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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*

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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

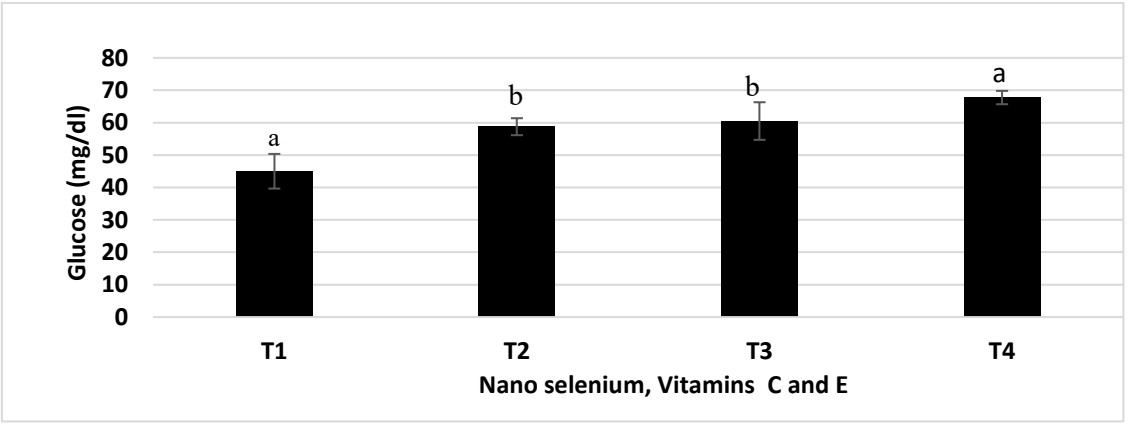
Biochemical indices

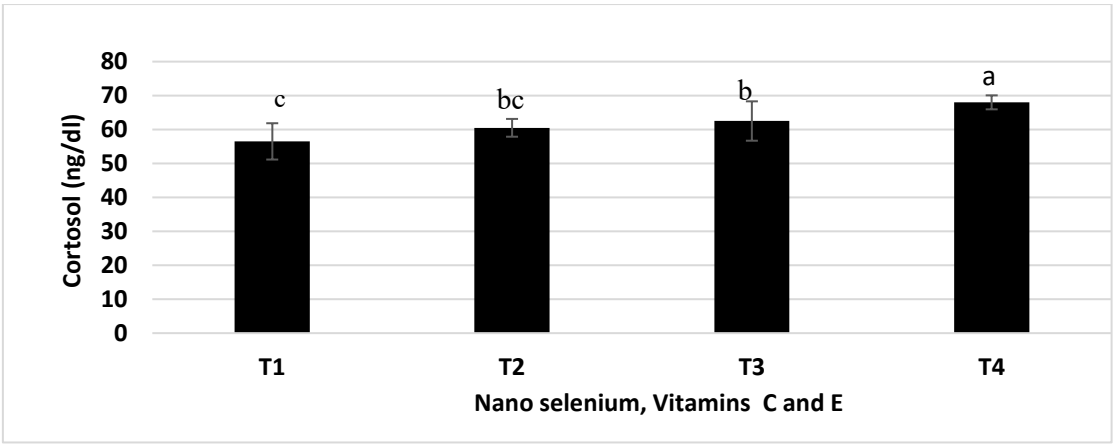
The highest levels of glucose and cortisol were observed in the control group and

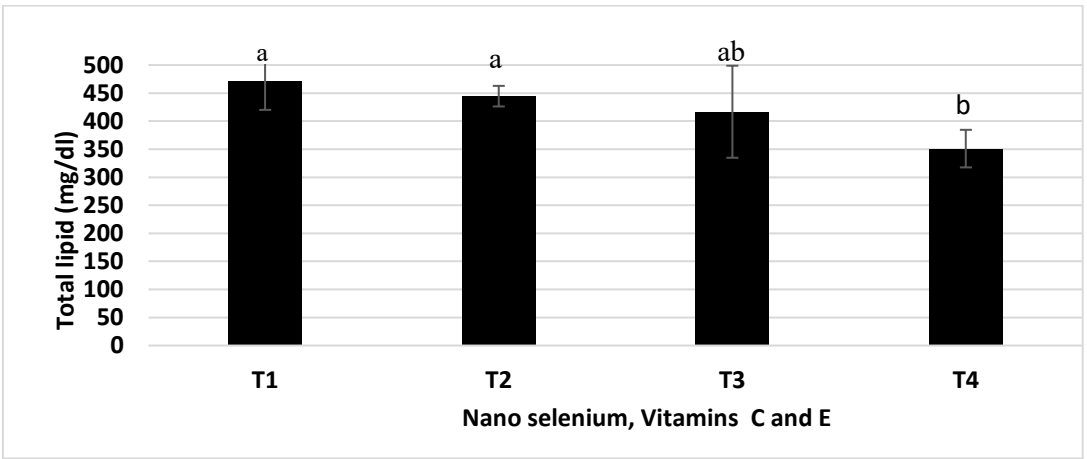
supplementation resulted in a decrease in glucose in the T1, T2, and T3 groups. The lowest levels of cortisol and glucose were observed in fish fed T1 and T2, which were significantly different from T4 (control) (p<0.05). In contrast to the control group (T4), levels of cholesterol, total lipids, albumin, 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).

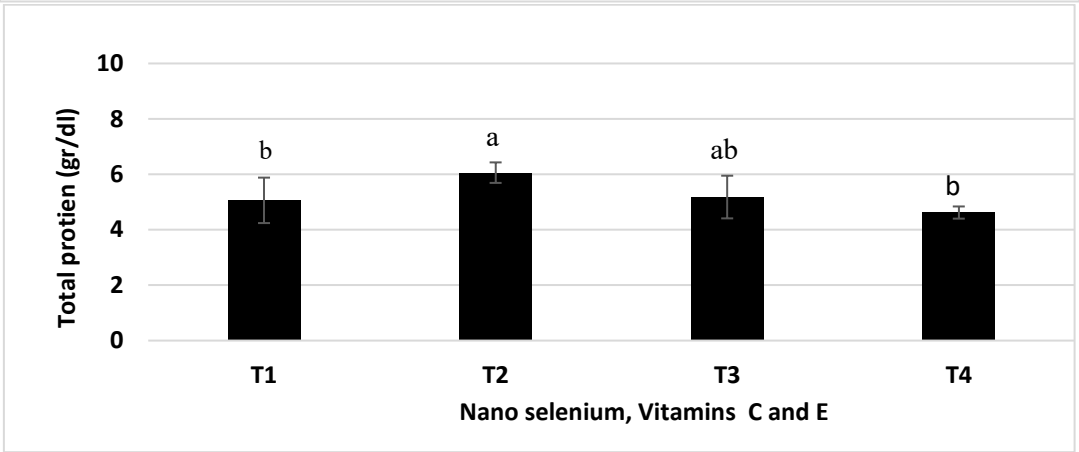
Table 3: Effect of microencapsulated selenium, vitamins C and E on biochemical indices of *Huso huso* (n=3, mean standard deviation).

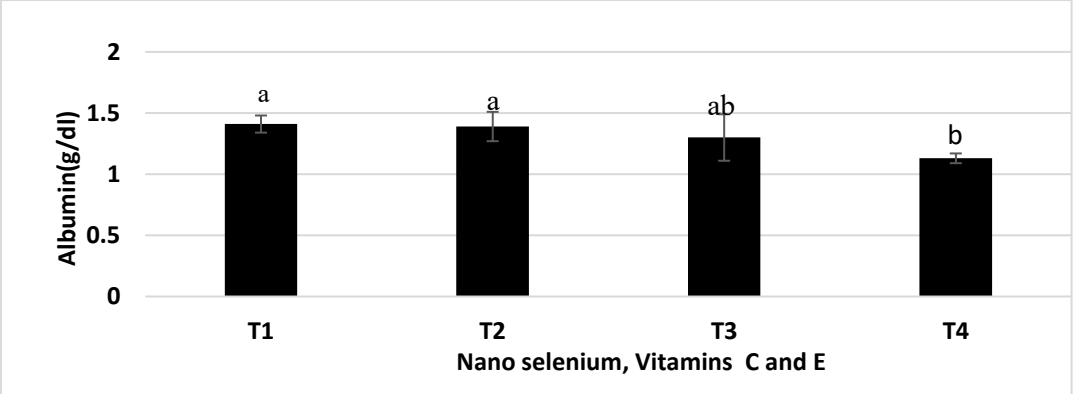
Indicators / Diet	T_1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg)	T_2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg)	T_3: Nano selenium, vitamins C and E (0.3, 90, and 300 mg/kg)	T_4: Control
Glucose (mg/dl)	45.00±5.35^{c}	58.75±2.62^{b}	60.50±5.8^{b}	67.75±2.06^{a}
Cortisol (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}

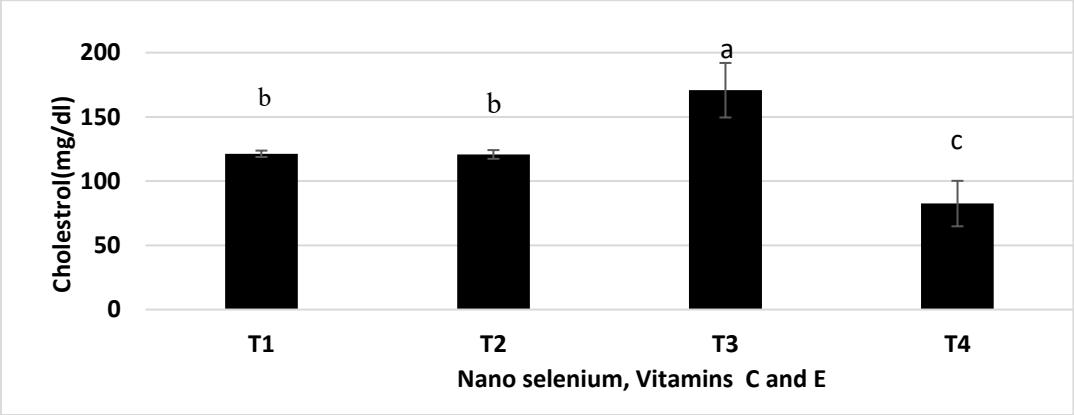


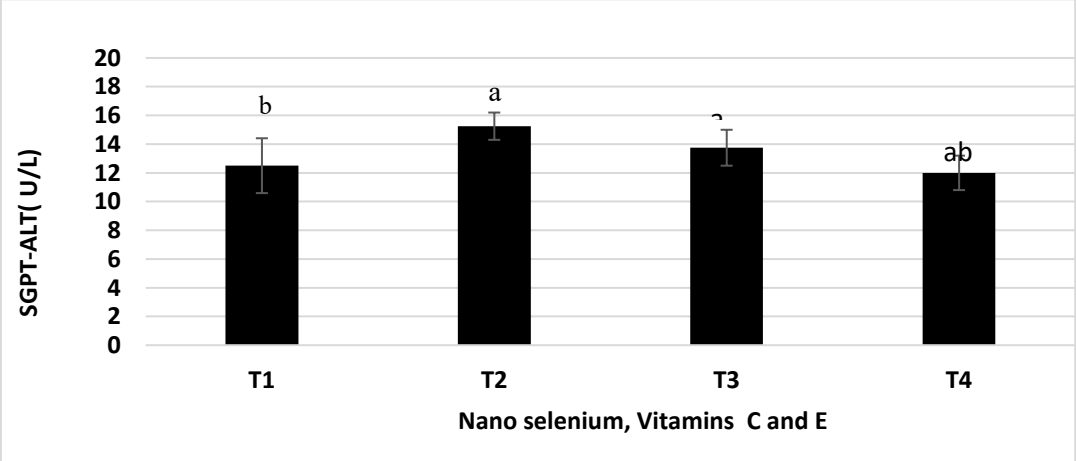


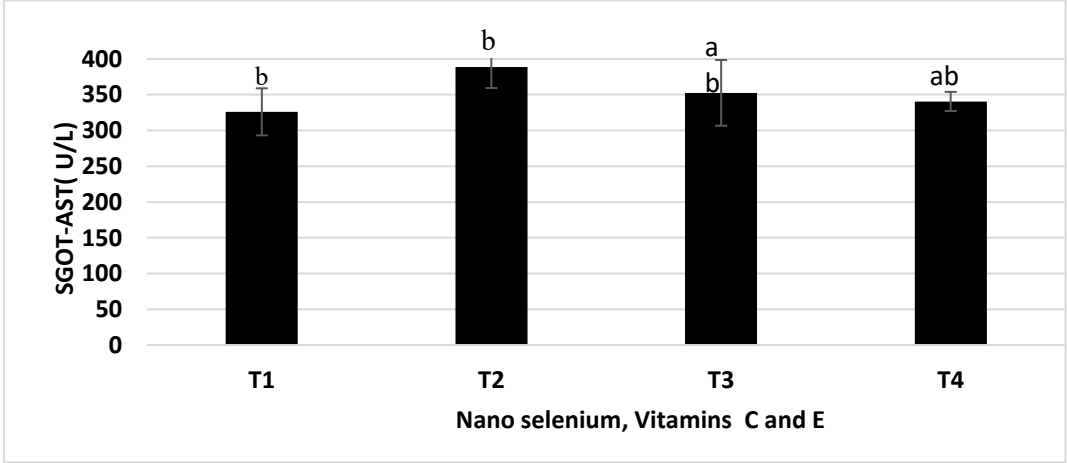












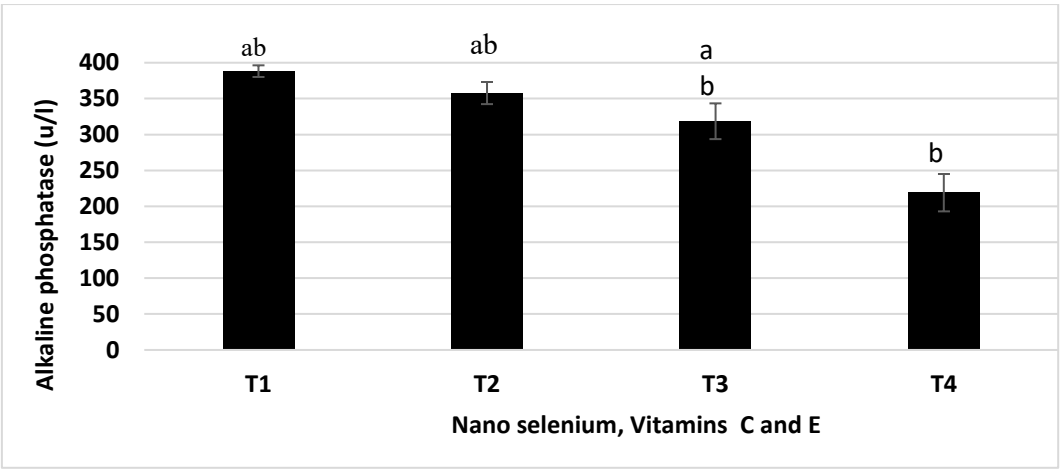
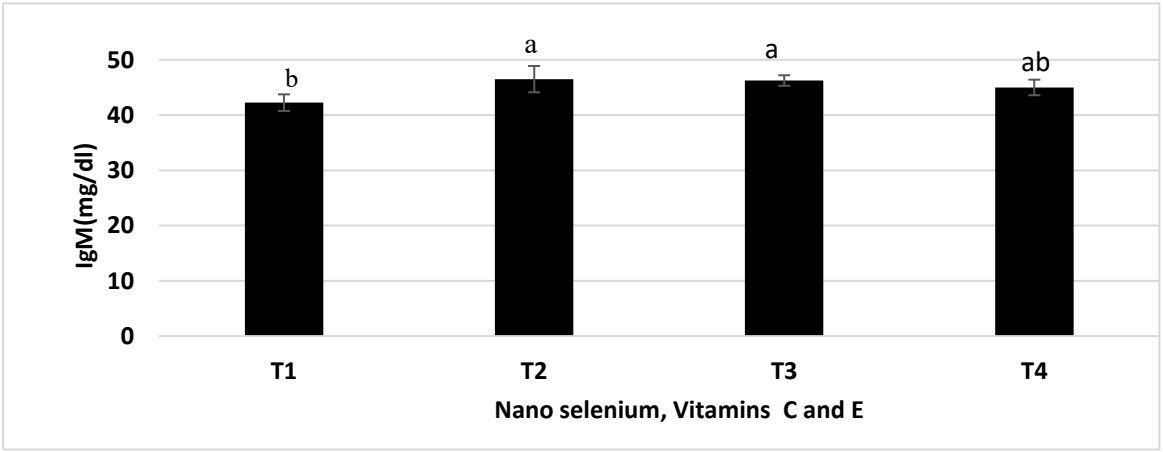
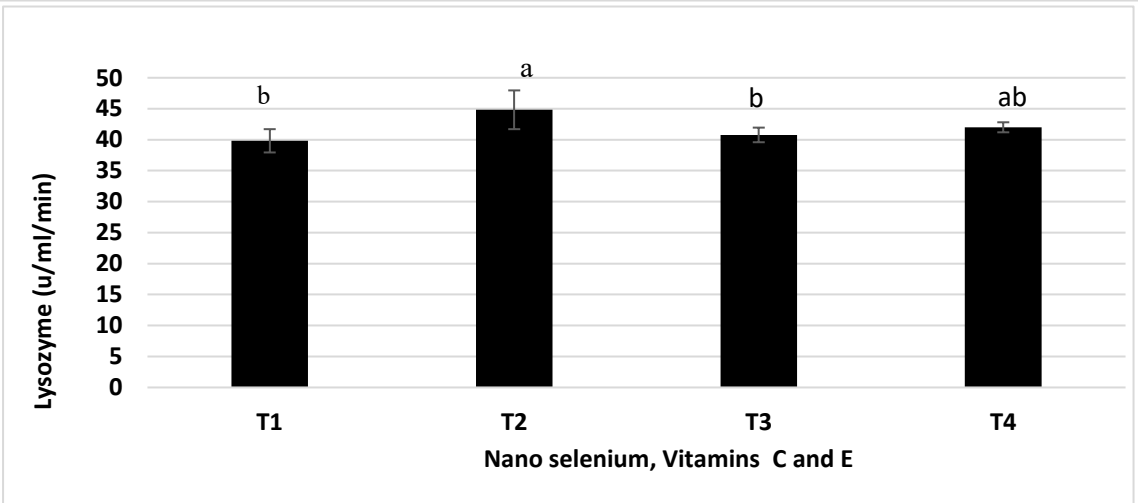


Figure 1: Effect of microencapsulated selenium, vitamins C and E on biochemical index of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg), T3: Nano selenium, vitamins C and E (0.3,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





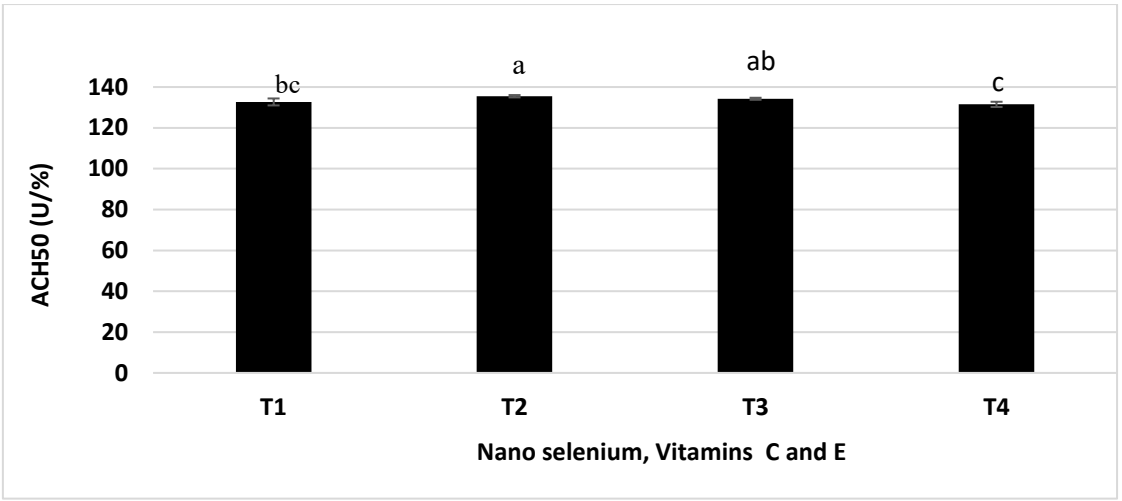


Figure 2: Effect of microencapsulated selenium, vitamins C and E on Imunological index of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60, and 200 mg/kg),T3: Nano selenium, vitamins C and E (0.3,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

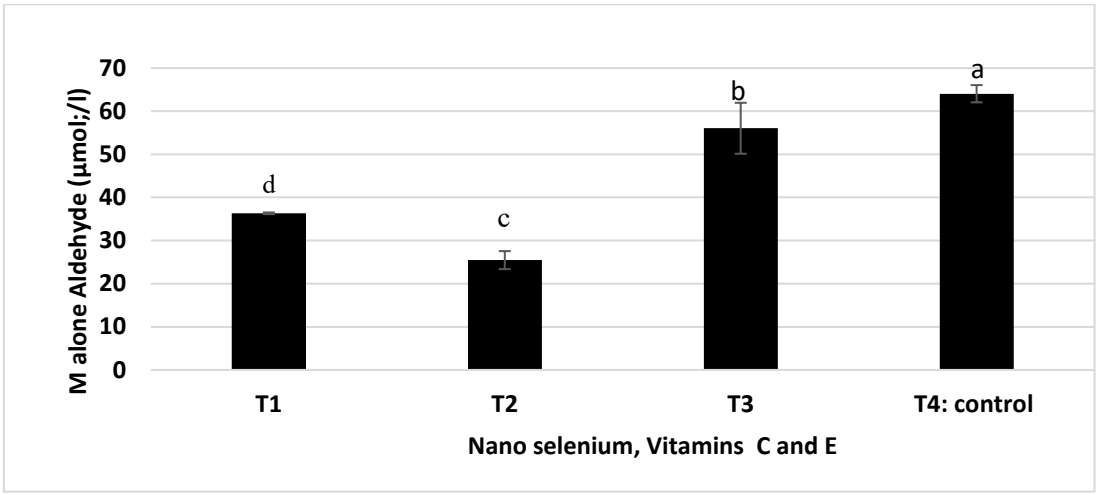
Antioxidant enzymes

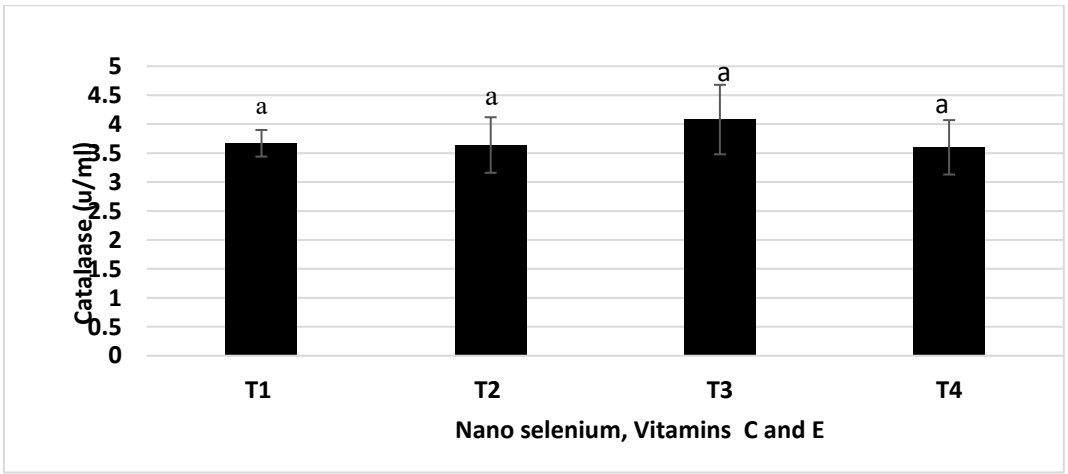
The antioxidant enzyme activities are shown in Table 5 and Figure 3. In this study, a reduction in malondialdehyde activity in fish was observed with inclusion of the supplement in the diet. The lowest amount of malondialdehyde was observed in fish of group T2 and in the next ranks were located T3 and 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]





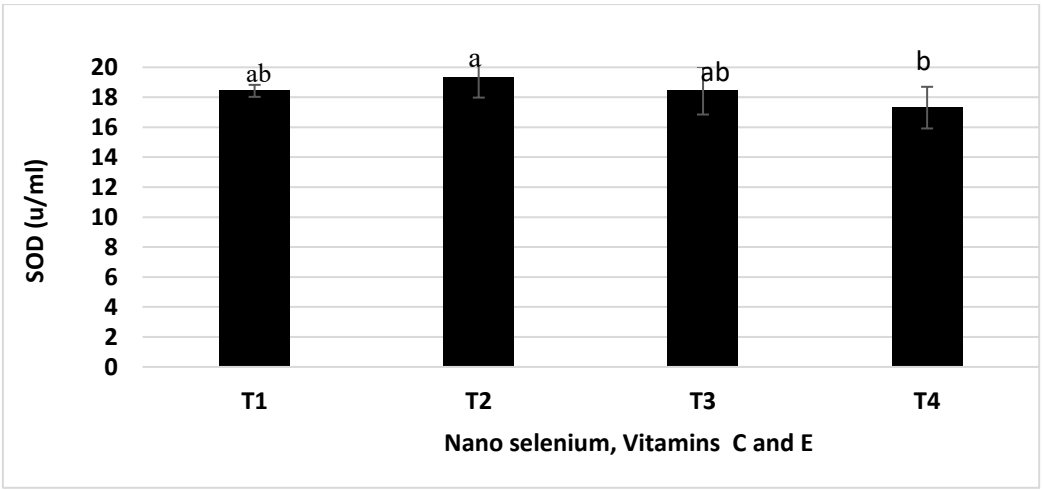


Figure 3: Effect of microencapsulated selenium, vitamins C and E on Antioxidant Enzyme of *Huso huso* (n=3, mean standard deviation) where T1: Nano selenium, vitamins C and E (0.1, 30, and 100 mg/kg),T2: Nano selenium, vitamins C and E (0.2, 60 and 200 mg / kg),T3: Nano selenium, vitamins C and E (0.3,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

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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