Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134040 2025, 24(4) 1015-1040

Research Article

Effect of an encapsulated blend of nano-selenium and vitamins C and E on growth performance, blood, immunity, and oxidative indices in *Huso huso*

Hosseinpour Zelaty A.[1*], Sohrabi T.[1], Seyed Hassani M.H.[1*]; Adineh H.[2], Monsef Shokri M.[1], Mirnabibaboli S.S.[3]

1International Sturgeon Research Institute, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran.
2Department of Fisheries, Faculty of Agriculture and Natural Resources, Gonbad Kavous University, Gonbad Kavous, Iran.
3Department of Fisheries, Faculty of Animal Science and Fisheries, University of Agriculture and Natural Resources, Sari, Iran.
*Correspondence: ahpour.z@gmail.com; mirhamedhassani@yahoo.com

Keywords

Nano-selenium,
Vitamin C,
Vitamin E,
Huso huso,
Growth,
Blood parameters,
Immune function

Article info

Received: December 2024
Accepted: February 2025
Published: July 2025

Abstract

A 10-week feeding trial was conducted to investigate the effect of dietary supplementation with encapsulated Nano-selenium (NanoSe) and vitamins C and E at varying dosages (Control: free from the supplementations; T1: 0.1, 30, and 100 mg/kg; T2: 0.2, 60, and 200 mg/kg; T3: 0.3, 90, and 300 mg/kg, respectively) on the growth performance, hematology, immunity and antioxidant indices of juvenile *Huso huso*. The fish, with an average initial weight of 78.54±0.90 g, were stocked in 12 fiberglass tanks and fed with the respective diets for 10 weeks. The fish fed with diets T1 and T2 exhibited significantly higher final weight, feed conversion ratio, and protein efficiency ratio compared to the fish fed with T3 and the control diets (p<0.05). Additionally, the white blood cell count (WBC), hemoglobin (Hb), and neutrophil levels increased in the fish fed with T1 and T2 diets compared to the control group (p<0.05). The alternate complement activity (ACH50) was higher in T2 and T3 groups compared to the control group (p>0.05). The serum lysozyme activity showed no significant difference across groups T1, T2, and T3 (p>0.05). The result showed that IgM levels in the fish fed with the supplemented diets (T1-T3) showed no significant difference compared to the control group (p>0.05). The total lipids and albumin in T1 and T2, total protein in T2, and cholesterol in all treated-groups were increased compared to the control group (p>0.05). The fish fed with supplemented diets had significantly lower levels of serum cortisol and glucose compared to the control group (p<0.05). Supplementation resulted in improved antioxidant status, as demonstrated by increased superoxide dismutase levels in the T2 group and decreased malondialdehyde levels in all supplemented groups (T1, T2, and T3) compared to the control group (p<0.05). This study suggests that dietary supplementation with a moderate mixture of NanoSe (0.2 mg/kg) and vitamins C (60 mg/kg) and E (200 mg/kg) positively promotes growth, improves some blood indices, stimulates the immune system, and reduces oxidative stress in juvenile *Huso huso*.

Introduction

The Beluga (*Huso huso*) is the most significant farmed species in Iran, typically raised in high densities within concrete ponds (Kalbassi *et al.*, 2013). High-density rearing can lead to stress, decreased food absorption, reduced growth (Rafatnezhad *et al.*, 2008), and lower resistance to infectious diseases (Li *et al.*, 2012) in farmed sturgeon. Currently, routine treatments, including accepted antibiotics and chemical medications, are often ineffective. Therefore, researchers aim to enhance fish growth by improving the non-specific defense system through the utilization of the non-specific immune system and growth stimulants (Wang *et al.*, 2013). Additionally, they aim to reduce the sensitivity of aquatic animals to pathogens, improve the quality of the aquatic environment, and decrease the use of drugs in aquatic animals (Nayak, 2010). Nano selenium is a pseudo-metal with antioxidant properties. It enhances antioxidant activity by increasing the activity of glutathione peroxidase (GPx) and glutathione reductase (GR). It reduces oxidative stress by lowering malonaldehyde (MDA) levels, while potentially supporting the activity of catalase and superoxide dismutase (SOD) (Monteiro *et al.*, 2009; Jamil, 2013; Khan *et al.*, 2016). Additionally, it improves the immune and hematological systems (Keen *et al.*, 2004; Khan *et al.*, 2017; Naderi *et al.*, 2017) and enhances growth while reducing feed conversion ratios in aquatic animals (Naderi *et al.*, 2017; Longbaf Dezfouli *et al.*, 2019; Abd El-Kader *et al.*, 2020; Dawood *et al.*, 2020; Harsij *et al.*, 2020). Vitamin C is a crucial micronutrient for fish, necessary for optimal growth and physiological health. Since many teleost fish are unable to synthesize ascorbic acid (Lin and Shiau, 2005; Khan *et al.*, 2015), and sturgeon cannot synthesize these vitamins under farm conditions (Falahatkar *et al.*, 2006), it is essential to regularly add this vitamin to fish diets. In addition to vitamin C, vitamin E plays a crucial role in various physiological processes, including growth, reproduction, endocrine function, resistance to oxidation, cellular death, and the immune system (Zhao *et al.*, 2018). The inclusion of vitamin E in the diet has been shown to improve carcass quality (Zanon *et al.*, 2018), fatty acid profiles (Chen *et al.*, 2018), and safety (Lu *et al.*, 2016) in aquatic animals. Selenium has been found to have a synergistic effect with trace elements and other nutrients (Khan *et al.*, 2017). This nutrient has a strong nutritional interaction with vitamins E and C in fish (NRC, 2011; Khan *et al.*, 2017). Studies have demonstrated the synergistic effects of nanoselenium and vitamin E in various fish species. For example, in rainbow trout (*Onchorhynchus mykiss*), the combination of 1 mg/kg nanoselenium and 500 mg/kg vitamin E resulted in improved growth, stress, and safety indices compared to individual supplements. Similarly, in masher fish (*Tor putitora*), the combination of 68 mg/kg nanoselenium and 300 mg/kg vitamin C led to improved growth indices, hematology, and lysozyme levels (Naderi *et al.*, 2019). Furthermore, the combination of nanoselenium, vitamins C and E (0.2 mg, 200 mg, and 600 mg) caused improvements in growth parameters, antioxidant capacity, and immune response in rainbow trout (Harsij *et al.*, 2020). Additionally, the

combination of vitamin E (50 ppm) and selenium (0.35 ppm) led to increased weight and improved body composition in rainbow trout (Rodríguez and Rojas, 2014) and olive flounder (*Paralichthys olivaceus*) (Moniruzzaman *et al.*, 2017). Recent studies have demonstrated that selenium nanoparticles exhibit greater bioavailability for fish compared to organic and inorganic selenium forms (Zhang *et al.*, 2008; Jamil, 2013). Additionally, nanoselenium has been found to have a stronger synergistic effect with vitamins C and E compared to organic and inorganic forms (Dawood *et al.*, 2020). However, there is a lack of research on the impact of combining different levels of nanoselenium, vitamins C and E on growth indices, hematology, antioxidant enzymes, and immunity in beluga. Therefore, this study aimed to investigate this combination's effects on these parameters.

Materials and methods

Preparation of encapsulated nanoselenium, vitamins C, E and the expreimental diets

The Nano selenium used had a purity of 99.99% and a particle size of 30-50 nm, and was obtained from Pishgaman Nanomaterials Company in Khorasan, Iran. The Vitamin E used had a purity of 99% and was obtained from Health Leads UK, while the Vitamin C used was polyphosphate ascorbate from Omid Parsa Damavand in Tehran, Iran. To prepare Microencapsulated Nano selenium, Vitamins C and E, a mixture of 30 g maltodextrin and 10 g Arabic gum was homogenized in 60 mg of distilled water at 60-70°C using an IKA T 25 digital ULTRA homogenizer at a round of 1000g for 3 min. The mixture was then stored in a Ben-Marie (Memert. WNB 14, Germany) at 60°C for 24 hours. The coating materials and the mixture of nanoselenium, vitamins C and E (in a 3:1 ratio) were mixed for 4 min. The solid mixture was dried for 24 hours using a Freeze dryer (Alpha-2 LD plus, Germany) and subsequently crushed in a porcelain mortar. The control group consisted of wall materials without any antioxidant supplements (Adineh *et al.*, 2021). The study employed a commercial diet from Faradaneh Industrial Group (2025), specifically the Sturgeon Commercial Diet (GFS1), with a size of 2 mm. The diet contained 36-44% protein, 1.5% crude fiber, 12-16% crude lipid, 7-10% ash, and 6-11% moisture (According to the manufacturer's announcement; Faradaneh Industrial Group, IRAN). The digestible energy was 4300 kcal/kg and the phosphorus content was 1-1.5%. Four diets were prepared, including T1, T2, T3, and T4. T1, T2, and T3 contained crushed and encapsulated Nano selenium (Nano Se), vitamins C, and E at doses of (0.1, 30, 100 mg/kg), (0.2, 60, 200 mg/kg), and (0.3, 90, 300 mg/kg), respectively. T4 was wall coated without nano-selenium, vitamins C, and E. The diets were milled and passed through an industrial meat grinder (Pars Esfahan, GM32, Esfahan, Iran) with a 2 mm diameter to produce spaghetti-like strands. The pellets were spread on grill trays and then transferred to a convection dryer at 45°C for 24 hours until the moisture content was reduced to less than 10% (Hardy and Barrows, 2003). They were then packaged, labeled, and stored in a freezer at -20°C until use.

Fish culture and blood sampling

A total of 120 fish, with an initial average body weight of 77.34±1.38 g, were stocked in 12 fiberglass tanks (500-L; water flow 0.38 L/min). The flow-through water was supplied from a mix of well water and Sepidrood River at a rate of 4.75 L/min throughout the experiment. In each tank, 12 fish were stocked without any significant differences in biomass (p>0.05). During a 10-week period, fish were fed experimental diets at a rate of 2% of their body weight at 8.00 p.m, 15.00 and 21.00 a.m. Daily measurements were taken of temperature, oxygen, and pH levels (24.90±0.53°C, 6.90±0.21 mg/l and 7.92±0.09, respectively) during the rearing period. Biometry was carried out at 15-day intervals (Luo *et al.*, 2006). The growth performance of the tested fish was evaluated by calculating various parameters using the following formulas (Hunt *et al*., 2011):

Body Weight Increase (BWI) = Final body weight - Initial body weight

Specific Growth Rate (SGR) = (ln(Final weight) - ln(Initial weight)) / Time (days) × 100

Feed Conversion Ratio (FCR) = Total feed consumed (dry weight) / Total weight gain

Protein Efficiency Ratio (PER) = Weight gain / Protein intake

Condition Factor (K) = (Weight / Length^3) × 100

At the end of the nutrition period, 30% of the fish population (3 fish from each tank) were randomly selected, and blood was taken to prepare plasma. Blood samples were centrifuged (Labfuge 200, Frankfurt, Germany) at 3000g for 10 min and transferred to the laboratory (Harsij *et al.*, 2020).

Hematological, biochemical, imunological and antioxidant indices

The red and white blood cell counts, as well as the differential white blood cell counts (lymphocytes, neutrophils, and monocytes), were measured using a neobar hemocytometer slide (Bain *et al.*, 2016). The blood hemoglobin level was calculated using the cyanometric hemoglobin calorimetric method with a reagent solution and a spectrophotometer (Genoa, 6505-UCV/VIS, England) and Kit (Pars Azmoun, Karaj, Iran) in g/dL. Mean erythrocyte corpuscular volume (MCV), hemoglobin concentration (MCH) and hemoglobin concentration of erythrocytes (MCHC) were calculated based on the following formula (Potki *et al.*, 2018):

MCV (fl) = 10 × (Hct / RBC)

MCH (pg/cell) = 10 × (Hb / RBC)

MCHC (g/ dL) = 100 × (Hb / Hct)

To determine hematocrit levels, a capillary tube centrifuge was used to measure the ratio of red blood cells to whole blood within the tube. Cortisol and glucose levels were measured using enzymatic/colorimetric methods and a commercial kit (Pars Azmoon, Karaj, Iran), following the manufacturer's protocols (Bain *et al.*, 2016). Protein, albumin, lipid, and cholesterol levels were determined using Pars Azmoon kits (Pars Azmoon Company, Tehran, Iran) following the manufacturer's protocols. Lysozyme activity was measured using the turbidimetric method of Ellis (1990) approach. Complement activity was

assessed using the rabbit erythrocyte hemolysis (RaRBC) method protocol (Yano, 1992). ALT, AST, and ALP were measured using Pars Azmon kits and the spectrophotometric method (IRMA, Tokyo, Japan). The levels of immunoglobulin M (IgM) were measured using the nephelometric technique, as described by Yeh *et al.* (2008).

Malondialdehyde (MDA) levels in serum were determined using the thiobarbituric acid (TBA) method as described by Esterbauer and Cheeseman (1990), with 1, 1, 3, 3-tetramethoxypropane used as the standard. The spectrophotometric measurement of samples and standards was used to determine their absorbance at 532 nm. The concentration of MDA was calculated using the linear equation extracted from the standard curve. The CAT assay was performed using the method described by Aebi (1984), in which the rate of H_2O_2 decomposition was monitored at 240 nm.

Superoxide dismutase (SOD) enzyme activity was measured using the method described by Marklund and Marklund (1974), which is based on the inhibition of pyrogall autooxidation. Enzyme activity was quantified by monitoring the absorbance at 420 nm for 3 min. One unit of enzyme activity was defined as the amount of enzyme that inhibits pyrogall autooxidation by 50%.

Statistic analysis

The data obtained were recorded and processed in an Excel program. Statistical analysis was performed using SPSS 20.0 (SPSS, Chicago, USA). First, the normality of the data was measured by Kolmogorov-Smirnov, then one-way analysis of variance (ANOVA) accompanied by Duncan's test was used to compare the means between each treatment, and variations were analyzed at the $p<0.05$ level. Quantitative analyses of data were presented as mean±SD.

Results

Growth parameters and feed efficiency ratio

Growth parameters and feed conversion ratios of the fish fed with the control diet (T4) and microencapsulated antioxidant nanosellenium, vitamins C and E diet (T1) (0.1, 30, and 100 mg/kg), (T2) (0.2, 60, and 200 mg/kg) and (T3) (0.3, 90, and 300 mg/kg) are presented in Table 1. The final weight of fish fed T1 and T2 with microencapsulated antioxidant nanoselenium, vitamins C and E at levels (0.1, 30, and 100 mg/kg) and (0.2, 60, and 200 mg/kg) was higher than that of fish fed T4 (with wall, without nanoselenium, vitamins C and E) ($p<0.05$). Fish condition factor in different treatments showed no significant difference ($p>0.05$), but the highest percentage of weight gain (BWI%) and specific growth rate (SGR) was in the fish fed diets T1 and T2 that were higher than fish fed T3 and diet control (T4) ($p<0.05$). Feed conversion ratio (FCR) was also affected by the dietary treatments: the lowest FCR was observed in fish fed T1 and T2, while fish fed T4 had the highest FCR ($p<0.05$). The best performance was observed in fish fed with T1 and T2, which was higher than the fish fed with T3 and T4 diets ($p<0.05$) (Table 1).

Hematological indices

The hematological indices of fish fed with the control diet (T4) and different levels of inclusion of micro-coated antioxidant nanoselenium, vitamins C and E in the diet are presented in Table 2. Inclusion of nanoselenium, vitamins C and E in T1 and T2 groups led to an increase in WBC and RBC in fish, however, WBC and RBC in fish in two later groups (T3 and T4) showed a decreasing trend and similarly, the amount of hemoglobin in fish belonging to T1 and T2 groups was higher than fish treated by T4 (p<0.05). Although no significant difference in Hct was recorded for T1, T2, and T3 diets, Hct in T1 was higher and had a significant difference compared to T4 (control) (p<0.05). Also, nanoselenium, vitamins C and E inclusion had signs of a significant increase in neutrophils in fish fed T1 and T2 compared to T3 and T4, respectively (p<0.05).

Table 1: Growth performance of *Huso huso* fed with a mixture of nanoselnium micronutrients, vitamins C and E (n = 3, mean standard deviation)

Indicators / Diet	T_1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T_2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T_3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T_4: control
Initial weight (g)	78.79±0.72	79.67±0.52	77.35±1.22	78.37±1.16
Final weight (g)	351.70±3.81^{a}	370.8±3.12 ^{a}	311.88±5.18^{b}	286.11±4.59^{b}
Initial length (cm)	27.93±0.65	27.99±0.50	27.92±0.59	28.15±0.31
Final length (cm)	42.75±0.62^{ab}	43.99±0.99 ^{a}	41.82±0.68 ^{b}	39.5±0.42^{c}
Condition factor	0.45±0.01	0.43±0.01	0.42±0.01	0.46±0.03
BWI (percentage during the period)	394.14±4.67^{ab}	362.52±19.09^{a}	306.78±0.4^{bc}	276.24±25.3^{c}
SGR (percentage per day)	2.11±0. 14 ^{a}	2.15±0.05 ^{a}	1.97±0. 1^{bc}	1.86±09^{c}
Amount of food consumed per fish (g)	2691.4	2810.30	2598.12	2504.02
FCR	0.98±0.015^{c}	0.96±0.05^{c}	1.1±0.03^{b}	1.19±04^{a}
PER	2.53±0.01^{a}	2.58±0.14^{a}	2.26±0.06^{b}	2.09±08^{b}

Table 2 : Effect of microencapsulated selenium, vitamins C and E on Hematology index of *Huso huso* (n = 3, mean standard deviation)

Indicators / Diet	T_1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T_2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T_3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T_4: control
WBC (mm3)	10037.5±390.24^{a}	10700.00±293.43 ^{a}	6525.00±531.50^{b}	7250.00±387.29^{b}
RBC (mm3)	639500.00±2257.79^{a}	621500.00±1615.12 ^{a}	616750.00±29341.38 ^{b}	570000.00±18384.77 ^{b}
Hb (g/dl)	6.07±0.2^{ab}	6.22±0.18^{a}	5.65±0.41^{bc}	5.30±0. 8 ^{C}
Hct (%)	27.75±0.95^{a}	26.50±2.08 ^{ab}	26.25±1.25^{ab}	24.00±0.81 ^{b}
MCV (fl)	433.75±2.65	435.00±3.7	431.75±4.11	431.69±4.92
MCH (pg)	95.00±0.35	96.82±2.26	95.95±0.83	94.50±1.11
MCHC g/dl)	21.87±0.15	22.25±0.50	22.37±0.20	21.82±0.26
Neut (%)	21.50±1.29^{a}	22.00±0.80 ^{a}	15.00±0.81^{b}	14.50±1.91^{b}
Lymphocyte (%)	77.25±2.67	72.00±0.81	79.75±1.25	75.00±2.96
Mono (%)	5.00±1.41	5.5±1.29	4.25±0.95	4.5±0.57

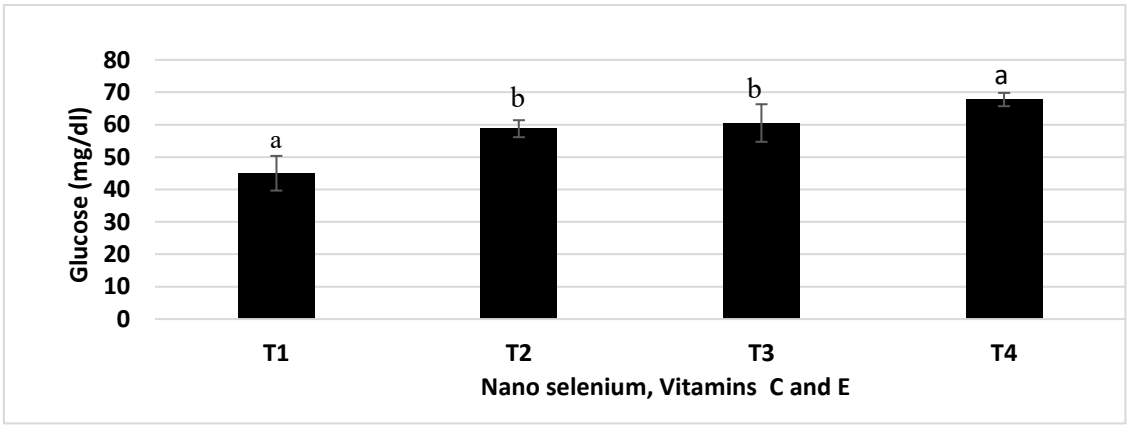
Biochemical indices

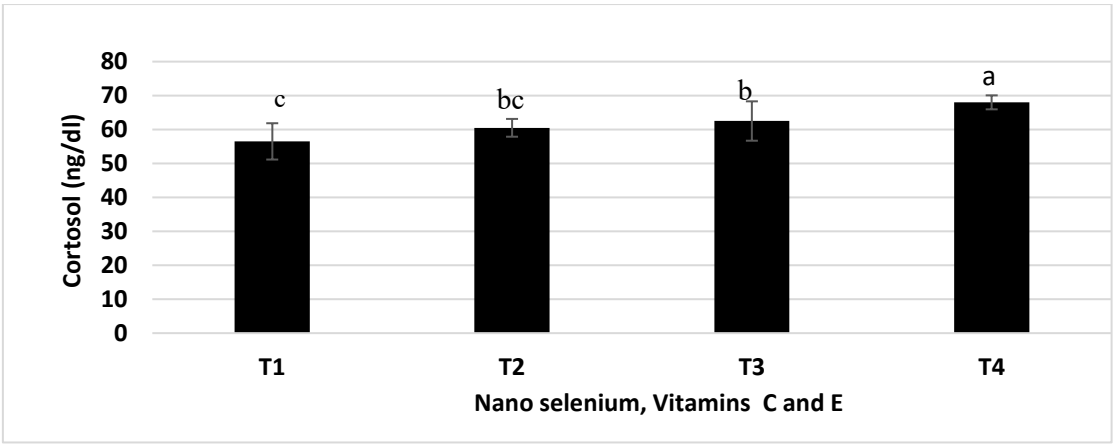
The highest levels of glucose and cortisol were observed in the control group and

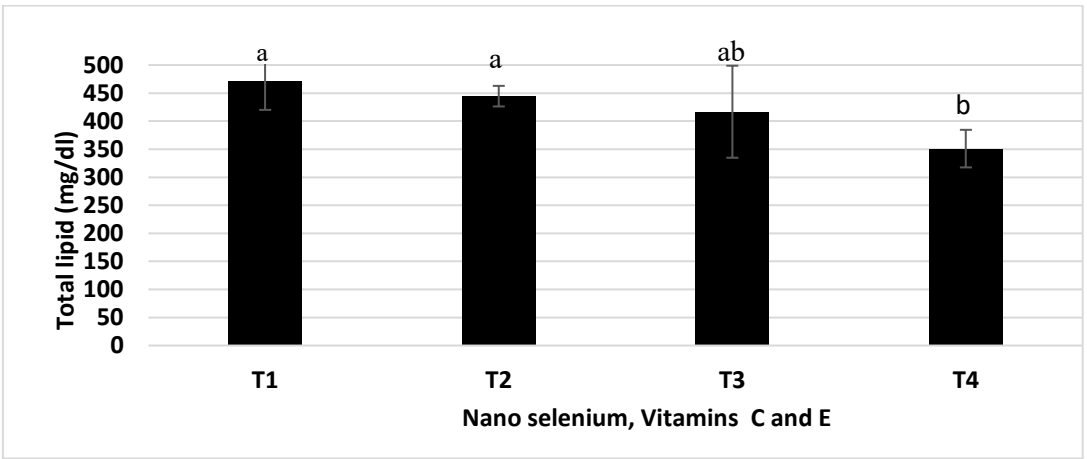
supplementation resulted in a decrease in glucose in the T1, T2, and T3 groups. The lowest levels of cortisol and glucose were observed in fish fed T1 and T2, which were significantly different from T4 (control) (p<0.05). In contrast to the control group (T4), levels of cholesterol, total lipids, albumin, and total protein were significantly increased in groups T1, T2, and T3 ($p < 0.05$). The liver enzyme activities of fish fed the different diets are presented in Table 3. The ALT and AST contents increased in the fish fed diets containing 0.2, 60, and 200 mg/kg of nanoselenium, vitamins C and E (T2), but no significant difference was observed compared to T3 and T4 groups (p>0. 05), serum alkaline phosphatase was affected by the different dietary treatments and the highest level was observed in fish T1 and T2 (388.25± 8.1 and 357.75± 15.39 units), which were significantly higher than fish fed the T4 group (reared without supplementation) (p<0.05) (Fig. 1).

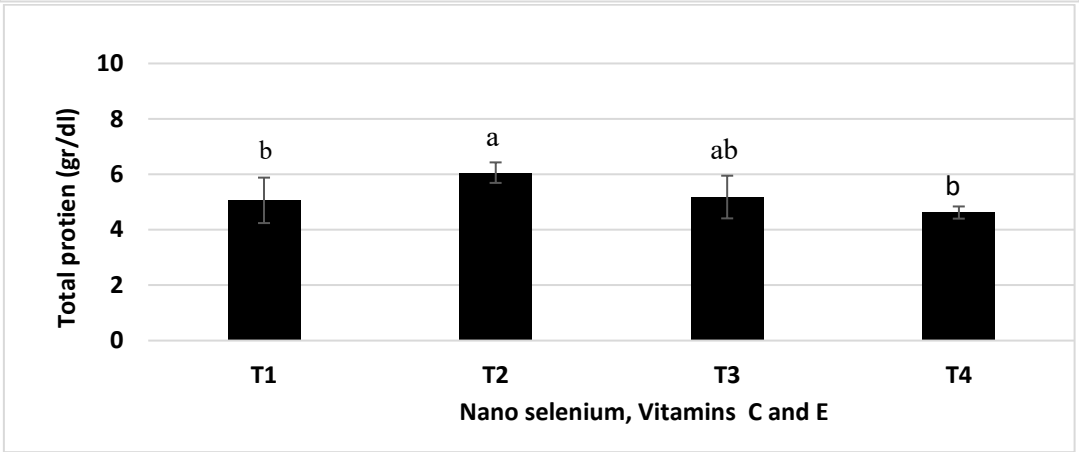
Table 3: Effect of microencapsulated selenium, vitamins C and E on biochemical indices of *Huso huso* (n=3, mean standard deviation).

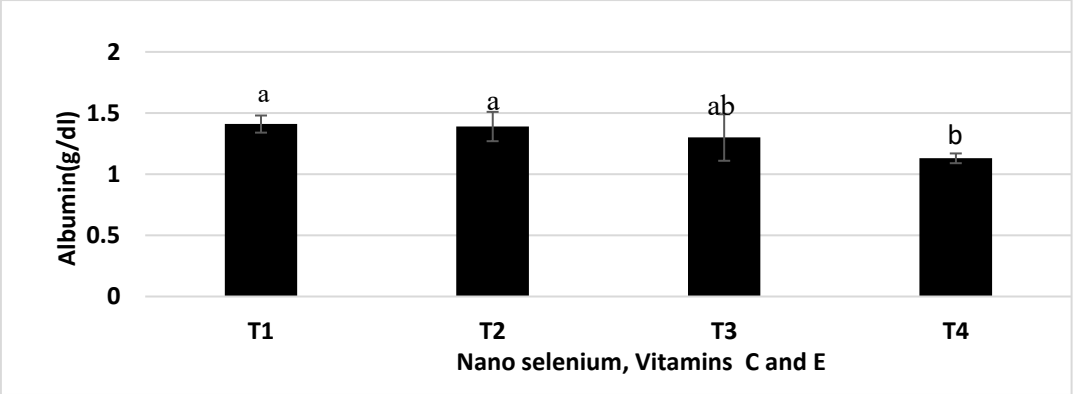
Indicators / Diet	T_1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T_2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T_3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T_4: Control
Glucose (mg/dl)	45.00±5.35^{c}	58.75±2.62^{b}	60.50±5.8^{b}	67.75±2.06^{a}
Cortisol (ng/mL)	56.50±4.23^{c}	60.50±3.10^{bc}	62.50±2.8^{b}	68.00±1.41^{a}
Total lipid (mg/dl)	472.25±52.18^{a}	444.5±18.37^{a}	416.75±82.02^{ab}	351.00±33.49^{b}
Total protein (gr/dl)	5.06±0.82^{b}	6.06±0.37^{a}	5.18±0.77^{ab}	4.62±0.22^{b}
Albumin (gr/dl)	1.41±0.07^{a}	1.39±0.12^{a}	1.30±0.19^{ab}	1.13±0.04^{b}
Cholesterol (mg/dl)	121.25±2.5^{b}	120.75±3.4^{b}	170.75±21.23^{a}	82.50±17.71^{c}
SGPT-ALT (u/lit)	12.5±1.91^{b}	15.25±0.95^{a}	13.75±1.25^{ab}	12.00±1.55^{ab}
SGOT –AST (u/lit)	326.00±32.87^{b}	388.5±29.27^{b}	352.50±45.94^{ab}	340.50±13.40^{ab}
Alkaline phosphatase (u/lit)	388.25±8.1^{a}	357.75±15.39^{a}	318.50±24.89^{a}	219.00±26.01^{b}

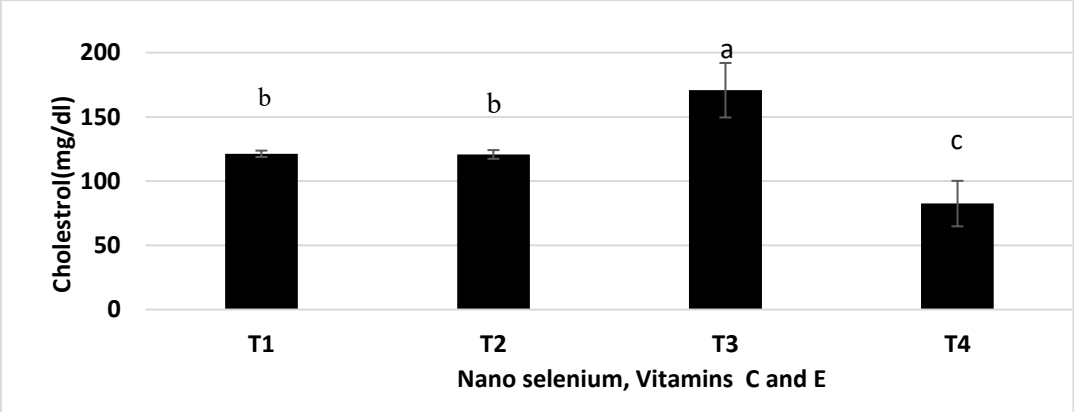


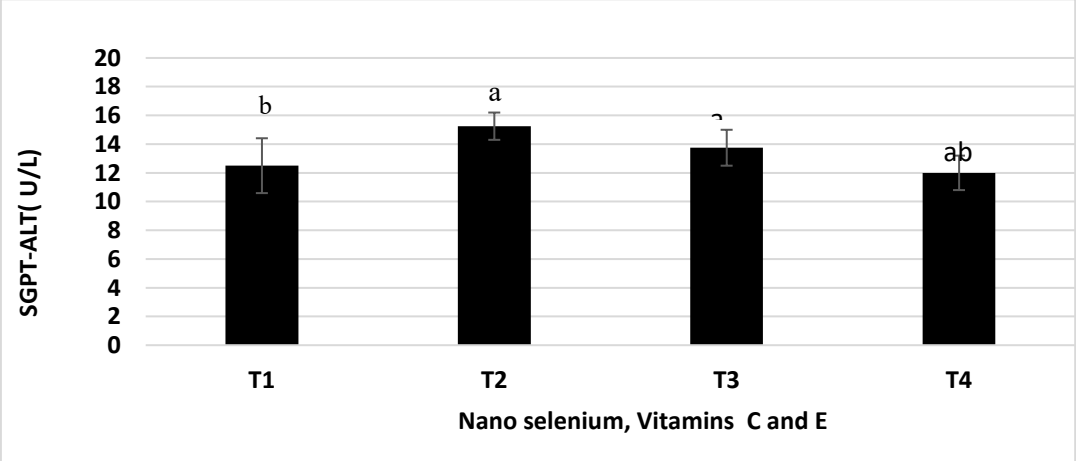


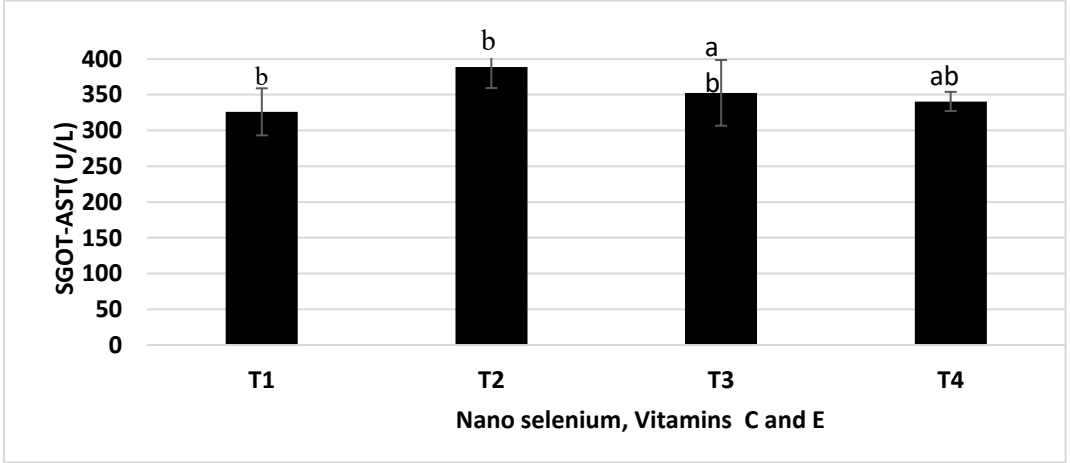












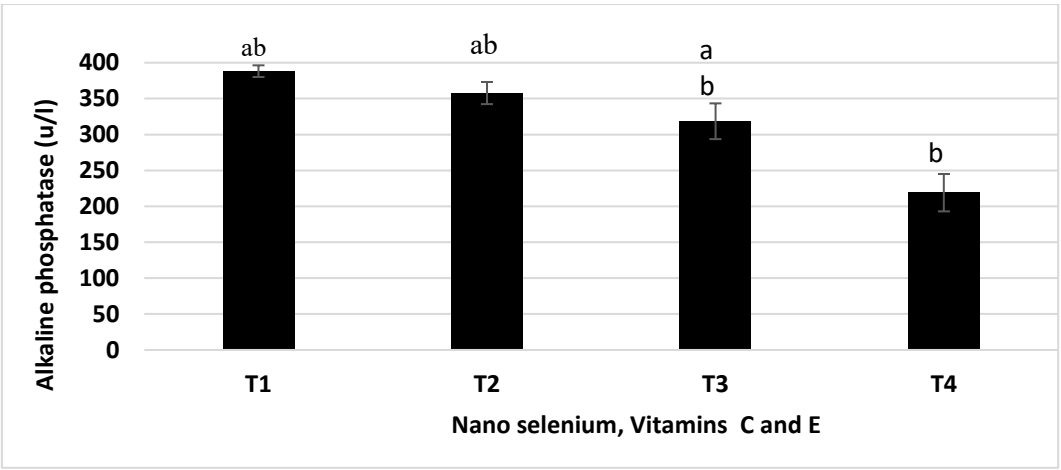
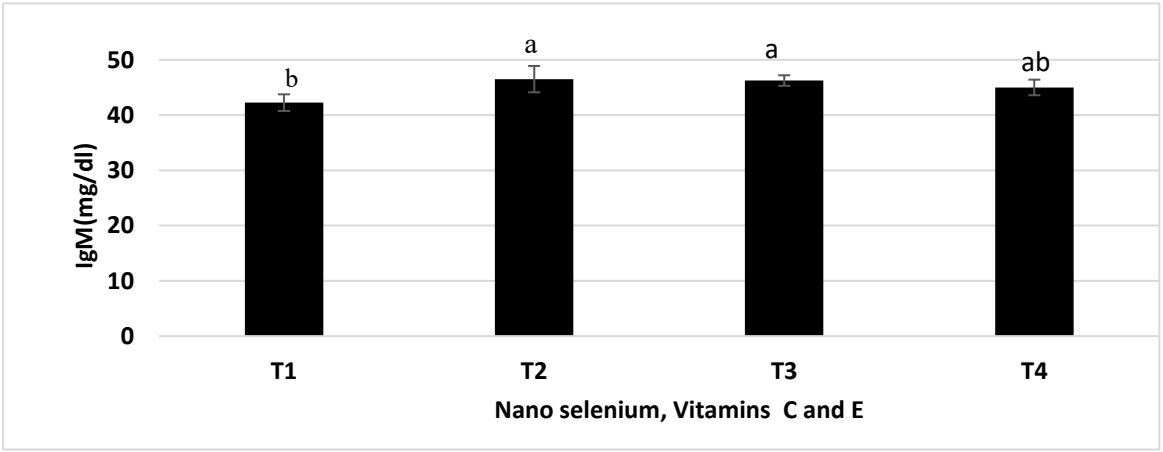
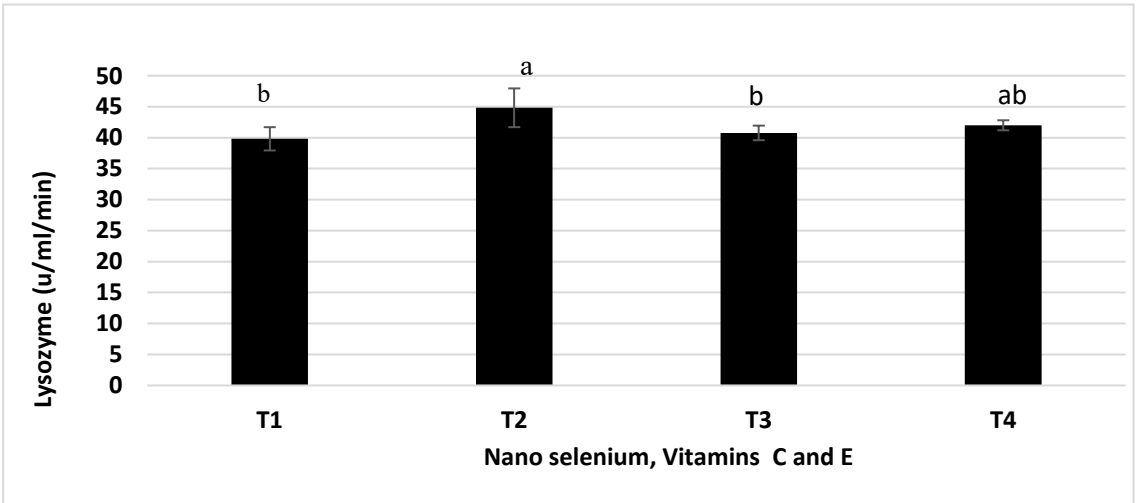


Figure 1: Effect of microencapsulated selenium, vitamins C and E on biochemical index of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg), T3: Nano selenium, vitamins C and E (0.3, 90 and 300 mg / kg),T4: control.

Imunological indices

Fish Immunological index presented in Table 4 and Figure 2. There was no significant difference for serum lysozyme in fish fed (T1) (42.00±0.81un /mL/min) T2 and T3 groups (44.83±3.13 and 40.77±1.18 un/mL/min) (p> 0.05), but serum lysozyme in fish fed (T2) was higher than serum lysozyme in fish belonging to T1 (39.82±1.88 un/mL/min) (p<0.05).





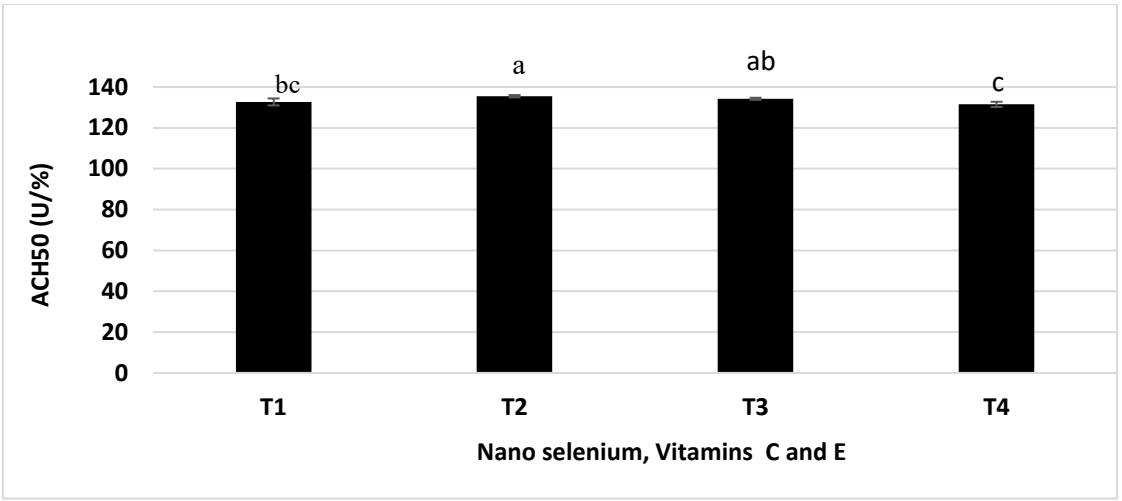


Figure 2: Effect of microencapsulated selenium, vitamins C and E on Imunological index of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg),T3: Nano selenium, vitamins C and E (0.3, 90 and 300 mg / kg),T4: control.

The result showed that incorporation of microcoated supplements of nanoselenium, vitamins C and E (0.2, 60, 200 and 0.3, 69, 300 mg/kg) increased IgM compared to diet T4 (no supplement), but the difference was not significant (p>0.05). However, IgM levels in T1 group fish were significantly lower than in T2 and T3 groups (p<0.05), but by adding 0.2, 60, 200 mg/kg and 0.3, 90, 300 mg nanoselenium, vitamins C and E to the diet (T2 and T3), complement activity was significantly increased compared to fish in T4 group (p<0.05) (Table 4).

Table 4 : Effect of microencapsulated selenium, vitamins C and E on Imunological index of *Huso huso* (n=3, mean standard deviation).

Indicators / Diet	T_1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T_2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T_3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T_4: control
IgM (mg/dl)	42.25 ± 1.5^{b}	46.50 ± 2.38^{a}	46.25 ± 0.95^{a}	45.00 ± 1.41^{ab}
ACH_{50} (u/%)	132.7 ± 1.70^{bc}	135.5 ± 0.57^{a}	134.25 ± 0.50^{ab}	131.5 ± 1.29^{c}
Lysozyme (u/ml/min)	39.82 ± 1.88^{b}	44.83 ± 3.13^{a}	40.77 ± 1.18^{b}	42.00 ± 0.81^{ab}

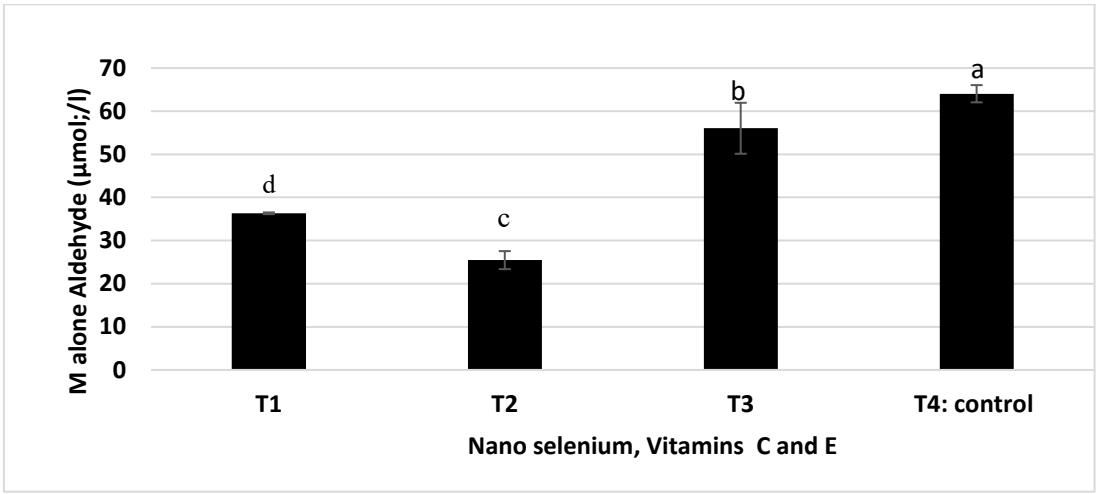
Antioxidant enzymes

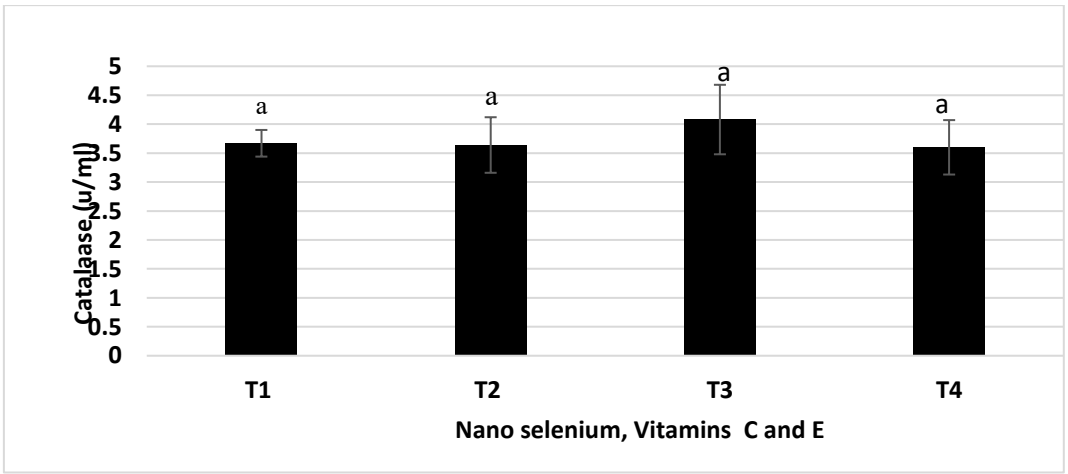
The antioxidant enzyme activities are shown in Table 5 and Figure 3. In this study, a reduction in malondialdehyde activity in fish was observed with inclusion of the supplement in the diet. The lowest amount of malondialdehyde was observed in fish of group T2 and in the next ranks were located T3 and T1 that their enzyme activity was significantly reduced compared to control group (without supplementation) (p<0.05). Conversely, we observed an increasing trend in the activity of catalase, although no difference was observed between treatments, the highest level of catalase belonged to the T3 group (p>0.05). Serum SOD was significantly higher in T2 and T3 than in T4 and the

highest activity belonged to the T2 group (p<0.05).

Table 5 : Effect of nanoencapsulated of selenium, vitamins C and E on Antioxidant Enzyme of *Huso huso* (n=3, mean standard deviation).

Indicators / Diet	T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T4: Control
Malone Aldehyde (µmol/l)	36.32±0.2[d]	25.46±2.09[c]	56.04±5.91[b]	64.05±2.00[a]
Catalase (u/mL)	3.67±0.23	3.64±0.48	4.08±0.60	3.60±0.47
SOD (u/mL)	18.43±0.4[ab]	19.31±1.33[a]	18.44±1.59[ab]	17.31±1.35[b]





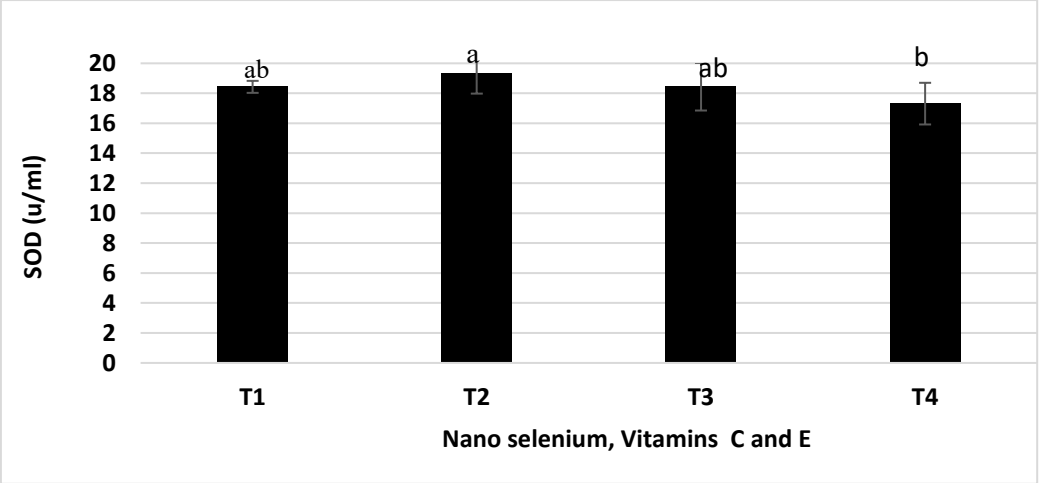


Figure 3: Effect of microencapsulated selenium, vitamins C and E on Antioxidant Enzyme of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60 and 200 mg / kg),T3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg),T4: control.

Discussion

Although in this study, fish were not cultured at high densities (3300 ± 110.42g/m^2) (Rafatnezhad *et al*., 2008), the positive and synergistic effects of nano-selenium, vitamins C and E on increased growth indices were significant. FW, BWI, SGR, FCR and PER in beluga fed with T1 and T2 treatment (0.1, 30 and 100 and 0.2, 60 and 200 mg / kg nano selenium, vitamins C and E) were higher than control fish (p<0.05).

The study aligns with prior research emphasizing the functions of the specific roles of selenium, vitamins E and C in fish growth and development (Harsij *et al.,* 2020; Aramli *et al*., 2023). Selenium has been shown to facilitate the secretion of growth hormone from the pituitary gland, accelerate the production of selenoproteins in intestinal epithelial cells, enhance digestibility, and act as a coenzyme in the production of digestive enzymes in fish (Muller *et al*., 1999; Wassef *et al*., 2001; Wassef *et al.,* 2005; Wang *et al*., 2013). The results of this study, where fish fed with T1 and T2 treatments showed improved food intake, Well-being, and growth performance compared to the control group, were consistent with previous research conducted on various fish species. These studies have demonstrated the positive effects of nano-selenium on growth indices and feed conversion ratio in masher fish (Jamil, 2013; Khan *et al*., 2016), Common carp (Ashouri *et al*., 2015), Catfish (Chris *et al.,* 2018), Meagre (Mansour *et al*., 2017), European cypress (Abd El-Kader *et al*., 2020), Nile tilapia (Dawood *et al.,* 2020), and rainbow trout (Hunt *et al*., 2011). The addition of vitamins C and E to selenium has been shown to enhance its efficiency. Vitamins C and E have been reported to improve the nonspecific immune system, exhibit antioxidant effects, increase fish resistance to stress, and enhance growth and food efficiency (Roberts *et al*., 1995; Taveekijakarn *et al.,* 1996; Wassef *et al*., 2001; Zahra *et al.,* 2012; Chen *et al*., 2015; Kim *et al*., 2015). The synergistic effects of these nutrients have been observed in

various studies, including those on juvenile masher fish (Khan *et al.,* 2017) and rainbow trout (Naderi *et al.*, 2017; Harsij *et al.,* 2020). While the study did not compare the separate effects of nano-selenium, vitamins C and E on growth indices and feed conversion ratio, the significant improvement in fish performance compared to the control treatment suggests a synergistic effect of the combined nutrients.

In this study, we found a decreasing trend in growth performance in T3 treatment, compared to fish in T1 and T2 treatments (p<0.05). However, studies have shown that selenium is essential for maintaining fish health, but excess selenium accumulates in muscle, liver, and kidneys (Rodríguez and Rojas, 2014) and leads to gastrointestinal disorders, deficiencies in the immune system and ultimately, it results in a reduction in fish growth (Clark *et al.,* 1996; Gasmi *et al.,* 1997; Raza, 2012). The tolerance threshold of selenomethionine in beluga with 8 g weight was reported between 11.56 and 20.26 µg/g (Arshad *et al.,* 2011).

Although the amount of selenium used in this study did not exceed 0.3 mg/kg and was in nano form, the species' tolerance depended on various factors such as physiological status, diet interactions, supplementation procedures, and absorption conditions (Khan *et al.,* 2017). It appears that under the experimental conditions, dietary supplementation with encapsulated nano-selenium, vitamin C, and vitamin E at moderate levels (0.2 mg/kg selenium, 60 mg/kg vitamin C, and 200 mg/kg vitamin E) significantly improved growth performance, enhanced specific blood parameters, and reduced oxidative stress. However, higher dosages (0.3 mg/kg selenium and above) did not yield additional benefits, suggesting the importance of optimal dosage for maximizing the benefits of dietary supplementation in juvenile Huso huso.

The study examined various hematological parameters in fish, including hematocrit (Hct), hemoglobin concentration (Hb), erythrocytes (RBC), white blood cells (WBC), platelet count (PLT), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These parameters are essential for monitoring fish health and response to environmental stresses or nutritional conditions (Schütt *et al.,* 1997; Řehulka, 2000). Selenium and vitamin C have been demonstrated to be effective antioxidants and stimulants of the immune system, leading to increased survival and blood cell counts (Brown and Arthur, 2001; Molnár *et al.,* 2012; Raza, 2012; Ashouri *et al.,* 2015; Khan *et al.,* 2016; Saffari *et al*., 2018; Harsij *et al*., 2020). In this study, significant increases were observed in WBC, RBC, Hb, and neutrophils in fish fed with T1 and T2 treatments compared to the control group (p<0.05). The increase in hematocrit, total red blood cell count, and white blood cell count in the serum indicates a heightened nonspecific immune system response, characterized by increased phagocytosis and antibody production (Sakai, 1999; Tukmechi *et al*., 2011). Additionally, red blood cells, hematocrit, and hemoglobin play a crucial role in oxygen transport, which is essential for

high-density conditions (Srivastava and Sahai, 1987; Yarahmadi *et al*., 2015; Naderi *et al*., 2019). The increased blood cell counts, hematocrit, hemoglobin, and neutrophils in beluga fish suggest a high tolerance to stress and high-density conditions, as well as improved health and safety indices (Lamas *et al*., 1994; Sakai, 1999; Talpur and Ikhwanuddin, 2012). The findings of this study are consistent with previous research on rainbow trout (Naderi *et al*., 2019), Nile tilapia (El-Hammady *et al*., 2007; Molnár *et al*., 2012), Mahseer fish (Khan *et al*., 2017), and European sea bass (Abd El-Kader *et al*., 2020), which have all reported similar hematological indices in response to selenium supplementation.

Stress in fish can be categorized into acute and chronic forms, with cortisol and glucose being key indicators of primary and secondary stress (Barton and Dwyer, 1997; Wendelaar Bonga, 1997). In response to stress, fish exhibit three sets of compensatory mechanisms, one of which involves cortisol secretion which can lead to increased metabolism and energy mobilization (Barton and Schreck, 1987; Vijayan *et al*., 1997). In this study, cortisol and glucose levels were significantly reduced in fish fed with T1 and T2 treatments compared to the control group (p<0.05). Vitamin C has been shown to modulate physiological reactions to stress, and the fish fed diets with higher vitamin C content are better able to reduce their responses to stressful conditions (Jiménez-Fernández *et al*., 2015; Dawood *et al*., 2016). Vitamin E plays a crucial role in regulating glucose levels under acute stress and preventing their increase (Naderi *et al*., 2017). Selenium is considered an anti-stress factor and has been shown to reduce cortisol and glucose levels in fish under stress (Küçükbay *et al*., 2009; Rider *et al*., 2009; Adineh *et al*., 2021). The study also found that serum total lipid and albumin levels were significantly increased in fish fed with T1 and T2 treatments compared to the control group (p<0.05). Total protein concentration in plasma is used as a baseline index of innate immune system activity, health, and nutritional status in fish (Martins *et al*., 2004). Albumin and globulin measurements can indicate the general fish nutritional and health position, as well as liver functioning (Abdel-Tawwab *et al*., 2007; Khan *et al*., 2017). The findings of the study are consistent with previous research on the effects of selenium, vitamins C and E on fish health and stress response. For instance, similar results were obtained in studies on common carp (Ashouri *et al*., 2015), Nile tilapia (Abdel-Tawwab *et al*., 2007; Dawood *et al*., 2020), and European sea bass (Abd El-Kader *et al*., 2020), which attributed the increases in total protein to immune system enhancement and normal liver function. However, the study found that ALT and AST enzymes increased in the fish fed diets with 0.2, 60, and 200 mg/kg nano-selenium, vitamins C and E (T2), but no significant difference was observed compared to T3 and T4 groups (p>0.05). This is consistent with previous studies on rainbow trout that showed minimal damage to normal liver function and red blood cell damage (Loeb and Quimby, 1999; Harsij *et al*., 2020). Finally, the study found that serum alkaline phosphatase was affected by different dietary treatments, with the highest amount

observed in fish T1 and T2. ALP is considered an indicator of defense system mechanism, and reduction in blood serum is considered a degradation of the immune system. The findings of the study are consistent with previous research on the effects of vitamin E and selenium on ALP activity in common carp (Li *et al*., 2014), rainbow trout (Loeb and Quimby, 1999; Naderi *et al*., 2017; Harsij *et al*., 2020), and Nile tilapia (Neamat-Allah *et al*., 2019).

In fish, the defense system is organized into two levels: (1) Intrinsic defense system and (2) acquired defense system (through adaptation to the environment). The intrinsic defense system includes epithelial barriers, lectin, lysozyme, C-reactive protein, interferon, complement system, and inflammatory reactions that provide prophylactic protection against disease outbreaks in fish (Van Muiswinkel and Vervoorn-Van Der Wal, 2006). Selenium has been shown to enhance the innate immune system and defense systems of fish, with synergistic effects when combined with vitamin E (Gatlin III *et al*., 1986; Fonseca *et al*., 2013; Khan *et al*., 2016; Khan *et al*., 2017). Vitamin E has a positive effect on various immune system processes and provides protection against oxidative stress (Bernabucci *et al*., 2002; El-Shenawy *et al*., 2015). In this study, there was no significant difference in serum lysozyme and IgM levels among fish fed T1, T2, and T3 compared to the control group (p<0.05). However, ACH50 activity was significantly higher in the T2 group compared to the control group (p<0.05). The complement system plays a crucial role in the body's innate immunity, facilitating chemotaxis and the elimination of pathogens (Holland and Lambris, 2002). It is also associated with the acquired immune system and enhances B cell proliferation upon activation (Morgan *et al*., 2005). Additionally, the complement system provides protection against nano-selenium-induced oxidative stress (Yu *et al*., 2005). The study suggests that the inclusion of nano-selenium (Khan *et al*., 2017), vitamins C and E (Khara *et al*., 2016) in the diet leads to the stimulation of immune responses, including increased macrophage activity, cell proliferation, and complement activity.

Today, high-density fish leads to hypoxia (Yu *et al*., 2020) and oxidative stress (Abdel-Tawwab *et al*., 2007) in fish. Oxidative stress is produced by the production of oxygen free radicals (ROS), and antioxidant enzymes such as catalase and superoxide dismutase are activated to convert free radicals to H_2O_2 and oxygen, preventing the accumulation of free radicals and cell membrane destruction (Linhua *et al*., 2009). Selenium is a component of glutathione peroxidase and plays a crucial role in activating the antioxidant defense system by stimulating, synthesizing, and expressing several important selenoenzymes and selenoproteins (Khan *et al*., 2017; Kumar *et al*., 2019). Selenium reduces the effects of oxidative stress and protects cell membranes (Mansour *et al*., 2017; Dawood *et al*., 2020). Natural antioxidants such as vitamins C and E can eliminate ROS and protect cell membranes from damage (Agarwal *et al*., 2005). In this study, serum SOD levels were significantly increased in fish fed with nano-selenium, vitamins C and E supplementation at 0.2, 60, and 200

mg/kg compared to the control groups (p<0.05). Similar results were reported in various fish species, including Gilthead bream (Saleh *et al*., 2015), Nile tilapia (Dawood *et al*., 2020), common carp (Ashouri *et al*., 2015; Jovanović *et al*., 2015), and Mahseer fish (Khan *et al*., 2017), indicating improved immune system function and resistance to oxidative stress (Li *et al*., 2014). Malondialdehyde (MDA) is a product of lipid peroxidation and an important indicator of oxidative stress (Dotan *et al*., 2004; Liu *et al*., 2018). In this study, the amount of MDA in the blood serum of fish fed with nano-selenium and vitamins C and E supplementation was significantly lower than the control diet. However, under chronic stress conditions, MDA content can be increased, but the addition of nano-selenium and vitamin E supplements can reduce MDA levels by removing oxygen free radicals (ROS). It seems that the presence of high iron levels in the culture environment of beluga led to oxidative stress, and antioxidants, including nano-selenium, vitamin C, and vitamin E, were used to eliminate the free radicals produced in the beluga. The supplementation also led to a decrease in malondialdehyde (MDA) levels, indicating reduced oxidative damage in the fish. Similar results were reported by Küçükbay *et al*. (2009), Rider *et al*. (2009), and Yarahmadi *et al*. (2016) in trout species.

Conclusion

Results of this study revealed that adding a mixture of 0.2, 60, and 200 mg/kg nano selenium, vitamin C and vitamin E increased growth, improved hematologic indices, immune system stimulation and reduced oxidative stress in juvenile beluga. However, the potential interaction among these nutrients could be further investigated.

Acknowledgments

The authors would like to acknowledge the support of the International Sturgeon Research Institute and special thanks to Saajad Ghasemian and Hooshang Yeganeh for rearing and feeding the fish.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

Abd El-Kader, M.F., El-Bab, A.F.F., Shoukry, M., Abdel-Warith, A.W.A., Younis, E.M., Moustafa, E.M. and Dawood, M.A., 2020. Evaluating the possible feeding strategies of selenium nanoparticles on the growth rate and wellbeing of European seabass (*Dicentrarchus labrax*). *Aquaculture Reports,* 18, 100539. DOI: 10.1016/j.aqrep.2020.100539

Abdel-Tawwab, M., Mousa, M.A. and 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. DOI:10.1016/J.AQUACULTURE.2007.09.004

Adineh, H., Naderi, M., Nazer, A., Yousefi, M. and Ahmadifar, E., 2021. Interactive effects of stocking density and dietary supplementation with Nano

selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon idella*. *Journal of the World Aquaculture Society,* 52(**4**), 789-804. http:// doi.org/10.1111/jwas.12747

Aebi, H., 1984. Catalase *in vitro*. *Methods in Enzymology,* 105, 121-126.

Agarwal, A., Prabakaran, S.A. and Said, T.M., 2005. Prevention of oxidative stress injury to sperm. *Journal of Andrology,* 26(**6**), 654-660.

Aramli, M.S., Sarvi Moghanlou, K. and Imani, I. 2023. Effect of dietary antioxidant supplements (selenium forms, alpha-tocopherol, and coenzyme Q10) on growth performance, immunity, and physiological responses in rainbow trout (*Oncorhynchus mykiss*) using orthogonal array design. *Fish and Shellfish Immunology*, 134, 108615. DOI:10.1016/j.fsi.2023.108615.

Arshad, U., Takami, G., Sadeghi, M., Bai, S., Pourali, H. and 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. DOI:10.1111/J.1439-0426.2010.01639.X

Ashouri, S., Keyvanshokooh, S., Salati, A.P., Johari, S.A. and 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. DOI:10.1016/J.AQUACULTURE.2015.04.021

Bain, B.J., Bates, I., Laffan, M.A. and Lewis, S.M., 2016. Dacie and Lewis practical haematology: expert consult: online and print. Elsevier Health Sciences, United Kingdom. 600 P.

Barton, B. and Dwyer, W., 1997. Physiological stress effects of continuous-and pulsed-DC electroshock on juvenile bull trout. *Journal of Fish Biology,* 51(**5**), 998-1008.

Barton, B.A. and Schreck, C.B., 1987. Metabolic cost of acute physical stress in juvenile steelhead. *Transactions of the American Fisheries Society,* 116(**2**), 257-263.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *Journal of Dairy Science,* 85(**9**), 2173-2179.

Brown, K.M. and Arthur, J., 2001. Selenium, selenoproteins and human health: a review. *Public Health Nutrition,* 4(**2b**), 593-599.

Chen, Y.J., Yuan, R.M., Liu, Y.J., Yang, H.J., Liang, G.Y. and Tian, L.X., 2015. Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture,* 435, 431-436. DOI:10.1016/J.AQUACULTURE.2014.10.013

Chen, Y., Liu, W., Wang, X., Li, E., Qiao, F., Qin, J.G. and Chen, L., 2018. Effect of dietary lipid source and vitamin E on growth, non-specific immune response and resistance to *Aeromonas hydrophila*

challenge of Chinese mitten crab *Eriocheir sinensis*. *Aquaculture Research,* 49(**5**), 2023-2032. DOI:10.1111/ARE.13659

Chris, U.O., Singh, N. and Agarwal, A., 2018. Nanoparticles as feed supplement on Growth behaviour of Cultured Catfish (*Clarias gariepinus*) fingerlings. *Materials Today: Proceedings,* 5(**3**), 9076-9081. DOI:10.1016/J.MATPR.2017.10.023

Clark, R.F., Strukle, E., Williams, S.R. and Manoguerra, A.S., 1996. Selenium poisoning from a nutritional supplement. *Jama,* 275(**14**), 1087-1088.

Dawood, M.A., Koshio, S., Ishikawa, M. and Yokoyama, S., 2016. Immune responses and stress resistance in red sea bream, Pagrus major, after oral administration of heat-killed *Lactobacillus plantarum* and vitamin C. *Fish and shellfish immunology,* 54, 266-275. DOI:10.1016/j.fsi.2016.04.017

Dawood, M.A., Moustafa, E.M., Elbialy, Z.I., Farrag, F., Lolo, E.E., Abdel-Daim, H.A. and Van Doan, H., 2020. *Lactobacillus plantarum* L-137 and/or β-glucan impacted the histopathological, antioxidant, immune-related genes and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *Research in Veterinary Science,* 130, 212-221. DOI:10.1016/j.rvsc.2020.03.019

Dawood, M.A., Zommara, M., Eweedah, N.M. and Helal, A.I., 2020. The evaluation of growth performance, blood health, oxidative status and immune-related gene expression in Nile tilapia (*Oreochromis niloticus*) fed dietary nanoselenium spheres produced by lactic acid bacteria. *Aquaculture,* 515, 734571. DOI:10.1016/j.aquaculture.2019.734571

Dotan, Y., Lichtenberg, D. and Pinchuk, I., 2004. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Progress in lipid research,* 43(**3**), 200-227. DOI:10.1016/J.PLIPRES.2003.10.001

El-Hammady, A., El-Kasheif, M. and Ibrahim, S., 2007. Synergistic reactions between vitamin eand selenium in diets of hybrid tilapia (*Oreochromis nilot1cus* × *Oreochromis aureus*) and their effect on the growth and liver histological structure. *Egyptian Journal of Aquatic Biology and Fisheries,* 11(**1**), 53-81. DOI:10.21608/EJABF.2007.1914

Ellis, A.I., 1990. Lysozyme assays. *Techniques in fish immunology,* 1, 101-103.

El-Shenawy, N.S., AL-Harbi, M.S. and Hamza, R.Z., 2015. Effect of vitamin E and selenium separately and in combination on biochemical, immunological and histological changes induced by sodium azide in male mice. *Experimental and Toxicologic Pathology,* 67(**1**), 65-76. DOI:10.1016/j.etp.2014.10.005

Esterbauer, H. and Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. In: Lester P. and Alexander N.G. (eds) Methods in enzymology. Academic Press, United States of America. 186, pp 407-421. DOI:10.1016/0076-6879(90)86134-H

Falahatkar, B., Soltani, M., Abtahi, B., Kalbassi, M. and Pourkazemi, M.,

2006. Effects of dietary vitamin C supplementation on performance, tissue chemical composition and alkaline phosphatase activity in great sturgeon (*Huso huso*). *Journal of Applied Ichthyology,* 22, 283-286. DOI:10.1111/j.1439-0426.2007.00969.x

Faradaneh Industrial Group., 2025. Feed Analysis. Faradaneh Industrial Group. Avaiable at: https://www.faradaneh.net (Accessed on January 30, 2025).

Fonseca, S.B.D., Silva, J.H.V.D., Beltrão Filho, E.M., Mendes, P.D.P., Fernandes, J.B. K., Amancio, A.L.L. and Silva, F.R.P.D., 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. DOI:10.1590/S0101-20612013000500017

Gasmi, A., Garnier, R., Galliot-Guilley, M., Gaudillat, C., Quartenoud, B., Buisine, A. and Djebbar, D., 1997. Acute selenium poisoning. *Veterinary* and *Human Toxicology,* 39(**5**), 304-308.

Gatlin III, D., Poe, W., Wilson, R., Ainsworth, A. and Bowser, P., 1986. Effects of stocking density and vitamin C status on vitamin E-adequate and vitamin E-deficient fingerling channel catfish. *Aquaculture,* 56(**3-4**), 187-195. DOI:10.1016/0044-8486(86)90334-0

Hardy, R.W. and Barrows, F.T., 2003. Diet formulation and manufacture. In: John E. Halver, Ronald W. Hardy, Fish nutrition (Third Edition), Academic Press, United States of America. pp 505-600.

Harsij, M., Kanani, H.G. and Adineh, H., 2020. Effects of antioxidant supplementation (nano-selenium, vitamin C and E) on growth performance, blood biochemistry, immune status and body composition of rainbow trout (*Oncorhynchus mykiss*) under sub-lethal ammonia exposure. *Aquaculture,* 521, 734942. DOI:10.1016/j.aquaculture.2020.734942

Holland, M.C.H. and Lambris, J.D., 2002. The complement system in teleosts. *Fish and Shellfish Immunology,* 12(**5**), 399-420.

Hunt, A.O., Berkoz, M., Ozkan, F., Yalin, S., Ercen, Z., Erdogan, E. and Gunduz, S.G., 2011. Effects of organic selenium on growth, muscle composition, and antioxidant system in rainbow trout. *Israeli Journal of Aquaculture-Bamidgeh,* 63(**562**), 10.

Jamil, Z., 2013. Effects of inorganic and nanoform of selenium on growth performance and biochemical indices of mahseer (*Tor putitora*). *Journal of the World Aquaculture Society,* 35, 245-252.

Jiménez-Fernández, E., Ponce, M., Rodriguez-Rúa, A., Zuasti, E., Manchado, M. and Fernández-Díaz, C., 2015. Effect of dietary vitamin C level during early larval stages in Senegalese sole (*Solea senegalensis*). *Aquaculture,* 443, 65-76. DOI:10.1016/J.AQUACULTURE.2015.03.013

Jovanović, I.B., Velickovic, M., Milanovic, S., Valcic, O., Gvozdic, D. and Vranješ-Đurić, S., 2015. Supplemental selenium reduces the

levels of biomarkers of oxidative and general stress in peripartum dairy cows. *Acta Veterinaria, Beograd,* 65(**2**), 191-201. DOI:10.1515/acve-2015-0016

Kalbassi, M.R., Abdollahzadeh, E. and Salari-Joo, H., 2013. A review on aquaculture development in Iran. *Ecopersia,* 1(**2**), 159-178.

Keen, C.L., Uriu-Adams, J.Y., Ensunsa, J.L. and Gershwin, M.E., 2004. Trace elements/minerals and immunity. *Handbook of nutrition and immunity* (pp. 117-140): Springer. DOI:10.1007/978-1-59259-790-1_6

Khan, K.U., Zuberi, A. and Ullah, I., 2015. Effects of Graded Level of Dietary L-Ascorbyl-2-Polyphosphate on Growth Performance and Some Hematological Indices of Juvenile Mahseer (*Tor putitora*). *International Journal of Agriculture and Biology,* 17(**4**). DOI:10.17957/IJAB/14.0023

Khan, K.U., Zuberi, A., Nazir, S., Fernandes, J.B.K., Jamil, Z. and Sarwar, H., 2016. Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile Tor putitora. *Turkish Journal of Zoology,* 40(**5**), 704-712. DOI:10.3906/zoo-1510-5

Khan, K.U., Zuberi, A., Fernandes, J.B.K., Ullah, I. and 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(**6**), 1689-1705. DOI:10.1007/s10695-017-0402-z

Khara, H., Sayyadborani, M. and Sayyad-Borani, 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(**2**), 385-393. DOI:10.4194/1303-2712-v16_2_18

Kim, K., Bae, O.N., Koh, S.H., Kang, S., Lim, K.M., Noh, J.Y. and Chung, J.H., 2015. High-dose vitamin C injection to cancer patients may promote thrombosis through procoagulant activation of erythrocytes. *Toxicological Sciences,* 147(**2**), 350-359. DOI:10.1093/toxsci/kfv133

Kumar, N., Brahmchari, R.K., Bhushan, S., Thorat, S.T., Kumar, P., Chandan, N.K. and Singh, N.P., 2019. Synergistic effect of dietary selenium nanoparticles and riboflavin on the enhanced thermal efficiency of fish against multiple stress factors. *Journal of Thermal Biology,* 85, 102417. DOI:10.1016/j.jtherbio.2019.102417

Küçükbay, F., Yazlak, H., Karaca, I., Sahin, N., Tuzcu, M., Cakmak, M. and 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. DOI:10.1111/j.1365-2095.2008.00624.x

Lamas, J., Santos, Y., Bruno, D., Toranzo, A. and Anadon, R., 1994. Non-specific cellular responses of rainbow trout to *Vibrio anguillarum* and its extracellular products (ECPs). *Journal of Fish Biology,* 45(**5**), 839-854.

Li, D., Liu, Z. and Xie, C., 2012. Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, *Acipenser schrenckii*. *Fish physiology and biochemistry,* 38(**2**), 511-520. DOI:10.1007/s10695-011-9531-y

Li, J., Liang, X.F., Tan, Q., Yuan, X., Liu, L., Zhou, Y. and Li, B., 2014. Effects of vitamin E on growth performance and antioxidant status in juvenile grass carp *Ctenopharyngodon idellus*. *Aquaculture,* 430, 21-27. DOI:10.1016/j.aquaculture.2014.03.019

Lin, M.F. and Shiau, S.Y., 2005. Dietary L-ascorbic acid affects growth, nonspecific immune responses and disease resistance in juvenile grouper, *Epinephelus malabaricus*. *Aquaculture,* 244(**1-4**), 215-221. DOI:10.1016/j.aquaculture.2004.10.026

Linhua, H., Zhenyu, W. and Baoshan, X., 2009. Effect of sub-acute exposure to TiO2 nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (*Cyprinus carpio*). *Journal of Environmental Sciences,* 21(**10**), 1459-1466. DOI:10.1016/S1001-0742(08)62440-7

Liu, L., Liang, X.F., Li, J., Fang, J., Yuan, X. and Alam, M., 2018. Effects of dietary selenium on growth performance and oxidative stress in juvenile grass carp *Ctenopharyngodon idellus*. *Aquaculture Nutrition,* 24(**4**), 1296-1303. DOI:10.1111/anu.12667

Loeb, W.F. and Quimby, F.W., 1999. *The clinical chemistry of laboratory animals*.

Longbaf Dezfouli, M., Ghaedtaheri, A., Keyvanshokooh, S., Salati, A.P., Mousavi, S.M. and Pasha-Zanoosi, H., 2019. Combined or individual effects of dietary magnesium and selenium nanoparticles on growth performance, immunity, blood biochemistry and antioxidant status of Asian seabass (*Lates calcarifer*) reared in freshwater. *Aquaculture Nutrition,* 25(**6**), 1422-1430. DOI:10.1111/anu.12962

Lu, Y., Liang, X.P., Jin, M., Sun, P., Ma, H.N., Yuan, Y. and Zhou, Q.C., 2016. Effects of dietary vitamin E on the growth performance, antioxidant status and innate immune response in juvenile yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture,* 464, 609-617. DOI:10.1016/j.aquaculture.2016.08.009

Luo, L., Xue, M., Wu, X., Cai, X., Cao, H. and Liang, Y., 2006. Partial or total replacement of fishmeal by solvent-extracted cottonseed meal in diets for juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition,* 12(**6**), 418-424. DOI:10.1111/j.1365-2095.2006.00443.x

Mansour, A.T.E., Goda, A.A., Omar, E.A., Khalil, H.S. and Esteban, M.Á., 2017. Dietary supplementation of organic selenium improves growth, survival, antioxidant and immune status of meagre, *Argyrosomus regius*, juveniles. *Fish and shellfish immunology,* 68, 516-524. DOI:10.1016/j.fsi.2017.07.060

Marklund, S. and Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry,* 47(**3**), 469-474. DOI:10.1111/j.1432-1033.1974.tb03714.x

Martins, M., Tavares-Dias, M., Fujimoto, R., Onaka, E. and Nomura, D., 2004. Haematological alterations of *Leporinus macrocephalus* (Osteichtyes: Anostomidae) naturally infected by *Goezia leporini* (Nematoda: Anisakidae) in fish pond. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* 56, 640-646. DOI:10.1590/S0102-09352004000500011

Molnár, T., Biró, J., Balogh, K., Mézes, M. and Hancz, C., 2012. Improving the nutritional value of Nile tilapia fillet by dietary selenium supplementation. Israeli Journal of Aquaculture-Bamidgeh, 64. DOI:10.46989/001c.20641

Moniruzzaman, M., Park, G., Yun, H., Lee, S., Park, Y. and Bai, S.C., 2017. Synergistic effects of dietary vitamin E and selenomethionine on growth performance and tissue methylmercury accumulation on mercury-induced toxicity in juvenile olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture Research,* 48(**2**), 570-580. DOI:10.5958/0974-181X.2016.00014.7

Monteiro, D.A., Rantin, F.T. and Kalinin, A.L., 2009. The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxã, Brycon cephalus exposed to organophosphate insecticide Folisuper 600 BR®(methyl parathion). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology,* 149(**1**), 40-49. DOI:10.1016/j.cbpc.2008.06.012

Morgan, B.P., Marchbank, K.J., Longhi, M.P., Harris, C.L. and Gallimore, A.M., 2005. Complement: central to innate immunity and bridging to adaptive responses. *Immunology letters,* 97(**2**), 171-179. DOI:10.1016/j.imlet.2004.11.010

Muller, E.E., Locatelli, V. and Cocchi, D. 1999. Neuroendocrine control of growth hormone secretion. *Physiological reviews,* 79(**2**), 511-607. DOI: 10.1111/j.1651-2227.1991.tb17962.x

Naderi, M., Keyvanshokooh, S., Salati, A.P. and Ghaedi, A., 2017. Effects of dietary vitamin E and selenium nanoparticles supplementation on acute stress responses in rainbow trout (*Oncorhynchus mykiss*) previously subjected to chronic stress. *Aquaculture,* 473, 215-222. DOI: 10.1016/j.aquaculture.2017.02.020

Naderi, M., Keyvanshokooh, S., Ghaedi, A. and Salati, A.P., 2019. Interactive effects of dietary Nano selenium and vitamin E on growth, haematology, innate immune responses, antioxidant status and muscle composition of rainbow trout under high rearing density. *Aquaculture Nutrition,* 25(**5**), 1156-1168. DOI:10.1111/anu.12931

Nayak, S.K., 2010. Probiotics and immunity: a fish perspective. *Fish and shellfish immunology,* 29(**1**), 2-14. DOI:10.1016/j.fsi.2010.02.017

NRC., 2011. *Nutrient Requirements of Fish and Shrimp*: The National Academy Press, Washington, D. C.

Neamat-Allah, A.N., Mahmoud, E.A. and Abd El Hakim, Y., 2019. Efficacy of dietary Nano-selenium on growth, immune response, antioxidant, transcriptomic profile and resistance of Nile tilapia, *Oreochromis niloticus*

against *Streptococcus iniae* infection. *Fish and Shellfish Immunology,* 94, 280-287. DOI:10.1016/j.fsi.2019.09.019

Potki, N., Falahatkar, B. and Alizadeh, A., 2018. Growth, hematological and biochemical indices of common carp *Cyprinus carpio* fed diets containing corn gluten meal. *Aquaculture International,* 26(**6**), 1573-1586. DOI:10.1007/s10499-018-0304-9

Rafatnezhad, S., Falahatkar, B. and Tolouei Gilani, M.H., 2008. Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. *Aquaculture Research,* 39(**14**), 1506-1513. DOI:10.1111/j.1365-2109.2008.02020.x

Raza, A., 2012. Effects of graded levels of dietary selenium supplementation on the growth of juvenile mahseer *(Tor putitora).* M. Phil. Thesis, Quaid-i-Azam University, Islamabad Pakistan. DOI:10.17957/IJAB/14.0023

Řehulka, J., 2000. Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture,* 190(**1-2**), 27-47. DOI:10.1016/S0044-8486(00)00383-5

Rider, S.A., Davies, S.J., Jha, A.N., Fisher, A.A., Knight, J. and 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. DOI:10.1016/j.aquaculture.2009.07.003

Roberts, M., Davies, S. and Pulsford, A., 1995. The influence of ascorbic acid (vitamin C) on non-specific immunity in the turbot (*Scophthalmus maximus* L.). *Fish and Shellfish Immunology,* 5(**1**), 27-38. DOI:10.1016/S1050-4648(05)80004-X

Rodríguez, H. and Rojas, S., 2014. Efecto de dietas enriquecidas con vitamina ey selenio orgánico en el comportamiento productivo y calidad funcional del filete de trucha arco iris (*Oncorhynchus mykiss*). *Revista de Investigaciones Veterinarias del Perú,* 25(**2**), 213-225. DOI:10.15381/rivep.v25i2.8494

Saffari, S., Keyvanshokooh, S., Zakeri, M., Johari, S.A., Pasha-Zanoosi, H. and 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*(4), 1087-1097. DOI: 10.1007/s10695-018-0496-y

Sakai, M. 1999. Current research status of fish immunostimulants. *Aquaculture,* 172(**1-2**), 63-92.

Saleh, R., Betancor, M., Roo, J., Benítez-Dorta, V., Zamorano, M.J., Bell, J.G. and Izquierdo, M., 2015. Effect of krill phospholipids versus soybean lecithin in microdiets for gilthead seabream (*Sparus aurata*) larvae on molecular markers of antioxidative metabolism and bone development. *Aquaculture Nutrition,* 21(**4**), 474-488. DOI:10.1111/anu.12177

Schütt, D.A., Lehmann, J., Goerlich, R. and Hamers, R., 1997. Haematology of

swordtail, *Xiphophorus helleri*. I: blood parameters and light microscopy of blood cells. *Journal of Applied Ichthyology, 13*(2), 83-89. DOI: 10.1111/j.1439-0426.1997.tb00106.x

Srivastava, N. and Sahai, R., 1987. Effects of distillery waste on the performance of Cicer arietinum L. *Environmental Pollution,* 43(**2**), 91-102.

Talpur, A.D. and Ikhwanuddin, M., 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against Vibrio harveyi infection in Asian sea bass, Lates calcarifer (Bloch). *Aquaculture,* 364, 6-12. DOI:10.1016/j.aquaculture.2012.07.035

Taveekijakarn, P., Miyazaki, T., Matsumoto, M. and Aral, S., 1996. Histopathological and haematological changes in amago salmon, *Oncorhynchus rhodurus* (Jordan and McGregor), fed a vitamin-D-free diet. *Journal of Fish Diseases,* 19(**4**), 289-294. DOI:10.1046/j.1365-2761.1996.d01-85.x

Tukmechi, A., Andani, H.R.R., Manaffar, R. and Sheikhzadeh, N., 2011. Dietary administration of beta-mercapto-ethanol treated Saccharomyces cerevisiae enhanced the growth, innate immune response and disease resistance of the rainbow trout, *Oncorhynchus mykiss*. *Fish and Shellfish Immunology,* 30(**3**), 923-928. DOI:10.1016/j.fsi.2011.01.016

Van Muiswinkel, W.B. and Vervoorn-Van Der Wal, B., 2006. 18 The Immune System of Fish.

Vijayan, M.M., Pereira, C., Grau, E.G. and Iwama, G.K., 1997. Metabolic responses associated with confinement stress in tilapia: the role of cortisol. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* 116(**1**), 89-95. DOI:10.1016/S0742-8413(96)00124-7

Wang, Y., Yan, X. and Fu, L., 2013. Effect of selenium nanoparticles with different sizes in primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio*. *International Journal of Nanomedicine,* 8, 4007. DOI:10.2147/IJN.S43691

Wassef, E., El Masry, M. and Mikhail, F., 2001. Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research,* 32, 315-322. DOI:10.1046/j.1355-557x.2001.00043.x

Wassef, E.A., El-Sayed, A.F.M., Kandeel, K.M. and Sakr, E.M., 2005. Evaluation of Pterocla Dia (Rhodophyta) and Ulva (Chlorophyta) meals as additives to gilthead seabream Sparus aurata diets. *Egyptian Journal of Aquatic Research,* 31, 321-332.

Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiological reviews,* 77(**3**), 591-625.

Yano, T., 1992. Assays of hemolytic complement activity. *Techniques in Fish Immunology*, 131-141.

Yarahmadi, P., Miandare, H.K., Hoseinifar, S.H., Gheysvandi, N. and Akbarzadeh, A., 2015. The effects of stocking density on hemato-

immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International,* 23(**1**), 55-63. DOI: 10.1007/s10499-014-9797-z

Yarahmadi, P., Miandare, H.K., Fayaz, S. and Caipang, C.M.A., 2016. Increased stocking density causes changes in expression of selected stress- and immune-related genes, humoral innate immune parameters and stress responses of rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology,* 48, 43-53. DOI:10.1016/j.fsi.2015.11.007

Yeh, S.P., Chang, C.A., Chang, C.Y., Liu, C.H. and Cheng, W., 2008. Dietary sodium alginate administration affects fingerling growth and resistance to *Streptococcus* sp. and iridovirus, and juvenile non-specific immune responses of the orange-spotted grouper, *Epinephelus coioides*. *Fish and shellfish immunology, 25*(1-2), 19-27. DOI: 10.1016/j.fsi.2007.11.011

Yu, H.J., Liu, J.q., Bock, A., Li, J., Luo, G.m. and Shen, J.c., 2005. Engineering glutathione transferase to a novel glutathione peroxidase mimic with high catalytic efficiency: incorporation of selenocysteine into a glutathione-binding scaffold using an auxotrophic expression system. *Journal of Biological Chemistry,* 280(**12**), 11930-11935. DOI:10.1074/jbc.M408574200

Yu, H., Zhang, C., Zhang, X., Wang, C., Li, P., Liu, G., Yan, X., Xiong, X., Zhang, L., Hou, J. and Liu, S., 2020. Dietary nano-selenium enhances antioxidant capacity and hypoxia tolerance of grass carp *Ctenopharyngodon idella* fed with high-fat diet. *Aquaculture Nutrition,* 26(**2**), 545-557. DOI:10.1111/anu.13016

Zahra, F., Kidwai, S.S., Siddiqi, S.A. and Khan, R.M., 2012. Frequency of newly diagnosed diabetes mellitus in acute ischaemic stroke patients. *J Coll Physicians Surg Pak,* 22(**4**), 226-229.

Zanon, R.B., Silva, T.S.d.C., Cerozi, B.d.S. and Cyrino, J.E.P., 2018. Effects of graded levels of dietary vitamin E on striped surubim *Pseudoplatystoma reticulatum*. *Aquaculture Research,* 49(**4**), 1423-1429. DOI:10.1111/are.13594

Zhang, J., Wang, X. and Xu, T., 2008. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with se-methylselenocysteine in mice. *Toxicological Sciences,* 101(**1**), 22-31. DOI:10.1093/toxsci/kfm221

Zhao, H., Zhu, H., Huang, J., Zhu, Y., Hong, M., Zhu, H., Zhang, J., Li, S., Yang, L., Lian, Y. and Wang, S., 2018. The synergy of Vitamin C with decitabine activates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid leukemia. *Leukemia Research,* 66, 1-7. DOI:10.1016/j.leukres.2017.12.009