{cd}	279.67 ± 0.88^{d}
MCH (pg)	47.83 ± 0.03^{e}	47.67 ± 0.07^{e}	47.40 ± 0.1^{e}	49.5 ± 0.15^{a}	48.63 ± 0.03^{c}	49.13 ± 0.03^{b}	48.67 ± 0.13^{c}	48.1 ± 0.12^{d}	48.1 ± 0.06^{d}
MCHC (g/dL)	16.44 ± 0.01^{f}	17.03 ± 0.01^{c}	17.05 ± 0.01^{c}	17.05 ± 0.08^{c}	16.93 ± 0.01^{d}	17.31 ± 0.02^{a}	16.64 ± 0.04^{e}	17.23 ± 0.01^{ab}	17.21 ± 0.02^{b}

Different letters indicate significant differences in each group ($p < 0.05$).

WBCs differential count

The results obtained from the differential count of WBCs are detailed in Table 9. The highest significant number of neutrophils was observed in T_3 ($p < 0.05$). The control group exhibited the highest significant number of lymphocytes ($p < 0.05$). As shown in Table 9, the lowest significant number of monocytes was observed in the control group, T_2 and T_5 ($p < 0.05$). The percentage of eosinophils in all groups was calculated to be less than 1% and did not exhibit a significant difference ($p > 0.05$).

Carcass analysis

According to the carcass composition analyses in Fig. 1, the highest carcass fat rate (%) was observed in the control group ($p < 0.05$). Additionally, the lowest significant levels of ash, protein, and moisture rate (%) in the carcass were measured in the control group ($p < 0.05$). The highest significant level of carcass protein rate was observed in T_1 and T_2 ($p < 0.05$). Based on the results of the factorial design analysis, it can be asserted that there is a mutualistic relationship between the two supplements, SelMet and Dip^+ ($p < 0.05$).

IL-1 and TNF-α relative gene expression

The impacts of using SelMet and Dip^+ in *S. caspius* on the relative expression of IL-1 and TNF-α genes are illustrated in Fig. 2. In T_8, a remarkable increase of approximately 16 times in the IL-1 gene expression and about 22 times in the TNF-α gene expression was observed, displaying the most significant difference compared to the

control group ($p < 0.05$). While no significant difference was observed when examining the interaction between SelMet and Dip^+ for the IL-1 gene ($p > 0.05$), a significant difference was noted when evaluating the mutualistic relationship between SelMet and Dip^+ for TNF-α relative gene expression ($p < 0.05$).

Table 9. Average levels of WBCs differential count in *S. caspius* fed different levels of SelMet and Dip^+ for 60 days.

	Control	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**
Neutrophil (%)	15.33 ± 0.33^{cd}	16.33 ± 0.33^{c}	15.33 ± 0.33^{cd}	18.67 ± 0.33^{a}	14.67 ± 0.33^{d}	16 ± 0^{c}	17.33 ± 0.33^{b}	17.67 ± 0.33^{b}	16.33 ± 0.33^{c}
Lymphocyte (%)	81 ± 0.58^{a}	77.33 ± 0.33^{de}	80.33 ± 0.67^{ab}	75.67 ± 0.33^{f}	$79 \pm 0.58bc^{d}$	$79.67 \pm 0.33a^{bc}$	77.67 ± 0.88^{de}	76.67 ± 0.33^{ef}	78.33 ± 0.33^{cde}
monocyte (%)	$4.33 \pm 0.33_{b}$	$4.67 \pm 0.33_{ab}$	4.33 ± 0.33^{b}	5.67 ± 0.33^{a}	4.67 ± 0.33^{ab}	4.33 ± 0.33^{b}	5.67 ± 0.33^{a}	5 ± 0^{ab}	5.33 ± 0.33^{ab}
Eosinophil (%)	0.33 ± 0.33^{a}	0.67 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}	0.33 ± 0.33^{a}

Different letters indicate significant differences in each group ($p < 0.05$).

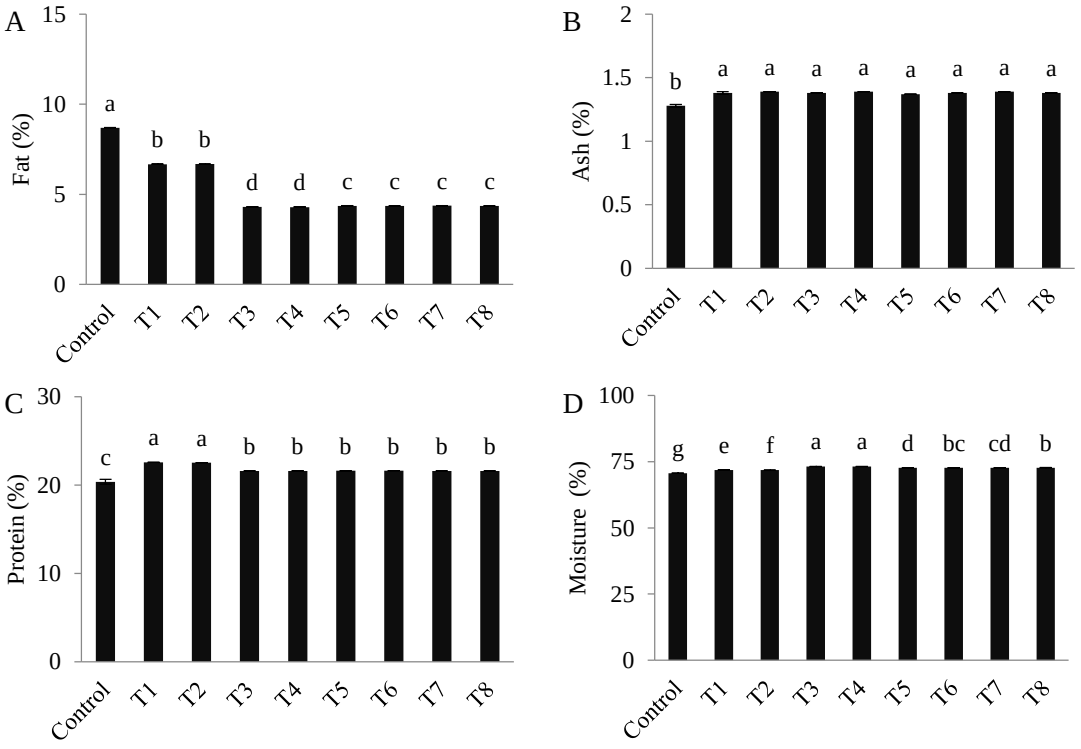

Fig. 1. The results of the carcass composition analyses of *S. caspius* fed different levels of SelMet and Dip^+ for 60 days. A: Fat (%). B: Ash (%). C: Protein (%). D: Moisture (%). Different letters indicate significant differences in each group ($p < 0.05$).

DISCUSSION

Growth performance

In recent decades, the utilization of immune stimulants in aquaculture has gained popularity for enhancing the activity of non-specific defense mechanisms and establishing relative resistance to diseases (Sakai 1999; Faruk *et al.* 2021). These substances have been recognized as compounds with the potential to reduce reliance on antibiotics (Farooqi & Qureshi 2018). The efficacy of supplements is contingent on factors such as the age and size of living organisms, environmental conditions, ambient temperature, and the mode of supplement administration (Khan *et al.* 2017). Among the various immune stimulants, synbiotic supplements have demonstrated effectiveness in promoting the growth and immunity of aquatic organisms (Sewaka *et al.* 2019; Safari *et al.* 2021; Rohani *et al.* 2022; Mohammadi *et al.* 2022). Furthermore, the presence of microelements, including Se, in the body of living organisms is essential for executing vital functions within the host organism

(Çiçek & Özoğul 2021 & Wang *et al.* 2022). Several studies on Se in aquatic animals have indicated that Se deficiency can result in decreased appetite, reduced growth, lipid peroxidation damage to cell membranes, and increased mortality (Watanabe *et al.* 1997; Dawood *et al.* 2019; Lin *et al.* 2021). High levels of Se in fish diets have also been shown to reduce feed efficiency, leading to decreased reproduction, tissue damage, and organ malformations (Lemly 2002; Wang *et al.* 2019). Therefore, determining the optimal level of Se required in the diet for different farmed fish species is crucial.

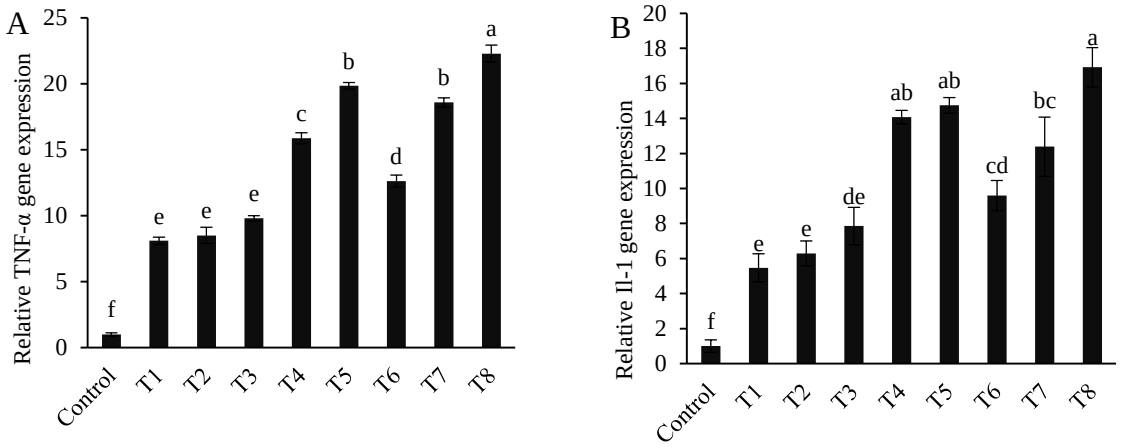

Fig. 2. Relative gene expression in *S. caspius* fed different levels of SelMet and Dip$^+$ for 60 days. A: TNF-α. B: IL-1. Different letters indicate significant differences in each group ($p < 0.05$).

The presence of Se is vital for muscle tissue growth, immune system fortification, and genomic stability (Surai 2002; Miezeliene *et al.* 2011). Se plays a preventive role in the peroxidation of arachidonic acid, thereby shielding cells and tissues from free radical-induced damage (Arthur *et al.* 2003). Selenomethionine (SelMet), an organic form of Se, exhibits higher absorbability than inorganic Se. Additionally, SelMet boasts a superior biological value and absorbability compared to Se (Lorentzen *et al.* 1994; Wang & Lovell 1997; Wang *et al.* 2007). The presence of Se in various organs may also contribute to the storage of vitamin E (Lin & Shiau 2005). Both compounds have demonstrated antioxidant activity (Lin & Shiau 2005). Various studies have been conducted to calculate the optimal amount of Se to enhance growth in different fish species. Han *et al.* (2011) reported optimal Se amounts for *Carassius auratus* as 0.7 mg Se/kg diet and for common carp as 1 mg Se/kg diet. Other studies have demonstrated that optimal Se levels can significantly improve digestibility and enhance antioxidant status (Küçükbay *et al.* 2009; Lin 2014). The use of Se in the diet of common carp (Ashouri *et al.* 2015; Saffari *et al.* 2017), *C. auratus* (Zhou *et al.* 2009), Nile tilapia (Lee *et al.* 2016), grouper (Lin & Shiau 2005) and rainbow trout (Wang *et al.* 2018) has been shown to improve growth. In the present study, using SelMet and Dip+ in T_6 resulted in the highest growth rate and the best performance of growth indices compared to other groups. However, the consumption of Dip$^+$ or SelMet alone did not significantly increase the growth rate. This may be attributed to not using the optimal concentration of Se, since the optimal amount of Se for *S. caspius in vitro* may exceed 4 mg, and concentrations lower than the optimal level may not significantly affect growth. Synbiotics, such as Dip$^+$, can enhance host growth by influencing the microbial flora of the intestine. In the present research, Dip$^+$ consisted of lactic acid bacteria and MOS. However, receiving 2 and 3 g of Dip$^+$ through feed did not significantly increase growth. On the other hand, Rohani *et al.* (2022) and Dawood *et al.* (2020) reported that the use of synbiotic supplements caused a significant increase in the growth of Nile tilapia.

This discrepancy could be attributed to differences in supplement concentration, duration of supplement use, and fish species (Heidarieh *et al.* 2013; Hassaan *et al.* 2018). The combined use of SelMet and Dip+ decreased the FCR and increased SGR, confirming the synergistic effect of using 2 g of Dip+ and 4 mg of SelMet. Se is an essential component of the deiodinase enzyme and indirectly affects growth hormone secretion (Muller *et al.* 1999). Se accelerates the production of selenoproteins in intestinal lining cells, improving digestion (Wang *et al.* 2013) and modulating the activity of digestive enzymes (Shenkin 2006; Dawood *et al.* 2020). The increase in the population of beneficial intestinal bacteria due to probiotic consumption (Rohani *et al.* 2022) can play a crucial role in producing extracellular enzymes (Sandhya & Vijayan 2019).

Se in feed and wastewater

The present study revealed that the Se levels measured in the outlet water of the experimental groups that received the same amount of SelMet did not significantly differ. The impact of a mineral supplement on growth parameters and the immune system can be influenced by the different forms in which the constituent elements of that supplement are bonded (Authman *et al.* 2015; Ali 2020). For instance, SelMet is more absorbable and accessible to tissues compared to inorganic Se (Lorentzen *et al.* 1994; Wang & Lovell 1997; Wang *et al.* 2007). Therefore, it can be inferred that the release rate of Se from the body to the environment is reduced. Additionally, all groups with similar nutritional conditions likely experienced the same level of Se absorption.

Immune indices

The vertebrate immune system consists of two main components, the innate and adaptive immune system, which is activated through adaptation to the surrounding environment and pathogens (Muiswinkel & Vervoorn-Van Der Wal 2006). In fish, non-specific immunity becomes more crucial due to limitations in acquired immunity, such as a scarcity of antibodies and slow proliferation of lymphocytes (Cipriano & Austin 2011). Total Ig and lysozyme are associated with non-specific immune responses (Hoseinifar *et al.* 2019; Yousefi *et al.* 2020), and they are considered proteins in the innate immune system (Magnadóttir 2006). As the innate immune system in aquatic organisms primarily consists of protein and enzyme components (Sadat Hoseini Madani *et al.* 2018), variations in these parameters can indicate the organism's health status or stress levels. Several studies have reported positive effects on increasing lysozyme activity and total Ig levels when using synbiotic supplements alone (Ai *et al.* 2011; Mouriño *et al.* 2017; Devi *et al.* 2019), which contrasts with the results of the present research. This discrepancy may arise from differences in the synbiotic consumed and the species studied (Zakariaee *et al.* 2021). Lysozyme is an antibacterial enzyme in the non-specific immune system that hydrolyzes glycosidic bonds in the bacteria's cell wall, effective against gram-negative and gram-positive bacteria. In this study, the combined use of Dip+ and SelMet significantly increased serum lysozyme. Research has shown that Se, as a micronutrient element, may enhance innate immune indices when combined with other dietary supplements (Gatlin *et al.* 1986; Fonseca *et al.* 2013; Nugroho & Fotedar 2014; Ambas *et al.* 2017). The current use of immune stimulants, such as probiotics, prebiotics, synbiotics, and micronutrients in aquaculture, requires further research. Various factors should be considered, including the type and concentration of the immune stimulant, preparation, in addition to the diet, species, and the presence of stressors. Using the correct concentration of the immunostimulant is crucial, since an incorrect concentration may lead to economic losses on a large scale, and excessive amounts may suppress the immune response. In this study, the significant increase in the non-specific immune system may be related to upraised WBCs in the GALT due to the elevated intestinal bacterial load.

Blood indices

Changes in the hematological indices of fish can occur due to various factors, including temperature fluctuations, seasonal changes, diet, manipulation stress, genetic differences, age, sexual maturity, and gender (Knowles *et al.* 2006). This research demonstrated that adding 4 mg kg^{-1} SelMet to the diet and 3 mg kg^{-1} Dip+ significantly increased WBCs, RBCs, Hb, and Hct. The elevation in WBC count may be attributed to the stimulation of the fish's immune system by the components of Dip+ and their synergistic effect when combined with SelMet. Studies have reported that synbiotics can enhance not only the stimulation of the innate immune system in fish but also modulate the number of WBCs, leading to increased resistance to stress (Yarahmadi *et al.* 2014). The elevation in the number of RBCs, Hct, and Hb may result from the regulation of dietary iron absorption, the secretion of extracellular enzymes, and the improvement of digestion and absorption facilitated by beneficial intestinal bacteria (Jenkins *et al.* 1999; Aftabgard *et al.* 2019). SelMet in the diet can also act as a cofactor for enzymes, which is influential in digestion and absorption processes (Levander *et al.* 1983; Smith & Picciano 1987). Previous studies conducted by researchers such as Akrami *et al.* (2015), Hoseinifar *et al.* (2016), Mohammadian *et al.* (2019), and Çiçek & Özoğul (2021) support the findings of the present study regarding the improvement of blood indices in fish treated with Dip+ and SelMet immune stimulants. MCV, MCH, and MCHC changes can indicate oxidative stress (Yonar 2012). A decrease in the volume of RBCs may suggest the absence of inflammation, facilitating the movement and suspension of RBCs. Generally, researchers believe that the blood indices of fish are closely related to the conditions of the breeding environment, the size and age of the fish, the species type, and the quantity and quality of food (Tangestani *et al.* 2011). The studies conducted by Ates *et al.* (2008), Firouzbakhsh *et al.* (2014), Hao *et al.* (2014), and Mansour *et al.* (2017) support the findings of the present study. The differential count of

WBCs in the present study revealed that the highest number of neutrophils was observed in fish fed 2 mg SelMet/kg diet. Additionally, the highest significant level of lymphocytes was observed in the control group. The number of eosinophils in all treatments, including the control group, was calculated to be less than 1%. A significant increase in the number of neutrophils, lymphocytes, and monocytes indicates the effect of SelMet and Dip+ oral supplementation on fish innate immunity, facilitating the control of pathogens due to immune system stimulation (Devi *et al.* 2019). The studies conducted by Devi *et al.* (2019) on synbiotic effects, as well as those by Biller-Takahashi *et al.* (2015) and Iqbal *et al.* (2017) regarding the effect of Se on the differential increase of WBCs, are consistent with the results of the present study.

Carcass composition

The carcass's biochemical composition is influenced by various factors such as species, age, feeding rate, feed type, and feed composition (Dumas *et al.* 2007). In the present study, the control group exhibited the highest significant amount of fat and the lowest significant levels of ash, protein, and moisture in the carcass. Additionally, the groups receiving Dip^+ supplementation had significantly higher protein levels in the carcass. The significant increase in carcass protein may be attributed to the potential effect of the synbiotic composition containing *Bacillus subtilis*, *Pediacoccus acidilactici*, and MOS on reducing the catabolism of amino acids in the body, thereby improving the body's protein synthesis (Grisdale-Helland *et al.* 2013). Ye *et al.* (2011) reported a significant increase in protein and a significant drop in fat under synbiotic treatment (*B. subtilis* and MOS) on Japanese flounder, aligning with the present study's findings. Dip^+ and SelMet supplements moderate protein metabolism and improve protein storage (Mehrabi *et al.* 2012). Additionally, reducing carcass fat in the experimental treatments could result from fish consuming fat to meet their energy requirements.

IL-1 and TNF-α relative gene expression

Preventive measures need to be implemented to regulate the immune system, maintain health, prevent disease spread, avoid heavy casualties, and minimize economic losses due to diseases. Supplements are recognized as an easy and efficient way to strengthen the body's defense system (Vallejos-Vidal *et al.* 2016). The present study observed that the use of SelMet and Dip^+ oral supplements increased the relative expression of IL-1 and TNF-α genes in *S. caspius*. Limited data about the effect of Dip^+ on the relative expression of inflammatory cytokine genes in farmed fish have been published. Information related to the effect of Se on the relative expression of genes involved in immunity in aquatic animals is also very limited. However, several studies on probiotic, prebiotic, and micronutrient supplements have shown the modulation of inflammatory cytokines in various organisms (Niers *et al.* 2005). The current research results demonstrated the positive effect of both SelMet and Dip^+ oral supplements in increasing the relative expression of IL-1 and TNF-α genes. The highest significant level of relative expression of these genes was observed in fish fed a diet containing 4 mg SelMet/kg and 3 g Dip+/kg. In a synbiotic combination, the prebiotic part is consumed by probiotic bacteria, producing secondary compounds in the intestine that induce changes in the microbial flora of the digestive tract (Nayak 2010). Eventually, various metabolites are produced throughout the intestine (Ashouri *et al.* 2020). Research has shown that most of these metabolites, including short-chain fatty acids, can enhance the immune system's function (Ashouri *et al.* 2020). It is also possible that Se indirectly increases the relative expression of IL-1 and TNF-α genes by enhancing digestive enzymes (Ray *et al.* 2012). Studies have indicated that metabolites from the fermentation of prebiotics are rapidly absorbed into the bloodstream from the gut and distributed throughout the body. These metabolites serve as a source of energy in the body. For instance, propionate, a bacterial metabolite, has been shown to stimulate the immune system (Demigné & Rémésy 1985; Gómez & Balcázar 2008). Part of the non-specific defense system in vertebrates is associated with the lymphoid system and plays an influential role in modulating the immune system under the influence of food. In this study, the observed increase in the relative expression of IL-1 and TNF-α genes in *S. caspius* can be attributed to the effect of Dip^+ and SelMet food supplements on the microbial flora of the digestive tract, thereby enhancing the function of the immune system by affecting GALT (Montagne *et al.* 2003). Research conducted by Zhang *et al.* (2018), Devi *et al.* (2019), Zhang *et al.* (2020), and Khosravi-Katuli *et al.* (2021) has confirmed the increase in the relative expression of genes involved in the immune system in various aquatic species in connection with the use of MOS, probiotics, and Se.

CONCLUSION

The observed enhancements in growth and immune system modulation in *S. caspius* may be attributed to microbial flora influence in the digestive tract, leading to increased secretion of secondary metabolites and extracellular

enzymes. Improved intestinal absorption and modulation of microbial flora can enhance immune system functioning via the gut-associated lymphoid tissue. The results indicate that the simultaneous use of Dip^+ and SelMet yields better outcomes than using Dip^+ or SelMet alone. The combined application of Dip^+ and SelMet demonstrates the potential for enhanced growth and improved feed utilization in *S. caspius*. Moreover, the concurrent use of Dip^+ and SelMet can stimulate the non-specific immune system in *S. caspius*. Based on the findings, using 4 mg SelMet and 3 g Dip^+ simultaneously for consumption in *S. caspius* farms is recommended.

CRediT author statement

Maziyar Akbarabadi: Investigation, Methodology, Formal analysis, Writing – original draft. Hossein Khara: Conceptualization, Supervision, Writing – review & editing, project administration. Habib Vahaabzadeh Roudsari: Supervision, Writing– review, Data curation & editing. Mohaddeseh Ahmadnezad: Supervision, Writing– review & editing. Roghieh Safari: Supervision, Methodology, Writing– review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Ethical approval

All experimental procedures about the fish adhered to ethical standards and followed the guidelines for the ethical review of fish welfare outlined in ARRIVE (Animals in Research: Reporting *In Vivo* Experiments). The study's compliance with these guidelines can be accessed through the link https://doi.org/10.1371/journal.pbio.1000412.

REFERENCES

Aftabgard, M, Salarzadeh, A, Mohseni, M, Bahri Shabanipour, AH & Zorriehzahra, MEJ 2019, The combined efficiency of dietary isomaltooligosaccharides and *Bacillus* spp. on the growth, hemato-serological, and intestinal microbiota indices of Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). *Probiotics and Antimicrobial Proteins*, 11: 198-206.

Ai, Q, Xu, H, Mai, K, Xu, W, Wang, J & Zhang, W 2011, Effects of dietary supplementation of Bacillus subtilis and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. *Aquaculture*, 317: 155-161.

Ajdari, A, Ghafarifarsani, H, Hoseinifar, SH, Javahery, S, Narimanizad, F, Gatphayak, K & Van Doan, H 2022, Effects of dietary supplementation of primaLac, inulin, and biomin imbo on growth performance, antioxidant, and innate immune responses of common carp (*Cyprinus carpio*). *Aquaculture Nutrition*, 1-13.

Akrami, R, Nasri-Tajan, M, Jahedi, A, Jahedi, M, Razeghi Mansour, M & Jafarpour, SA 2015, Effects of dietary synbiotic on growth, survival, lactobacillus bacterial count, blood indices and immunity of beluga (*Huso huso* L innaeus, 1754) juvenile. *Aquaculture Nutrition*, 21: 952-959.

Ali, D, Almarzoug, MH, Al Ali, H, Samdani, MS, Hussain, SA & Alarifi, S 2020, Fish as bio indicators to determine the effects of pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay. *Journal of King Saud University-Science*, 32: 2880-2885.

Ambas, I, Fotedar, R & Buller N 2017, Synbiotic effect of Bacillus mycoides and organic selenium on immunity and growth of marron, *Cherax cainii*. *Aquaculture Research*, 48: 2729-2740.

AOAC. 1990, Official methods of analysis. 15th edn. Virginia, Association of Official Analytical Chemists.

Arthur, JR, McKenzie, RC & Beckett, GJ 2003, Selenium in the immune system. *The Journal of Nutrition*, 33: 1457S-1459S.

Ashouri, S, Keyvanshokooh, S, Salati, AP, Johari, SA & 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.

Ashouri, G, Soofiani, NM, Hoseinifar, SH, Jalali, SAH, Morshedi, V, Valinassab, T, Bagheri, D, Van Doan, H, Mozanzadeh, MT & Carnevali, O 2020, Influence of dietary sodium alginate and *Pediococcus acidilactici*

on liver antioxidant status, intestinal lysozyme gene expression, histomorphology, microbiota, and digestive enzymes activity, in Asian sea bass (*Lates calcarifer*) juveniles. *Aquaculture*, 518: 734638.

Ates, B, Orun, I, Talas, ZS, Durmaz, G & Yilmaz, I 2008, Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu2+. *Fish Physiology and Biochemistry*, 34: 53-59.

Authman, MM, Zaki, MS, Khallaf, EA & Abbas, HH 2015, Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development*, 6: 1-13.

Biller-Takahashi, JD, Takahashi, LS, Mingatto, FE & Urbinati, EC 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: 360-367.

Çiçek, S & Özoğul F 2021, Effects of selenium nanoparticles on growth performance, hematological, serum biochemical parameters, and antioxidant status in fish. *Animal Feed Science and Technology*, 281: 115099.

Cipriano, RC & Austin, B 2011, Furunculosis and other aeromonad diseases. In Fish diseases and disorders. Volume 3: viral, bacterial and fungal infections (pp. 424-483). Wallingford UK: CABI.

Dato-Cajegas, CRS & Yakupitiyage, A 1996, The need for dietary mineral supplementation for Nile tilapia, *Oreochromis niloticus*, cultured in a semi-intensive system. *Aquaculture*, 144: 227-237.

Dawood, MA, Koshio, S, Abdel-Daim, MM & Van Doan, H 2019, Probiotic application for sustainable aquaculture. *Reviews in Aquaculture*, 11: 907-924.

Dawood, MA, Eweedah, NM, Moustafa, EM & Shahin, MG 2020, Synbiotic effects of *Aspergillus oryzae* and β-glucan on growth and oxidative and immune responses of nile Tilapia, *Oreochromis niloticus*. *Probiotics and Antimicrobial Proteins*, 12: 172-183.

Demigné, C & Rémésy, C 1985, Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. *The Journal of Nutrition*, 115: 53-60.

Devi, G, Harikrishnan, R, Paray, BA, Al-Sadoon, MK, Hoseinifar, SH & Balasundaram, C 2019, Effect of symbiotic supplemented diet on innate-adaptive immune response, cytokine gene regulation and antioxidant property in *Labeo rohita* against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, 89: 687-700.

Dumas, A, De Lange, CF, France, J & Bureau, DP 2007, Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 273: 165-181.

Ellis, AE 1999, Immunity to bacteria in fish. *Fish & Shellfish Immunology*, 9: 291–308.

Farooqi, FS & Qureshi, WUH 2018, Immunostimulants for aquaculture health management. *Journal of Pharmacognosy and Phytochemistry*, 7:1441-1447.

Faruk, MAR, Begum, MM & Anka, IZ 2021, Use of Immunostimulants for Fish Health Management in Mymensingh District of Bangladesh. *SAARC Journal of Agriculture*, 19: 237-248.

Firouzbakhsh, F, Mehrabi, Z, Heydari, M, Khalesi, MK & Tajick, MA 2014, Protective effects of a synbiotic against experimental *Saprolegnia parasitica* infection in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, 45: 609-618.

Fonseca, SBD, Silva, JHVD, Beltrão Filho, EM, Mendes, PDP, Fernandes, JBK, Amancio, ALL, Jordão Filho, J, Lacerda, PBD & Silva, FRPD 2013, Influence of levels and forms of selenium associated with levels of vitamins C and E on the performance, yield and composition of tilapia fillet. *Food Science and Technology*, 33: 109-115.

Gao, Z, Wang, W, Abbas, K, Zhou, X, Yang, Y, Diana, JS, Wang, H, Wang, H & Li, Y 2007, Haematological characterization of loach *Misgurnus anguillicaudatus*: a comparison among diploid, triploid and tetraploid specimens. *Comparative Biochemistry and Physiology*, 147: 1001-1008.

Gatlin, DM, Poe, WE & Wilson, RP 1986, Effects of singular and combined dietary deficiencies of selenium and vitamin E on fingerling channel catfish (*Ictalurus punctatus*). *The Journal of Nutrition*, 116: 1061-1067.

Ghafari farsani, H, Rashidian, G, Bagheri, T, Hoseinifar, SH & Van Doan, H 2021, Study on growth enhancement and the protective effects of dietary prebiotic inulin on immunity responses of rainbow trout fry infected with. *Annals of Animal Science*, 21: 543-559.

Ghaniem, S, Nassef, E, Zaineldin, AI, 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*, 1-16.

Gómez, GD & Balcázar, JL 2008, A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunology & Medical Microbiology*, 52: 145-154.

Grisdale-Helland, B, Takle, H & Helland, SJ 2013, Aerobic exercise increases the utilization efficiency of energy and protein for growth in Atlantic salmon post-smolts. *Aquaculture*, 406: 43-51.

Hamilton, SJ 2004, Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment*, 326: 1-31.

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: e741-e749.

Hao, X, Ling, Q & Hong, F 2014, Effects of dietary selenium on the pathological changes and oxidative stress in loach (*Paramisgurnus dabryanus*). *Fish Physiology and Biochemistry*, 40: 1313-1323.

Hassaan, MS, Soltan, MA, Mohammady, EY, Elashry, MA, El-Haroun, ER & Davies, SJ 2018, Growth and physiological responses of Nile tilapia, *Oreochromis niloticus* fed dietary fermented sunflower meal inoculated with *Saccharomyces cerevisiae* and *Bacillus subtilis*. *Aquaculture*, 495: 592-601.

Heidarieh, M, Mirvaghefi, AR, Akbari, M, Sheikhzadeh, N, Kamyabi-Moghaddam, Z, Askari, H & Shahbazfar, AA 2013, Evaluations of H ilyses™, fermented Saccharomyces cerevisiae, on rainbow trout (*Oncorhynchus mykiss*) growth performance, enzymatic activities and gastrointestinal structure. *Aquaculture Nutrition*, 9: 343-348.

Hilton, JW, Hodson, PV & Slinger, SJ 1980, The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *The Journal of Nutrition*, 110: 2527-2535.

Hoseinifar, SH, Ringø, E, Shenavar Masouleh, A & Esteban, MÁ 2016, Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Reviews in Aquaculture*, 8: 89-102.

Hoseinifar, SH, Hosseini, M, Paknejad, H, Safari, R, Jafar, A, Yousefi, M, Van Doan, H & Mozanzadeh, MT 2019, Enhanced mucosal immune responses, immune related genes and growth performance in common carp (*Cyprinus carpio*) juveniles fed dietary Pediococcus acidilactici MA18/5M and raffinose. *Developmental & Comparative Immunology*, 94: 59-65.

Ilham, I & Fotedar, R 2016, Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi* Valenciennes 1883): selenium and temperature interaction. *Animal Feed Science and Technology*, 217: 76-86.

Iqbal, S. Atique, U, Mughal, MS, Khan, N, Haider, MS, Iqbal, KJ & Akmal, M 2017, Effect of selenium incorporated in feed on the hematological profile of tilapia (*Oreochromis niloticus*). *Journal of Aquaculture Research & Development*, 8: 1-6.

Jenkins, KJ, Collins, FW & Hidiroglou, M 1992, Research note: efficacy of various flavonoids and simple phenolics in prevention of nutritional myopathy in the chick. *Poultry Science*, 71: 1577-1580.

Khara, H, Sayyadborani, M & Sayyadborani, M 2016, Effects of α-tocopherol (vitamin E) and ascorbic acid (vitamin C) and their combination on growth, survival and some haematological and immunological parameters of Caspian brown trout, *Salmo trutta caspius* juveniles. *Turkish Journal of Fisheries and Aquatic Sciences*, 16: 385-393.

Khara. H 2016, Effect of successive milt collection on sperm quality and reproduction of wild and cultured endangered caspian brown trout, *Salmo trutta*. *Iranian Journal of Fisheries Science*, 15: 31-38.

Khan, KU, Zuberi, A, Fernandes, JBK, Ullah, I & Sarwar, H 2017, An overview of the ongoing insights in selenium research and its role in fish nutrition and fish health. *Fish Physiology and Biochemistry*, 43: 1689-1705.

Khosravi-Katuli, K, Mohammadi, Y, Ranjbaran, M, Ghanaatian, H, Khazaali, A, Paknejad, H & Santander, J 2021, Effects of mannan oligosaccharide and synbiotic supplementation on growth performance and immune response of Gilthead Sea Bream (*Sparus aurata*) before and after thermal stress. *Aquaculture Research*, 52: 3745-3756.

Knowles, TP, Mullin, RA, Hunter, JA & Douce, HF 2006, Effects of syringe material, sample storage time, and temperature on blood gases and oxygen saturation in arterialized human blood samples. *Respiratory Care*, 51: 732-736.

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

Lee, CS & Donaldson, EM 2001, Reproductive Biotechnology in Finfish Aquaculture. Proceedings of a Workshop Hosted by the Oceanic Institute. Elzevier, p. 11.

Lee, S, Nambi, RW, Won, S, Katya, K & Bai, SC 2016, Dietary selenium requirement and toxicity levels in juvenile Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 464: 153-158.

Lemly, AD 2002, Symptoms and implications of selenium toxicity in fish: The Belews Lake case example. *Aquatic Toxicology*, 57: 39-49.

Levander, OA, Alfthan, G, Arvilommi, H, Gref, CG, Huttunen, JK, Kataja, M, Koivistoinen, P & Pikkarainen, J 1983, Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *The American Journal of Clinical Nutrition*, 37: 887-897.

Li, P & Gatlin, DM 2003, Evaluation of brewer's yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid Striped Bass (*Moronechry sops* × *M. saxatilis*). *Aquaculture*, 219: 681–692.

Lin, YH & Shiau, SY 2005, Dietary selenium requirements of juvenile grouper, *Epinephelus malabaricus*. *Aquaculture*, 250: 356-363.

Lin, YH 2014, Effects of dietary organic and inorganic selenium on the growth, selenium concentration and meat quality of juvenile grouper *Epinephelus malabaricus*. *Aquaculture*, 430: 114-119.

Lin, W, Zhang, J, Xu, JF & Pi, J 2021, The advancing of selenium nanoparticles against infectious diseases. *Frontiers in Pharmacology*, 12: 2284.

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

Magnadóttir, B 2006, Innate immunity of fish (overview). *Fish & Shellfish Immunology*, 20: 137-151.

Mansourghanaei, A, Khara, H, Vahabzadeh Roudsari, H, Pajand & Z, Ahmadnezhad, M 2022, Alterations in Hematological indices, histopathology and p450 gene expression in stellate sturgeon (*Acipenser stellatus* Pallas, 1811) fingerlings exposed to different salinities levels and ammonia. *Iranian Journal of Fisheries Science*, 21: 1343–1366.

Mansour, ATE, Goda, AA, Omar, EA, Khalil, HS & Esteban, MÁ 2017, Dietary supplementation of organic selenium improves growth, survival, antioxidant and immune status of meagre, *Argyrosomus regius*, juveniles. *Fish & Shellfish Immunology*, 68: 516-524.

Mehrabi, Z, Firouzbakhsh, F & Jafarpour, A 2012, Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Journal of Animal Physiology and Animal Nutrition*, 96: 474-481.

Merrifield, DL, Dimitroglou, A, Foey, A, Davies, SJ, Baker, RT, Bøgwald, J, Castex, M & Ringø, E 2010, The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, 302: 1-18.

Miezeliene, A., Alencikiene, G., Gruzauskas, R., Barstys, T., 2011, 'The effect of dietary selenium supplementation on meat quality of broiler chickens', Biotechnol. Agron. Society and Environment, vol. 15, no. 1, pp. 61-69.

Mohammadi, G, Hafezieh, M, Karimi, AA, Azra, MN, Van Doan, H, Tapingkae, W, Abdelrahman, HA & Dawood, MA 2022, The synergistic effects of plant polysaccharide and *Pediococcus acidilactici* as a synbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, 120: 304-313.

Mohammadi, M & Khara, H 2015, Effect of different anesthetics agents (Clove oil, MS-222, Ketamine, Tobacco) on hematological parameters and stress indicators Rainbow Trout *Oncorhynchus mykiss*. *Comparative Clinical Pathology*, 24: 1039-1044.

Mohammadian, T, Nasirpour, M, Tabandeh, MR & Mesbah, M 2019, Synbiotic effects of β-glucan, mannan oligosaccharide and *Lactobacillus casei* on growth performance, intestine enzymes activities, immune-hematological parameters and immune-related gene expression in common carp, *Cyprinus carpio*: An experimental infection with *Aeromonas hydrophila*. *Aquaculture*, 511: 634197.

Montagne, L, Pluske, JR & Hampson, DJ 2003, A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal feed science and technology*, 108: 95-117.

Mouriño, JLP, Vieira, FN, Jatobá, A, Silva, BC, Pereira, GV, Jesus, GFA, Ushizima, TT, Seiffert, WQ & Martins, ML, 2017, Symbiotic supplementation on the hemato-immunological parameters and survival of the hybrid surubim after challenge with *Aeromonas hydrophila*'. *Aquaculture Nutrition*, 23: 276-284.

Muiswinkel, WV & Vervoorn-Van Der Wal, B 2006, The immune system of fish. In Fish diseases and disorders,. Volume 1: protozoan and metazoan infections (pp. 678-701). Wallingford UK: CABI.

Muller, EE, Locatelli, V & Cocchi, D 1999, Neuroendocrine control of growth hormone secretion. *Physiological Reviews*, 79: 511-607.

Naiel, MA, Negm, SS, Abd El-hameed, SA & Abdel-Latif, HM, 2021, Dietary organic selenium improves growth, serum biochemical indices, immune responses, antioxidative capacity, and modulates transcription of stress-related genes in Nile tilapia reared under sub-optimal temperature. *Journal of Thermal Biology*, 99: 102999.

Nayak, SK 2010, Probiotics and immunity: A fish perspective. *Fish & Shellfish Immunology*, 29: 2-14.

Nekoubin, H, Gharedashi, E, Imanpour, MR & Asgharimoghadam, A 2012a, The influence of synbiotic (Biomin Imbo) on growth factors and survival rate of Zebrafish (*Danio rerio*) larvae via supplementation with biomar. *Global Veterinaria*, 8: 503-506.

Nekoubin, H, Hatefi, S, Javahery, S & Sudagar, M 2012b, Effects of synbiotic (Biomin Imbo) on growth performance, survival rate, reproductive parameters of angelfish (*Pterophyllum scalare*). *Walailak Journal of Science and Technology*, 9: 327-332.

Niers, LE, Timmerman, HM, Rijkers, GT, van Bleek, GM, van Uden, NO, Knol, EF, Kapsenberg, ML, Kimpen, JL & Hoekstra, MO 2005, Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clinical & Experimental Allergy*, 35: 1481-1489.

Nugroho, RA & Fotedar, R 2014, Comparing the effects of dietary selenium and mannan oligosaccharide supplementation on the growth, immune function, and antioxidant enzyme activity in the cultured marron *Cherax cainii*. *Aquaculture international*, 22: 585-596.

Oliva-Teles, A 2012, Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, 35: 83-108.

Pacini, N, Abete, MC, Dörr, AJM, Prearo, M, Natali, M & Elia, AC 2012, Detoxifying response in juvenile tench fed by selenium diet. *Environmental Ttoxicology and Pharmacology*, 33: 46-52.

Poston, HA & Combs Jr, GF 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: 54-63.

Rahbar, M, Nezami, ShA, Khara, H, Rezvani, M, Khodadoust, A, Movahed, R, & Eslami, S 2011, Effect of age on reproductive performance in female Caspian brown trout (*Salmo trutta caspious*, Kessler 1877). *Caspian Journal of Environment Sciences*, 9: 97-103.

Ray, AK, Ghosh, K & Ringø, EJAN 2012, Enzyme-producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, 18: 465-492.

Rizzo, A, Minoia, G, Trisolini, C, Manca, R & Sciorsci, RL 2007, Concentrations of free radicals and beta-endorphins in repeat breeder cows. *Animal Rreproduction Science*, 100: 257-263.

Rohani, MF, Islam, SM, Hossain, MK, Ferdous, Z, Siddik, MA, Nuruzzaman, M, Padeniya, U, Brown, C & Shahjahan, M 2021, Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. *Fish & Shellfish Immunology*, 120: 569-589.

Roubach, R, Menezes, A, Oh, K & Dabbadie, L 2019, Towards guidelines on sustainable aquaculture. *FAO Aquaculture Newsletter*, 60: 55-56.

Sadat Hoseini Madani, N, Adorian, TJ, Ghafari Farsani, H & Hoseinifar, SH 2018, The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) postlarvae. *Aquaculture Research*, 49: 1926-1933.

Safabakhsh, M, Mohseni, M, Bahri, A, Mohammadizadeh, F 2020, Effect of dietary selenium on growth performance, survival rate and biochemical-blood profile of farmed juvenile beluga (*Huso huso*). *Iranian Journal of Fisheries Sciences*, 19 :2077-2088

Safari, O, Sarkheil, M, Shahsavani, D & Paolucci, M 2021, Effects of Single or Combined Administration of Dietary Synbiotic and Sodium Propionate on Humoral Immunity and Oxidative Defense, Digestive

Enzymes and Growth Performances of African Cichlid (*Labidochromis lividus*) Challenged with *Aeromonas hydrophila*. *Fishes*, 6: 63.

Saffari, S, Keyvanshokooh, S, Zakeri, M, Johari, SA & Pasha-Zanoosi, HJ 2017, Effects of different dietary selenium sources (sodium selenite, selenomethionine and nanoselenium) on growth performance, muscle composition, blood enzymes and antioxidant status of common carp (*Cyprinus carpio*). *Aquaculture Nutrition*, 23: 611-617.

Sakai, M 1999, Current research status of fish immunostimulants. *Aquaculture*, 172: 63-92.

Sandhya, SV & Vijayan KK 2019, Symbiotic association among marine microalgae and bacterial flora: a study with special reference to commercially important *Isochrysis galbana* culture. *Journal of Applied Phycology*, 31: 2259-2266.

Sewaka, M, Trullas, C, Chotiko, A, Rodkhum, C, Chansue, N, Boonanuntanasarn, S & Pirarat N 2019, Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis* spp.). *Fish & Shellfish Immunology*, 86, 260-268.

Shenkin, A 2006, Micronutrients in health and disease. *Postgraduate Medical Journal*, 82: 559-567.

Smith, AM & Picciano, MF 1987, Relative bioavailability of seleno-compounds in the lactating rat. *The Journal of Nutrition*,117: 725-731.

Surai, PF 2002,. Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. *World's Poultry Science Journal*, 58: 333-347.

Tangestani, R, Doughikollaee, E.A, Ebrahimi, E & Zare, P 2011, Effects of garlic essential oil as an immunostimulant on hematological indices of juvenile beluga (*Huso huso*). *Journal of Veterinary Research*, 66: 209-279.

Tseng, Y, Dominguez, D, Bravo, J, Acosta, F, Robaina, L, Geraert, P.A, Kaushik, S & Izquierdo, M 2021, Organic selenium (OH-MetSe) effect on whole body fatty acids and mx gene expression against viral infection in gilthead seabream (*Sparus aurata*) juveniles. *Animals*, 11: 2877.

Vaezi, M, Khara, H, Shenavar, AR 2016, Synbiotic (Biomin imbo) alters gut bacterial microflora of Russian sturgeon, *Acipenser gueldenstaedtii* in a Time-dependent pattern. *Journal of Parasitic Diseases*. 40: 1189–1192.

Vallejos-Vidal, E, Reyes-López, F, Teles, M & MacKenzie, S 2016, The response of fish to immunostimulant diets. *Fish & Shellfish Immunology*, 56: 34-69.

Wang, C & Lovell, RT 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; 223-234.

Wang, Y, Han, J, Li, W & Xu, Z 2007, Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Animal Feed Science and Technology*, 134: 243-251.

Wang, YD, Wang, X & Wong, YS 2013, Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. *Food Chemistry*, 141: 2385-2393.

Wang, J, Um, P, Dickerman, BA & Liu, J 2018, Zinc, magnesium, selenium and depression: a review of the evidence, potential mechanisms and implications. *Nutrients*, 10: 584.

Wang, Y, Zhao, F, Rijntjes, E, Wu, L, Wu, Q, Sui, J, Liu, Y, Zhang, M, He, M, Chen, P & Hu, S 2019, Role of selenium intake for risk and development of hyperthyroidism. *The Journal of Clinical Endocrinology & Metabolism*, 104: 568-580.

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.

Watanabe, T, Kiron, V, Satoh, S 1997, Trace minerals in fish nutrition. *Aquaculture*, 151: 185–207.

Yarahmadi, P, Miandare, HK, Farahmand, H, Mirvaghefi, A & Hoseinifar, SH 2014, Dietary fermentable fiber upregulated immune related genes expression, increased innate immune response and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, 41: 326-331.

Ye, JD, Wang, K, Li, FD & Sun, YZ 2011, Single or combined effects of fructo-and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralichthys olivaceus*. *Aquaculture Nutrition*, 17: 902-911.

Yonar, ME 2012, The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*, W.). *Fish & Shellfish Immunology*, 32: 994-1001.

Yousefi, S, Shokri, MM, Noveirian, HA & Hoseinifar, SH 2020, Effects of dietary yeast cell wall on biochemical indices, serum and skin mucus immune responses, oxidative status and resistance against *Aeromonas hydrophila* in juvenile Persian sturgeon (*Acipenser persicus). Fish & Shellfish Immunology*, 106: 464-472.

Zakariaee, H, Sudagar, M, Hosseini, SS, Paknejad, H Baruah, K 2021, In vitro Selection of Synbiotics and in vivo Investigation of Growth Indices, Reproduction Performance, Survival, and Ovarian Cyp19α Gene Expression in Zebrafish, *Danio rerio*. *Frontiers in Microbiology*, 12: 758-758.

Zargari, A, Nejatian, M, Abbaszadeh, S, Jahanbin, K, Bagheri, T, Hedayati, A & Sheykhi, M 2023, Modulation of toxicity effects of CuSO4 by sulfated polysaccharides extracted from brown algae (*Sargassum tenerrimum*) in *Danio rerio* as a model. *Scientific Reports*, 13: 11429.

Zargari, A, Mazandarani, M, Safari, R, Hoseinifar, H & Hedayati, A 2024, Modulation of toxic effects of ammonia on growth, pathology of liver and kidney tissues and relative expression of GH and IGF-1 Genes by CoQ10 Supplementation in *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry*, 50: 1711-1729

Zheng, L, Feng, L, Jiang, WD, Wu, P, Tang, L, Kuang, SY, Zeng, YY, Zhou, XQ & Liu, Y 2018, Selenium deficiency impaired immune function of the immune organs in young grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology*, 77: 53-70.

Zhang, M, Li, M, Li, X, Qian, Y, Wang, R & Hong, M 2020, The protective effects of selenium on chronic ammonia toxicity in yellow catfish (*Pelteobagrus fulvidraco*). *Fish & Shellfish Immunology*, 107: 137-145.

Zhou, X, Wang, Y, Gu, Q & Li, W 2009, Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). *Aquaculture*, 291: 78-81.