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Antioxidant, biochemical, hematological indices and handling stress resistance in rainbow trout (*Oncorhynchus mykiss*) fed with diets supplemented with nano-selenium and vitamin C

Ferdows Momeni[1], Saeid Meshkini[2]*, Ahmad Imani[3]

[1] PhD Candidate, Department of Fisheries, Faculty of Natural Resources, Urmia University, Urmia, Iran; [2] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [3] Department of Fisheries and Aquatic Sciences, Faculty of Natural Resources, Urmia University, Urmia, Iran.

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Abstract

Use of various nanoparticles and vitamins to strengthen the immune system of fish to improve growth and biochemical indice, and to mitigate the harmful effects of free radicals through enhanced antioxidant enzyme activity has become the subject of numerous studies. In this study, fish with an average weight of 55.20 ± 7.90 g, after a two-week acclimation period, were randomly distributed into 18 polyethylene 300-L tanks, with 25 fish per tank. The experiment consisted of six treatments with three replicates: Control, vitamin (Vit) C100, nano-selenium (N-Se)0.40, Vit C100 + N-Se0.10, Vit C200 + N-Se0.20, and Vit C400 + N-Se0.40. Blood samples were collected post-treatment for analysis of antioxidant enzyme activities, biochemical and hematological indices as well as post-handling stress. Results indicated significant differences in antioxidant enzyme activities among treatments. The highest catalase activity was observed in Vit C100 and Vit C100 + N-Se0.10 groups. Glutathione peroxidase activity was significantly higher in the control and Vit C200 + N-Se0.20 groups. Malondialdehyde was the highest in Vit C100 + N-Se0.10 group. Superoxide dismutase activity peaked in Vit C200 + N-Se0.20 group. Biochemical indices showed significant differences among treatments except for high-density lipoprotein. Hemoglobin and red blood cell counts were significantly different. The Vit C100 + N-Se0.10 group showed the highest serum cortisol and glucose levels post-handling stress. Overall, dietary N-Se and Vit C supplementation improved antioxidant, biochemical, hematological indices and stress resistance in rainbow trout.

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Introduction

Minerals and vitamins (Vits) are two essential micro-nutrients required for the maintenance of normal physiological functions. Among micronutrients, selenium (Se) and Vit C are both known as potent antioxidants.[1] Antioxidant defense is a complex system comprising enzymatic and non-enzymatic components. The non-enzymatic elements in fish depend on behavior, diet and feeding. Previous studies demonstrated that oxidative stress, antioxidant defenses and hematological parameters in aquatic species were influenced by dietary components.[2,3] It has been reported that stress increases the requirement for minerals and vitamins in fish due to enhanced mobilization and excretion of these nutrients.[4] Hence, mineral supplementation is considered a strategy to alleviate stress-related damages.[5] Selenium, as an essential trace element, plays a protective role against oxidative stress by forming selenoproteins such as glutathione peroxidase (GPX).[6] Nano (N)-Se, compared to other forms, offers higher bioavailability and reduced toxicity.[7] Studies have confirmed its positive effects on growth and oxidative stress resistance.[8,9] Similarly, Vit C is vital for growth and physiological health, as most bony fish cannot synthesize ascorbic acid and require dietary supplementation.[10-12] Vitamin C improves erythropoiesis and tissue oxygenation leading to better stress tolerance and immune responses. Given the potential synergistic effects of these two nutrients, their combined dietary application may benefit aquatic species.[13] However, limited information exists on their modulatory roles in aquaculture.[14] This study investigated the effects of Vit C and N-Se on antioxidant, biochemical, hematological indices and handling stress in rainbow trout.

***Correspondence:**

Saeid Meshkini. DVM, PhD
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
E-mail: s.meshkiny@urmia.ac.ir

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Materials and Methods

Treatments and experimental diets**.** Healthy rainbow trout were procured from a commercial farm and acclimated for 2 weeks while fed with a basal diet (Kimiyagaran, Tehran, Iran) consisted of extrude trout grower (EXG1) with the size of 4.00 ± 0.30 mm, proximate composition of basal diet (as percent of dry weight): Protein = 39.81, Fat = 18.39, Fiber = 4.00, Ash = 10.77, moisture = 6.02, phosphorus = 5.00. The fish (55.20 ± 7.90 g) were randomly assigned to six dietary treatments in 300-L tanks (25 fish per tank). Experimental treatments include: Control diet , Diet with 100 mg kg^{-1} vitamin C (Vit C100), Diet with 0.40 mg kg^{-1} N-Se(N-Se0.40), Diet with 100 mg kg^{-1} Vit C + 0.10 mg kg^{-1} N-Se (Vit C100 + N-Se0.10), Diet with 200 mg kg^{-1} Vit C + 0.20 mg kg^{-1} N-Se (Vit C200 + N-Se0.20), Diet with 400 mg kg^{-1} Vit C + 0.40 mg kg^{-1} N-Se (Vit C400 + N-Se0.40). The basal diet contained 0.16 mg kg^{-1} Se and 220 mg kg^{-1} Vit C. Diets were prepared weekly by spraying N-Se and Vit C on commercial feed followed by gelatin coating. Feeds were stored at 4.00˚C until use. Nano-selenium (10.00 - 45.00 nm; USNano, Houston, USA) and ascorbic acid (Sigma-Aldrich, St. Louis, USA) were used.

Experimental design. The 60-day trial involved feeding fish four times daily at 2.30% body weight. Water temperature (15.50 ± 0.50˚C), pH (8.00 ± 0.50) and dissolved oxygen (7.00 ± 0.50 mg L^{-1}) were monitored weekly. Tanks were cleaned daily and maintained under a 12 : 12 light : dark cycle. Sampling procedure**.** Feeding was stopped 24 hr before sampling. Three fish per tank were anesthetized using 250 mg L^{-1} clove oil.[15] Blood samples were collected—half for hematology, and the remainder centrifuged and stored at – 80.00˚C for biochemical assays.[16]

Hematological analysis. Red- and white blood cells (RBCs and WBCs) were counted based on the method of Lewis *et al.*[17] Hematocrit (HCT) was measured via microhematocrit.[18] Hemoglobin (Hb) was determined spectrophotometrically.[19]

Biochemical parameters. Total protein was measured calorimetrically.[20] Albumin, cholesterol and triglycerides were determined using Pars Azmoon kits (Tehran, Iran).[21,22]

Antioxidant enzyme activities. Superoxide dismutase (SOD) enzyme activity was measured using chemical kits from ZellBo GmbH (Lonsee, Germany), according to the method recommended by Bolann and Ulvik,[23] and using a spectrophotometer at a wavelength of 420 nm. Catalase (CAT) enzyme activity was determined by hydrogen peroxide decomposition at a wavelength of 240 nm according to the method of Góth.[24] Glutathione peroxidase enzyme activity was determined at a wavelength of 340 nm according to the method of Paglia and Valentine.[25] Serum malondialdehyde (MDA) was measured colorimetrically based on its reaction with thiobarbituric acid at a wavelength of 535 nm according to the method reported by Dawn-Linsley *et al.*[26]

Handling stress. Test Stress was induced by air exposure for 90 sec according to the guidelines provided by Eslamloo and Falahatkar.[27] Blood was sampled 3 hr post-stress for cortisol, glucose, sodium, and chloride assays. Cortisol: enzyme-linked immunosorbent assay.[28] Glucose: enzymatic method, 546 nm.[21] Electrolytes: electrolyte analyzer.[29]

Statistical analysis. Data normality was tested (Kolmogorov-Smirnov and Levene's Test). One-way ANOVA and Tukey's HSD test were used in SPSS Software (version 20.0; IBM Corp., Armonk, USA). Results are presented as Mean ± SE with significance at $p < 0.05$. GraphPad software was used for charting.

Results

Hematological parameters. The results of hematological profiles including red and WBC counts and erythrocyte indices in rainbow trout fed with diets supplemented with Vit C and N-Se are shown in Tables 1 and 2. A significant difference ($p < 0.05$) was observed in RBC count and Hb content with the highest values in the Vit C400 + N-Se0.40 group and the lowest in the N-Se0.40 group. No significant differences ($p > 0.05$) were found among treatments for HCT, mean corpuscular Hb, mean corpuscular Hb concentration and mean corpuscular volume. The highest WBC count was observed in the Vit C100 + N-Se0.10 group which was significantly different from the control. The highest neutrophil percentage was recorded in Vit C100 and the lowest in Vit C200 + N-Se0.20. Lymphocyte percentages were the highest in Vit C100 + N-Se0.10 and Vit C400 + N-Se0.40, while Vit C100 had the lowest. Monocyte and eosinophil percentages showed no significant differences among treatments ($p > 0.05$).

Biochemical parameters. According to the statistical analysis of serum biochemical indices (Table 3), all values showed significant differences ($p < 0.05$) except for high-density lipoprotein (HDL). The highest cholesterol levels were found in N-Se0.4 and Vit C400 + N-Se0.40 groups, while the lowest was in Vit C100 + N-Se0.10. Triglyceride levels were the highest in Vit C100 + N-Se0.10 and the lowest in Vit C100. Although HDL differences were not statistically significant, Vit C100 + N-Se0.10 showed the highest value. Low-density lipoprotein (LDL) levels were significantly affected, the highest in Vit C100 and Vit C400 + N-Se0.40 and the lowest in Vit C100 + N-Se0.10. Total protein was significantly higher in control and Vit C100 + N-Se0.10, the lowest in Vit C400 + N-Se0.40. Albumin was the highest in control and Vit C200 + N-Se0.20, and the lowest in N-Se0.40.

Antioxidant enzyme activities. Table 4 presents antioxidant enzyme activities and MDA levels. Catalase activity was significantly influenced, with the highest in Vit C100 + N-Se0.10 and the lowest in N-Se0.4 ($p < 0.05$). Glutathione peroxidase activity was peaked in the control and Vit C200 + N-Se0.20 groups, while was the lowest in N-Se0.40. The SOD activity was the highest in Vit C200 + N-Se0.20 and the lowest in Vit C400 + N-Se0.40. MDA levels were the highest in Vit C100 + N-Se0.10 and the lowest in Vit C400 + N-Se0.40.

Handling stress test. Table 5 summarizes the effects of N-Se and Vit C on resistance to handling stress. Before stress, the highest serum cortisol level was observed in Vit C100 + N-Se0.10, and the lowest in control. After stress, N-Se0.40 group had the highest cortisol level, and Vit C200 + N-Se0.20 the lowest ($p < 0.05$). Glucose levels before stress followed a similar pattern. Post-stress glucose was the highest in N-Se0.4 and the lowest in Vit C100. No significant differences were found in sodium levels before stress, but after stress, control had the highest and Vit C200 + N-Se0.20 had the lowest. Chloride levels were the highest in control and the lowest in Vit C200 + N-Se0.20 before stress. Post-stress, they were the highest in N-Se0.40 and the lowest in Vit C100 + N-Se0.10.

Table 1. Total and differential white blood cell counts.

Treatments	WBC ($\times 10^3$ mL^{-1})	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control	17.46 ± 1.19^{a}	15.00 ± 3.00^{ab}	79.67 ± 2.52^{ab}	5.00 ± 1.00^{a}	0.33 ± 0.58^{a}
Vit C100	18. 40 ± 0.90^{ab}	17.00 ± 1.73^{b}	77.00 ± 2.00^{a}	6.00 ± 1.00^{a}	0.00 ± 0.00^{a}
N-Se0.4	18.23 ± 1.30^{ab}	14.33 ± 2.08^{ab}	79.33 ± 1.53^{ab}	6.00 ± 1.00^{a}	0.33 ± 0.58^{a}
Vit C100 + N-Se0.1	22.26 ± 2.51^{b}	10.33 ± 2.08^{a}	84.00 ± 2.65^{b}	5.67 ± 0.58^{a}	0.00 ± 0.00^{a}
Vit C200 + N-Se0.2	21.16 ± 1.23^{ab}	9.67 ± 3.06^{a}	83.00 ± 2.65^{b}	6.67 ± 1.53^{a}	0.67 ± 1.15^{a}
Vit C400 + N-Se0.4	21.76 ± 1.19^{ab}	10.00 ± 1.00^{a}	84.00 ± 1.00^{b}	6.00 ± 1.00^{a}	0.00 ± 0.00^{a}

Vit: Vitamin, and N-Se: Nano-selenium

^{ab} Different letters in each column indicate significant differences ($p < 0.05$).

Table 2. Red blood cell counts and erythrocyte indices.

Treatments	RBC ($\times 10^6$ mL^{-1})	Hemoglobin (g dL^{-1})	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
Control	1.79 ± 0.03^{bc}	8.88 ± 0.22^{bc}	52.10 ± 1.25^{a}	290.33 ± 1.15^{a}	49.53 ± 0.25^{a}	17.04 ± 0.05^{a}
Vit C100	1.67 ± 0.03^{ab}	8.27 ± 0.16^{ab}	48.40 ± 1.05^{a}	288.33 ± 0.58^{a}	49.37 ± 0.15^{a}	17.09 ± 0.06^{a}
N-Se0.4	1.62 ± 0.02^{a}	7.99 ± 0.17^{a}	46.63 ± 1.15^{a}	287.00 ± 3.21^{a}	49.30 ± 0.50^{a}	17.13 ± 0.10^{a}
Vit C100 + N-Se0.1	1.72 ± 0.55^{abc}	8.53 ± 0.26^{abc}	50.07 ± 1.50^{a}	289.33 ± 0.58^{a}	49.37 ± 0.15^{a}	17.04 ± 0.03^{a}
Vit C200 + N-Se0.2	1.74 ± 0.08^{abc}	8.55 ± 0.39^{abc}	50.33 ± 2.26^{a}	289.33 ± 1.73^{a}	49.13 ± 0.40^{a}	16.99 ± 0.05^{a}
Vit C400 + N-Se0.4	1.84 ± 0.04^{c}	9.03 ± 0.16^{c}	50.30 ± 4.23^{a}	291.33 ± 2.65^{a}	49.00 ± 0.36^{a}	16.93 ± 0.12^{a}

Vit: Vitamin, N-Se: Nano-selenium, RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, and MCHC: Mean corpuscular hemoglobin concentration.

^{abc} Different letters in each column indicate significant differences ($p < 0.05$).

Table 3. Serum biochemical parameters.

Treatments	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	TP (g dL^{-1})	Albumin (g dL^{-1})
Control	195.53 ± 1.20^{ab}	177.13 ± 7.66^{a}	53.33 ±1.12^{a}	105.87 ± 2.17^{b}	4.00 ± 0.15^{ab}	1.05 ± 0.01^{d}
Vit C100	200.03 ± 0.97^{cd}	164.40 ± 9.20^{a}	53.87 ± 1.39^{a}	114.10 ± 1.85^{c}	3.59 ± 0.19^{cd}	0.97 ± 0.02^{b}
N-Se0.4	201.57 ± 1.10^{d}	179.73 ± 2.40^{a}	52.93 ± 1.75^{a}	112.87 ± 1.22^{c}	3.39 ± 0.17^{d}	0.89 ± 0.01^{a}
Vit C100 + NSe0.1	193.37 ± 1.60^{a}	193.37 ± 7.39^{b}	54.47 ± 0.78^{a}	99.43 ± 1.81^{a}	3.65 ± 0.27^{a}	0.99 ± 0.01^{bc}
Vit C200 + NSe0.2	197.73 ± 1.40^{bc}	182.30 ± 11.01^{a}	51.33 ± 0.96^{a}	109.07 ± 3.36^{bc}	3.62 ± 0.13^{bc}	1.01 ± 0.01^{c}
Vit C400 + NSe0.4	201.33 ± 0.96^{d}	166.10 ± 12.21^{a}	54.23 ± 0.72^{a}	113.97 ± 1.47^{c}	3.36 ± 0.04^{d}	0.98 ± 0.10^{bc}

Vit: Vitamin, N-Se: Nano-selenium, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, and TP: Total protein.

$^{a-d}$ Different letters in each column indicate significant differences ($p < 0.05$).

Table 4. Antioxidant enzyme activity.

Treatments	SOD (U mL^{-1})	CAT (U mL^{-1})	GPX (U mL^{-1})	MDA (nmol mL^{-1})
Control	72.17 ± 2.97^{ab}	48.73 ± 1.08^{d}	203.00 ± 7.79^{b}	125.00 ± 3.46^{b}
Vit C100	69.53 ± 1.42^{a}	42.27 ± 0.87^{ab}	198.67 ± 3.51^{ab}	111.00 ± 2.94^{a}
N-Se0.4	73.37 ± 0.75^{ab}	38.67 ± 2.75^{a}	186.33 ± 3.06^{a}	125.33 ± 3.06^{b}
Vit C100 + NSe0.1	76.77 ± 1.34^{bc}	48.50 ± 1.80^{cd}	191.67 ± 5.03^{ab}	128.00 ± 1.00^{b}
Vit C200 + NSe0.2	79.33 ± 1.85^{c}	42.60 ± 1.01^{ab}	200.33 ± 2.08^{b}	124.33 ± 2.08^{b}
Vit C400 + NSe0.4	69.20 ± 1.32^{a}	44.43 ± 0.75^{bc}	189.67 ± 1.32^{ab}	107.33 ± 2.52^{a}

Vit: Vitamin, N-Se: Nano-selenium, MDA: Malondialdehyde, GPX: Glutathione peroxidase, CAT: Catalase, and SOD: Superoxide dismutase.

$^{a-d}$ Different letters in each column indicate significant differences ($p < 0.05$).

Table 5. Comparing stress indices before and after manipulation (stress).

Treatments	Cortisol (ng mL^{-1})		Glucose (mg dL^{-1})		Sodium (mmol L^{-1})		Chloride (mmol L^{-1})	
	Before	After	Before	After	Before	After	Before	After
Control	50.97 ± 5.50^{a}	114.24 ± 11.32^{ab}	75.97 ± 2.80^{a}	109.13 ± 3.01^{ab}	145.17 ± 2.32^{a}	149.25 ± 0.07^{b}	134.93± 6.18^{b}	110.37± 6.61^{bc}
Vit C100	55.27 ± 3.90^{a}	119.43 ± 9.34^{ab}	79.73 ± 2.71^{ab}	102.27 ± 3.07^{a}	146.43 ± 1.07^{a}	147.40 ± 1.27^{ab}	130.43 ± 15.40^{ab}	101.73 ± 2.10^{ab}
N-Se0.4	51.27 ± 5.86^{a}	145.37 ± 7.80^{c}	80.50 ± 5.86^{ab}	165.00 ± 18.62^{d}	144.93 ± 1.50^{a}	146.20 ± 1.179^{a}	112.70 ± 7.41^{ab}	119.97 ± 2. 86^{c}
Vit C100 + NSe0.1	68.13 ± 4.75^{b}	135.17 ± 9.75^{b}	97.13 ± 3.61^{c}	124.23 ± 6.30^{abc}	146.87 ± 1.07^{a}	148.37 ± 0.61^{ab}	119.47 ± 5.16^{ab}	97.27 ± 1. 89^{a}
Vit C200 + NSe0.2	58.23 ± 4.61^{ab}	108.67 ± 8.84^{a}	88.93 ± 7.03^{bc}	130.43 ± 12.45^{bc}	143.20 ± 1.35^{a}	145.87 ± 0.45^{a}	109.10 ± 5.26^{a}	103.1 ± 2. 42^{ab}
Vit C400 + NSe0.4	59.97 ± 2.01^{ab}	112.93 ± 2.32^{ab}	90.73 ± 1.94^{bc}	147.63 ± 6.52^{cd}	145.97 ± 0.68^{a}	147.60 ± 1.10^{ab}	111.47 ± 3.25^{a}	100.77 ± 1. 99^{ab}

Vit: Vitamin, N-Se: Nano-selenium

$^{a-d}$ Different letters in each column indicate significant differences ($p < 0.05$).

Discussion

It is well known that hematological indices such as Hb and HCT values, red and WBC counts in fish are associated with their health and immune response.[30] Similar to other animals, elevated hematological indices in fish are vital for immune function and disease resistance.[2] In this study, the RBC count and Hb levels were increased in the Vit C400 + N-Se0.4 group. However, HCT, mean corpuscular Hb, mean corpuscular Hb concentration and mean corpuscular volume were not significantly different among treatments ($p > 0.05$). Vitamin C might protect phagocytic cells and surrounding tissues from oxidative damage.[31]

Others suggested that Vit C extends RBC life span and supports cellular respiration.[32] In the present study, the combination of N-Se and Vit C enhanced erythropoiesis and Hb concentration. Similar improvements have been reported in sea bass fed N-Se.[33] Nano-selenium antioxidant role helps maintain RBC membrane integrity, reducing hemolysis and increasing oxygen availability.[8,34]

In the present study, HCT, mean corpuscular Hb, mean corpuscular Hb concentration and mean corpuscular volume were not affected by Vit C and N-Se. Dietary N-Se increased the values of hematological indices, which increased oxygen availability in tissue cells, and subsequently regulated the rate of nutrient metabolism.[6] Increased Se increased the protection of RBC membranes against the harmful effects of oxygen free radicals and reduced hemolysis and degeneration.[35] Similarly, others noted that dietary N-Se improved RBC Hb, packed cell volume parameters in common carp.[9] Yellow tail fish fed with 2.00 mg of Se showed increased RBCs and HCT activity.[36] In a study conducted on tilapia by others, it was reported that Hb was increased in tilapia fed with N-Se and Se powder, while fish fed with 0.80 mg kg^{-1} diet of N-Se recorded the highest RBC, HCT, WBC.[35] Such differences may be explained by differences in the type and content of supplements, experimental period, feed composition or the combined synergistic effects of supplements with their different individual effects.[37]

Serum biochemical indices are critical indicators of physiological responses.[38,39] Serum protein functions in cell metabolism, enzyme activity and hormone secretion.[35] In our study, total serum protein was the highest in control and Vit C100 + N-Se0.1 and the lowest in Vit C400 + N-Se0.4. Higher protein content reflects enhanced physiological health due to antioxidant supplementation. HDL levels were the highest in Vit C100 + N-Se0.1, while LDL and MDA were the lowest in this group indicating reduced oxidative damage. Improved lipid profiles in carp fed with N-Se was also observed. The HDL apo-lipoprotein AI plays a key antioxidant role, reducing lipid peroxidation.[8]

In our study, HDL concentration was the highest in Vit C100 + N-Se0.1 treatment and the lowest in Vit C200 + N-Se0.2 treatment. Also, LDL concentration was the highest in Vit C100 treatment and the lowest in Vit C100 + N-Se0.1 treatment. In agreement with the results of this study, others showed that Se could significantly reduce serum total cholesterol in Wistar rats.[40] It has also been reported that organic Se can significantly reduce LDL and increase HDL.[41] Consistently, in a study conducted by others, supplementation with 2.00 mg kg^{-1} diet of N-Se was observed to reduce serum total cholesterol and LDL concentration and to significantly increase HDL levels.[8] Consistently, others reported an increase in total protein globulin and albumin content in catfish fed with 5.00 mg kg^{-1} diet of organic Se.[42] In the present study, the increase in serum HDL was accompanied by a decrease in LDL and MDA. These results might indicate that rainbow trout were protected when fed with a diet containing N-Se and Vit C.

Selenium has a large number of biological functions in animals including fish. The most important and well-known function is its antioxidant effect. As a form of selenoprotein, it is part of the active site in glutathione peroxidase(GSH-PX).[43] During the normal metabolism of organisms, the production and removal of reactive oxygen species maintain a dynamic balance. Antioxidant enzymes such as CAT, SOD, GPX can eliminate excessive ROS and reduce the damage caused by lipid oxidation.[32] The activities of CAT, GPX and SOD as important antioxidant enzymes can be considered as oxidative biomarkers, in addition to indicating the antioxidant capacity of aquatic organisms.[14] However, MDA is a toxic substance produced by the breakdown of lipid peroxides that can cause damage to the body and reflects the extent of cellular damage and lipid peroxidation in animal cells.[44] Antioxidants can protect organisms against free radicals

and ROS and reduce the progression of many chronic diseases as well as lipid peroxidation. The antioxidant defense system is a multi-component mechanism with enzymatic and non-enzymatic elements. The non-enzymatic antioxidant elements in fish depend on factors such as feeding behavior and feed. While dietary micronutrients are widely used in animal feed and contribute to the antioxidant defense system, negative perceptions and consumer caution limit their use.[43] In the present study, N-Se and Vit C supplementation successfully showed the highest SOD activity in Vit C200 + N-Se0.2 and Vit C100 + N-Se0.1 treatments, the highest GPX activity in C and Vit C200 + N-Se0.2 treatments and the highest CAT activity in C and Vit C100 + N-Se0.1 treatments. Also, the MDA level at the end of feeding showed the highest activity in Vit C100 + N-Se0.1 treatment and the lowest serum MDA activity in Vit C400 + N-Se0.4 treatment, indicating the role of Se and Vit C in protecting rainbow trout against lipid oxidation. In a study conducted by others, an increase in the antioxidant enzyme activities of CAT, SOD and GPX and a decrease in MDA levels were reported in European bass fed with N-Se.[33] Others also reported that MDA levels were reduced in rainbow trout fish fed with N-Se.[8] In a study conducted by others, GPX, GSH-PX and SOD activities were increased in fish fed with Se supplementation while MDA concentration was decreased.[43] Consistently, in a study conducted by others, improved antioxidant enzyme activities and lower MDA were observed in fish fed with 0.4 and 0.8 mg N-Se compared to Se powder and control groups.[35] Furthermore, others reported that common carp fed with N-Se had the highest hepatic CAT, SOD and GPX activities and liver MDA content was significantly lower in fish fed diets containing N-Se and selenocysteine compared to the control group.[9] Others reported the highest muscle CAT, SOD and GPX activities and the lowest MDA content in the combined 1.00 mg N-Se and 500 mg kg^{-1} Vit E group compared to the control treatment.[45] In another study conducted by others, N-Se and Vit C supplementation successfully increased the activities of CAT, SOD, GPX and reduced MDA at the end of feeding.[14] In the present study the increase in antioxidant parameters in fish following the consumption of N-Se and Vit C might be due to the role of Se in the formation of selenocysteine, which is located in the active center of the enzyme (GPX). Vitamin C is also among the vitamins that can protect cells against oxidative stress. Based on the above results, it seems that Vit C and N-Se are excellent stimulants in rainbow trout farming.[8,9,46,47]

High-density aquaculture practices increase fish exposure to physical stressors like sorting, transportation and vaccination leading to immunosuppression and physiological disruption. Cortisol and glucose are critical indicators of stress in fish.[48] In this study, pre-stress cortisol was the highest in Vit C100 + N-Se0.1 and the lowest in control. Post-stress, cortisol and glucose were significantly elevated in the N-Se0.4 group while the lowest levels were in Vit C200 + N-Se0.2 and Vit C100, respectively. These results suggested that antioxidant supplementation with N-Se and Vit C mitigated stress response by stabilizing cortisol and glucose levels and preserving osmotic balance.

Dietary supplementation is a key strategy in improving aquafeed quality. This study demonstrated that N-Se and Vit C significantly enhanced physiological parameters in rainbow trout, including hematological, antioxidant, biochemical and stress resistance indices. However, at higher doses (Vit C200 + N-Se0.2 and Vit C400 + N-Se0.4), these benefits declined. Therefore, optimal combinations such as Vit C100 + N-Se0.1 might be effective additives for improving health and productivity in rainbow trout farming.

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Conflict of interest

The authors declare no competing interests.

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