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Effects of dietary organic selenium, vitamin C, and vitamin E supplementation on growth performance, serum biochemistry, and antioxidant status in juvenile *Hypophthalmichthys molitrix*

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The study aimed to evaluate the effects of dietary supplementation of selenium (Se), vitamin C, and vitamin E on growth performance, meat quality, and antioxidant status in *Hypophthalmichthys molitrix* juveniles. A total of 480 juveniles (mean weight: 20 ± 0.29 g) were randomly assigned to 24 aquaria (20 fish per 100 L), which were allocated to eight dietary treatments (three aquaria per treatment) following a completely randomized design. The dietary treatments included: (1) a basal diet without supplementation (Control), (2) selenium supplementation (S), (3) vitamin C supplementation (C), (4) vitamin E supplementation (E), (5) selenium and vitamin C supplementation (SC), (6) selenium and vitamin E supplementation (SE), (7) vitamin C and vitamin E supplementation (EC), and (8) selenium, vitamin C, and vitamin E supplementation (SCE). The supplementation levels were 0.9 mg/kg Se, 300 mg/kg vitamin C, and 100 mg/kg vitamin E, respectively. After a one-week acclimatization period, a 10-week growth trial was conducted. Fish fed the SCE diet exhibited significantly higher ($p < 0.05$) final weight gain, body length gain, body weight gain percentage, and specific growth rates compared to other treatments. Selenium concentrations in the kidney, liver, pancreas, and muscle were significantly higher ($p < 0.05$) in the SCE, SC, SE, and EC groups compared to the Control and single-supplement groups. Hematological parameters, including WBC, RBC, HGB, HCT, and MCHC, were significantly higher ($p < 0.05$) in the SCE group compared to all other groups. Similarly, serum activities of ALT, AST, and ALP were significantly lower ($p < 0.05$) in the SCE group than in other treatments,

while blood glucose levels were unaffected ($p > 0.05$) by dietary treatments. Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were significantly enhanced ($p < 0.05$) in the whole body and muscle tissues of fish receiving Se, vitamin C, and E supplementation. Thiobarbituric acid reactive substances (TBARS) levels in muscle and serum were significantly higher ($p < 0.05$) in the Control group compared to all supplemented groups. The proximate composition of meat, including dry matter, fat, ash, and protein contents, was not significantly affected ($p > 0.05$) by the dietary treatments. In conclusion, dietary supplementation of selenium, vitamin C, and vitamin E significantly improved growth performance, selenium deposition in tissues, and antioxidant status of *Hypophthalmichthys molitrix* juveniles, without adversely affecting meat chemical composition.

KEYWORDS

juveniles, growth performance, blood analysis, liver enzymes, antioxidants

1 Introduction

Fish production systems range from extensive pond-based setups to highly controlled intensive rearing environments. Intensive aquaculture offers several advantages, including accelerated growth rates, enhanced management of the rearing environment, and early detection of diseases (Kumar and Engle, 2016). However, one significant drawback of intensive systems is the induction of stress, which often leads to oxidative stress through the overproduction of reactive oxygen species (ROS) (Mushtaq et al., 2022). Elevated ROS levels disrupt physiological processes and impair antioxidant defense mechanisms in fish, ultimately resulting in reduced performance, higher morbidity, and increased mortality (Naiel et al., 2020). The antioxidant defense system in animals comprises endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Mushtaq et al., 2022). Exogenous antioxidants, including vitamins C and E, and micro minerals such as selenium (Se), enhance this defense system by acting as cofactors for these enzymes or through structural stabilization of biomolecules (Flora et al., 2013). Thus, formulating a balanced diet with appropriate redox-regulating nutrients is essential for promoting optimal growth performance and ensuring animal welfare in intensive aquaculture systems.

Selenium is a vital trace mineral in aquaculture due to its interaction with cysteine to form selenocysteine, a key component of selenoproteins (Arshad et al., 2021). Selenoproteins, comprising 21 amino acids, are extracellular proteins with at least 25 identified forms in animal tissues, where selenocysteine (Se-Cys) serves as a crucial cofactor for enzymatic redox processes (Yang and Liu, 2017). Extensive research highlights the role of dietary selenium in enhancing fish fertility (Naderi et al., 2017), thyroid hormone metabolism (Kohrle et al., 2005), DNA synthesis, and cellular protection against oxidative damage (Khademzade et al., 2022).

Organic selenium forms, such as selenomethionine, are preferred due to their lower toxicity and higher bioavailability compared to inorganic selenium (Naderi et al., 2017).

Vitamin C, or ascorbic acid, is another essential exogenous antioxidant, recognized for its free radical scavenging properties and its role as a cofactor in various metabolic recycling pathways (Smirnoff, 2018). Dietary requirements for vitamin C vary depending on the fish species, growth stage, body size, and environmental conditions (Council et al., 2011). Furthermore, elevated levels of vitamin C can enhance immune responses beyond physiological needs, offering additional protection against stress (Trichet, 2010).

Vitamin E represents a group of lipid-soluble antioxidants, including tocopherols and tocotrienols. Among these, α -tocopherol (α -TOH) exhibits the highest biological activity (Hamre, 2011) and is preferentially retained in mammalian systems, including Atlantic salmon, likely due to its high affinity for liver tocopherol-binding proteins (Kayden and Traber, 1993). Vitamin E plays a critical role in stabilizing cellular membranes and mitigating ROS-induced oxidative stress. It interrupts lipid peroxidation chains by neutralizing fatty acid peroxide radicals, thereby preventing the oxidation of other fatty acids (Gutteridge, 1995). Numerous studies document the positive effects of vitamin E on fish growth, immune responses, health, and stress resistance (Naderi et al., 2017).

Selenium, vitamin E, and vitamin C function synergistically to enhance antioxidant defense and support immune health in fish (Harsij et al., 2020). Selenium plays a pivotal role in combating oxidative stress by targeting peroxides through selenium-dependent glutathione peroxidase (GPx), while vitamin E prevents lipid peroxidation, reducing the oxidative burden on selenium (Khademzade et al., 2022). Vitamin C complements this system by regenerating the active forms of vitamin E and selenium, ensuring sustained antioxidant activity (Gutteridge, 1995). Together, these

nutrients form an integrated defense network, protecting cellular integrity, optimizing enzymatic functions, and promoting overall health and growth in fish. Their combined action highlights the importance of balanced supplementation in fish nutrition to mitigate oxidative stress and support immune function. Although considerable research exists on dietary antioxidants such as selenium, vitamin C, and vitamin E in various fish species, there is a lack of comprehensive data on the synergistic effects of these supplements in intensively reared *Hypophthalmichthys molitrix*. This species, commonly known as silver carp, is a significant aquaculture commodity in Asian countries, including Pakistan (Mushtaq et al., 2022). The present study aims to evaluate the impact of dietary combinations of selenium, vitamin C, and vitamin E on the growth performance and antioxidant status of intensively reared *Hypophthalmichthys molitrix*.

2 Materials and methods

2.1 Experimental site, research design, and farming practices

2.2 The protocols and procedures

The protocols and procedures for this study were approved by the Animal Use and Care Committee of the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan (Approval No. DR/175, dated 05-04-2022). The trial was conducted at the Fish Seed Rearing Unit, C-Block, Ravi Campus, Pattoki, UVAS. A total of 480 *Hypophthalmichthys molitrix* juveniles (mean weight: 20 ± 0.29 g) were randomly allocated to 24 aquaria (20 fish per 100 L), and the aquaria were assigned to eight dietary treatments in a completely randomized design (three replicates per treatment). The dietary treatments included: (1) a

basal diet without supplementation (Control), (2) supplementation with selenium (S), (3) supplementation with vitamin C (C), (4) supplementation with vitamin E (E), (5) selenium and vitamin C supplementation (SC), (6) selenium and vitamin E supplementation (SE), (7) vitamin C and vitamin E supplementation (EC), and (8) selenium, vitamin C, and vitamin E supplementation (SCE). The supplementation levels were 0.9 mg/kg for selenium, 300 mg/kg for vitamin C, and 100 mg/kg for vitamin E, based on previous studies (Khan et al., 2017; Mushtaq et al., 2022). The detailed research design is given below in Figure 1. The selenium source was selenomethionine (Selisseo[®] 2% Se, Europe), vitamin C was provided as L-ascorbic acid-2-phosphate (Vibrell[™] C, Kemin, USA), and vitamin E was supplied as OXABIOL[®] E (Madrid, Spain). The dosage calculations were made by considering the purity of respective supplement claimed by manufactures. To prepare the experimental diets, feed ingredients were ground to a particle size of 0.05 mm using a Kenwood grinder (AT284). The ingredients were thoroughly mixed (Kenwood, AT283), and dough was prepared by adding water. The dough was then extruded through a meat mincer (ANEX, AG 3060) to form 3 mm diameter pellets. The pellets were shade-dried to reduce moisture content to approximately 10% and stored in plastic bags until use. Diet proximate composition and feed ingredient analysis were conducted following AOAC guidelines (Horwitz, 1975). Fish were acclimatized to the experimental aquaria for two weeks before the start of the 12-week feeding trial. During the trial, fish were maintained in a controlled environment with dissolved oxygen levels of 5.8–7.3 mg/L, water temperatures of 24.9–28.7°C, and pH ranging from 7.4 to 8.6. The diets were fed ad libitum twice daily at 07:00 and 16:00 hours. Feed orts were collected and managed according to the methodology of Mushtaq et al (Mushtaq et al., 2022). to calculate feed intake. The aquaria were refilled with fresh water daily to maintain consistent water quality. The basal diet composition was based on a previous study by Mushtaq et al

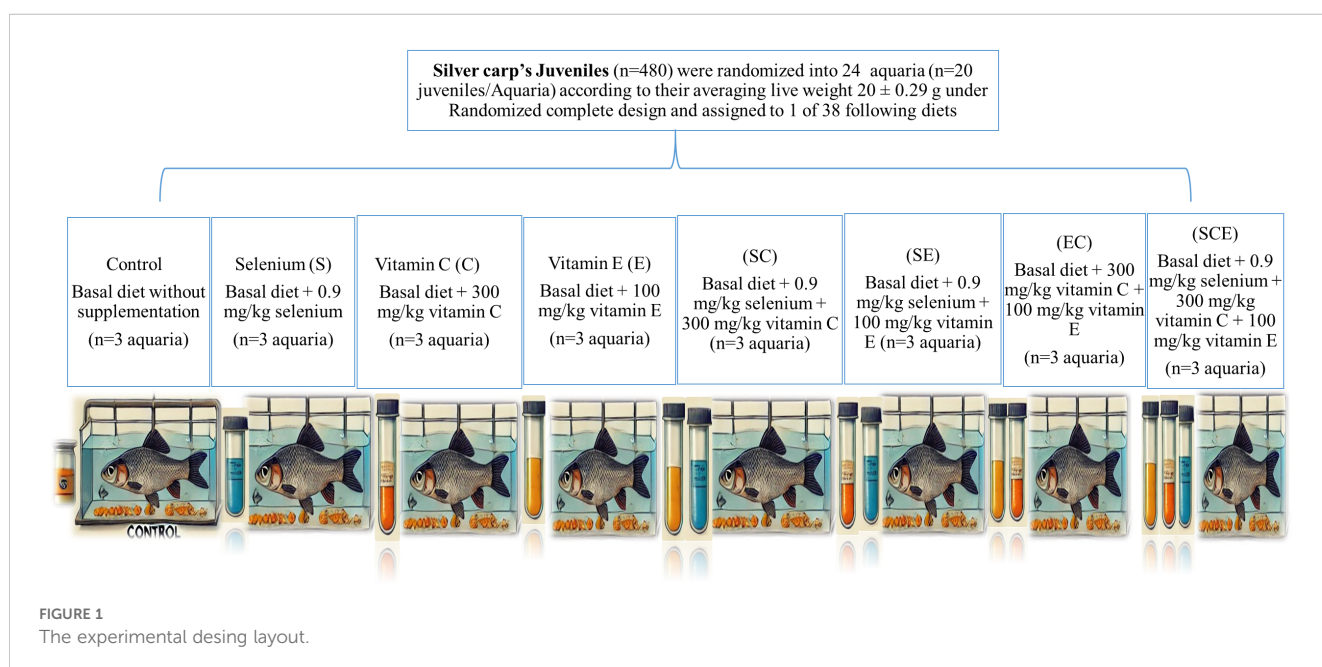


FIGURE 1 The experimental desing layout.

(Mushtaq et al., 2022), and the chemical composition on a dry matter basis is presented in Table 1. The basal diet consisted of the following ingredients on a dry matter basis: 20% fish meal, 20% soybean meal, 20% corn gluten meal-60, 15% rice polish, 14.5% wheat flour, 6% fish oil, 4% mineral-vitamin premixes, and 0.5% choline chloride. Growth performance

The fish length and live weight were recorded before the start, after every 2 weeks, and on the day of termination of the feeding trial. Growth parameters from the obtained values were calculated by using the Equations 1-4.

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)} \quad (1)$$

$$\text{Weight gain (\%)} = \left[\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \right] \times 100 \quad (2)$$

$$\text{Feed conversion ratio} = \left[\frac{\text{Feed intake on dry basis (g)}}{\text{Wet weight gain (g)}} \right] \quad (3)$$

$$\text{Specific growth rate} = \left[\frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{duration of trial (no. of days)}} \right] \times 100 \quad (4)$$

2.3 Sample collection, blood, and feed laboratory analysis

At the end of the feeding trial, 30 fish from each aquarium were randomly selected for weighing and anesthetized using tricaine methanesulfonate (MS-222) at a concentration of 150 mg/L, following the protocol described by Mushtaq et al. (Mushtaq et al., 2022). Blood samples were collected from 15 fish by puncturing the caudal vasculature. Samples intended for serum analysis were collected in plain vacutainers, centrifuged at 3000×g for 15 minutes, and the harvested serum was stored at -20°C for further analysis. Blood samples collected in EDTA-coated tuberculin syringes were analyzed for hematological parameters using an automated hematology analyzer (MEK-6550). Blood glucose concentrations were measured with commercial kits (21503, Biosystems, Barcelona, Spain). Ten fish from each aquarium were dissected to collect organs (kidney, liver, gills, and pancreas) for determining biological indices, while the remaining five fish were

minced (ANEX, AG 3060) for meat quality analysis. Collected organs and meat samples were sealed in labeled zipper bags and stored at -20°C until analysis. Dry matter contents of the feed and meat samples were determined by drying at 55°C for 48 hours in a forced-air oven. The dried samples were ground to a particle size of 1 mm using a Foss grinder (CT 293 Cyclotec, Denmark) and analyzed for crude protein content following AOAC Method 976.06. Fat content was determined using the Soxhlet procedure (Tecator, Hoganas, Sweden; AOAC Method 920.29). Ash content was measured by igniting the samples at 620°C for 3 hours in a muffle furnace.

2.4 Thiobarbituric acid reactive substances analysis

The muscle, liver, and serum samples were analyzed for TBARS following the protocol described in Mushtaq et al. (Mushtaq et al., 2022). Briefly, 1 g of each tissue sample was homogenized with 3 mL of holding solution (80 mM Tris-maleate and 11.5 g/L KCl; pH 7.4). The homogenate was mixed with 1 mL of 2 mM ascorbic acid and incubated at 37°C for 30 minutes to initiate lipid peroxidation. Subsequently, 5 mL of thiobarbituric acid (TBA) was added, followed by 5 mL of 200 g/L trichloroacetic acid. After boiling to develop the colorimetric reaction, the samples were cooled, refrigerated for 45 minutes, and centrifuged. The absorbance of the TBA complex was measured at 530 nm using a spectrophotometer.

2.5 Antioxidant enzymes assay

Two grams of muscle, liver, and whole-body tissues were sliced, homogenized with 6 mL of phosphate buffer (pH 7.4), filtered through Whatman No. 1 filter paper, and centrifuged at 10,000×g for 15 minutes at 4°C to collect the supernatant. TheSOD activity was measured by its ability to inhibit nitroblue tetrazolium (NBT) reduction, following the method of Giannopolitis and Ries (1977). Catalase activity was determined by the decomposition of hydrogen peroxide at 240 nm, as per Chance and Maehly (1955). TheGPx activity was assessed by measuring the residual hydrogen peroxide

TABLE 1 Basal diet compositional characteristics on a dry basis.

| Nutrients | Dietary treatments ¹ | | | | | | | |
|----------------------|---------------------------------|--------|--------|--------|--------|--------|--------|--------|
| | Control | S | C | E | SC | SE | EC | SCE |
| Dry matter (g/kg) | 896.91 | 898.63 | 896.25 | 897.23 | 894.01 | 892.03 | 895.51 | 898.21 |
| Crude protein (g/kg) | 315.90 | 316.01 | 315.85 | 316.00 | 315.87 | 315.90 | 315.99 | 316.02 |
| Crude fat (g/kg) | 123.01 | 123.05 | 123.21 | 123.25 | 123.03 | 123.02 | 123.18 | 123.12 |
| Crude fiber (g/kg) | 75.48 | 75.46 | 75.42 | 75.45 | 75.39 | 75.40 | 75.43 | 75.46 |
| Ash (g/kg) | 92.29 | 92.32 | 92.34 | 92.31 | 92.32 | 92.35 | 92.30 | 92.27 |

¹Treatments = Basal diet without supplementation (Control), supplementation with selenium (S), supplementation with vitamin C (C), supplementation with vitamin E (E), supplementation with both selenium and vitamin C (SC), supplementation with both selenium and vitamin E (SE), supplementation with both vitamin E and C (EC), and supplementation with selenium, vitamin C, and vitamin E (SCE). The supplementation levels for selenium, vitamin C, and vitamin E were 0.9 mg/kg, 300 mg/kg, and 100 mg/kg of the basal diet, respectively.

concentration at 470 nm. Serum ALT, AST, and ALP activities were analyzed using commercial kits (AL1205, AS3804, AP9764; Randox Laboratories Ltd).

2.6 Statistical analysis

The collected data were assessed for normality using QQ plots with IBM SPSS Statistics 20 (Chicago, USA). One-way analysis of variance (ANOVA) was conducted using the same software to evaluate the effects of dietary treatments. The statistical model included the fixed effects of treatments:

$$Y_{ij} = \mu + T_j + \epsilon_{ij}$$

Where Y_{ij} represents the observation, μ is the population mean, T_j denotes the treatment effect, and ϵ_{ij} is the residual error.

Statistical significance was determined at $p < 0.05$, with means compared using Tukey's test for multiple comparisons.

3 Results

3.1 Growth performance

The means and standard errors of the means for various growth performance parameters are summarized in Table 2. The recorded survival rates were 97% for the control group, 98% for the E-fed group, and 100% for all other treatments. As shown in Table 2, growth performance indicators, including final weight, body length gain, percentage body weight gain, and specific growth rate (SGR), were significantly higher ($p < 0.05$) in the SCE treatment compared to the other groups. In contrast, parameters such as feed intake,

initial body length, and initial body weight did not differ significantly among treatments ($p > 0.05$).

3.2 Hematology, serum enzymes, and blood metabolites

The means and standard errors of the means for hematological parameters, serum enzyme activities, and blood metabolites, including glucose concentrations, are presented in Table 3. The findings indicate that dietary supplementation with selenium (Se), vitamin C, and vitamin E significantly influenced ($p < 0.05$) hematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), and mean corpuscular hemoglobin concentration (MCHC) in juvenile *Hypophthalmichthys molitrix*. The SCE-fed group exhibited significantly higher ($p < 0.05$) values for these hematological parameters compared to other dietary treatments. Similarly, the activities of ALT, AST, and ALP varied significantly ($p < 0.05$) among treatments, with the lowest activities observed in the SCE-fed group. In contrast, blood glucose concentrations did not differ significantly ($p > 0.05$) across the treatments.

3.3 TBARS and antioxidant enzymes

As shown in Table 4, the means and standard errors of the means for TBARS values in muscle and serum were significantly higher ($p < 0.05$) in the control-fed fish group compared to those fed the other dietary treatments. Dietary supplementation with selenium, vitamin C, and vitamin E significantly enhanced ($p < 0.05$) the activities of SOD, CAT, and GPx in the whole body and

TABLE 2 Growth performance of juvenile *Hypophthalmichthys molitrix* fed on diets supplemented with methionine selenium, Vitamin C and E.

| Parameters | Dietary Treatments ¹ | | | | | | | |
|----------------------|---------------------------------|-------------------------------------|------------------------------|------------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|
| | Control | S | C | E | SC | SE | EC | SCE |
| Feed intake (g) | 29.53 | 31.20 | 31.52 | 31.44 | 31.23 | 31.67 | 31.28 | 31.35 |
| IBW ² (g) | 20.31 ± 0.31 | 20.26 ± 0.34 | 20.27 ± 0.31 | 20.29 ± 0.34 | 20.27 ± 0.26 | 20.30 ± 0.29 | 20.28 ± 0.36 | 20.31 ± 0.3 |
| FBW ³ (g) | 35.74 ± 2.14 ^{a,d} | 43.49 ± 3.14 ^b | 35.49 ± 3.14 ^{a,d} | 36.19 ± 3.14 ^{a,d} | 39.34 ± 3.14 ^c | 34.49 ± 3.14 ^a | 36.51 ± 3.14 ^d | 46.90 ± 3.11 ^e |
| Initial length (cm) | 9.89 ± 0.22 ^a | 9.79 ± 0.15 ^{a,b} | 9.77 ± 0.15 ^b | 9.76 ± 0.14 ^{b,c} | 9.79 ± 0.18 ^{a,b} | 9.79 ± 0.2 ^{a,b} | 9.83 ± 0.2 ^{a,b} | 9.86 ± 0.19 ^{a,b} |
| Final length (cm) | 17.19 ± 0.43 ^a | 19.49 ± 1.11 ^b | 16.26 ± 1.11 ^c | 18.06 ± 1.11 ^d | 20.96 ± 1.11 ^{e,f} | 21.15 ± 1.11 ^{e,f} | 20.65 ± 1.11 ^e | 21.45 ± 1.11 ^f |
| BWG ⁴ (g) | 15.44 ± 2.15 ^{a,d} | 23.23 ± 3.14 ^b | 15.21 ± 3.23 ^{a,d} | 15.90 ± 3.14 ^{a,d} | 19.07 ± 3.21 ^c | 14.19 ± 3.23 ^a | 16.23 ± 3.16 ^d | 26.59 ± 3.15 ^e |
| FCR ⁵ | 0.52 ± 0.07 ^a | .74 ^b ± 0.1 ^b | .48 ± 0.1 ^{a,d} | .051 ± 0.1 ^a | .61 ± 0.1 ^c | 0.45 ± 0.1 ^d | .52 ± 0.1 ^a | .85 ± 0.1 ^e |
| Weight gain (%) | 76.05 ± 10.76 ^{a,d} | 114.72 ± 15.7 ^b | 75.15 ± 16.37 ^{a,d} | 78.42 ± 15.59 ^{a,d} | 94.12 ± 16.19 ^c | 69.98 ± 16.28 ^a | 80.06 ± 15.83 ^d | 131.01 ± 15.91 ^e |
| SGR ⁶ (g) | 0.29 ± 0.03 ^{a,e,f} | .39 ± 0.04 ^b | .29 ± 0.05 ^{a,e,f} | 0.30 ± 0.05 ^{a,c} | .34 ± 0.04 ^d | 0.27 ± 0.05 ^e | 0.30 ± 0.05 ^{e,f} | .43 ± 0.04 ^f |
| Survival rate (%) | 97.00 | 100.00 | 100.00 | 98.00 | 100.00 | 100.00 | 100.00 | 100.00 |

¹Treatments = Basal diet without supplementation (Control), supplementation with selenium (S), supplementation with vitamin C (C), supplementation with vitamin E (E), supplementation with both selenium and vitamin C (SC), supplementation with both selenium and vitamin E (SE), supplementation with both vitamin E and C (EC), and supplementation with selenium, vitamin C, and vitamin E (SCE). The supplementation levels for selenium, vitamin C, and vitamin E were 0.9 mg/kg, 300 mg/kg, and 100 mg/kg of the basal diet, respectively.

²IBW, Initial body weight; ³FBW, Final body weight; ⁴BWG, Body weight gain; ⁵FCR, Feed conversion ratio; ⁶SGR, Specific growth rate.

^{a-f} superscripts within the same row show a significant difference between means and standard error of means presented in the row's cells at $p < 0.05$ while, cells within the same row containing shared subscript have no statistically significant difference ($p > 0.05$).

TABLE 3 Hematology, serum enzymes, and blood glucose analysis of juvenile *Hypophthalmichthys molitrix* fed on diets supplemented with methionine selenium, Vitamin C, and E.

| Parameters | Dietary Treatments ¹ | | | | | | | |
|-----------------------------------|---------------------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------|----------------------------|-----------------------------|-------------------------------|
| | Control | S | C | E | SC | SE | EC | SCE |
| White cells (10 ⁶ /μl) | 442.88 ± 0.97 ^a | 458.33 ± 2.08 ^a | 482.33 ± 0.58 ^b | 501.33 ± 0.58 ^c | 529.33 ± 0.58 ^d | 587 ± 0 ^e | 503.67 ± 0.58 ^c | 510.33 ± 16.17 ^c |
| Red cells (10 ⁶ /μl) | 1.77 ± 0.01 ^a | 2.05 ± 0.02 ^{b,d} | 1.88 ± 0.01 ^{a,b} | 2.36 ± 0.01 ^c | 1.95 ± 0.01 ^{a,b} | 2.08 ± 0.01 ^{b,d} | 1.94 ± 0.04 ^{a,b} | 2.23 ± 0.24 ^{c,d} |
| Hb (g/dl) ⁴ | 8.86 ± 0.01 ^{a,d} | 9.06 ± 0.01 ^a | 9.44 ± 0.01 ^b | 8.8 ± 0.07 ^{a,d} | 9.15 ± 0.01 ^{a,b} | 9.97 ± 0.02 ^c | 8.65 ± 0.28 ^d | 8.94 ± 0.2 ^{a,d} |
| HCT (%) ⁵ | 22.03 ± 0.01 ^a | 24.87 ± 0.01 ^{a,c} | 38.26 ± 0.01 ^{b,d} | 28.07 ± 0.01 ^{a,e} | 40.8 ± 0.01 ^b | 43.56 ± 0.01 ^b | 40.36 ± 0.11 ^b | 32.32 ± 7.35 ^{c,d,e} |
| MCHC (g/dl) | 26.51 ± 0.02 ^{a,c} | 26.16 ± 0.25 ^{a,c} | 27.06 ± 0.03 ^{a,d} | 28.44 ± 0.04 ^b | 26.71 ± 0.23 ^{a,c} | 28.17 ± 0.03 ^b | 25.65 ± 0.11 ^c | 27.91 ± 0.96 ^{b,d} |
| ALP (U/ml) | 15.33 ± 0.01 ^a | 17.72 ± 0.01 ^b | 24.21 ± 0.01 ^c | 24.18 ± 0.06 ^c | 23.57 ± 0.01 ^d | 23.33 ± 0.01 ^d | 21.26 ± 0.06 ^e | 24 ± 0.37 ^c |
| ALT (IU/ml) | 78.86 ± 0.02 ^a | 40.97 ± 0.01 ^b | 40.66 ± 0.01 ^b | 39.21 ± 0.01 ^c | 39.92 ± 0.01 ^d | 41.65 ± 0.02 ^e | 39.06 ± 0.03 ^c | 39.45 ± 0.41 ^c |
| AST (IU/ml) | 102.35 ± 0.02 ^a | 72.4 ± 0.05 ^b | 72.67 ± 0.34 ^{b,c} | 73.07 ± 0.02 ^{b,c,d} | 74.23 ± 0.01 ^{c,d} | 76.47 ± 0.01 ^e | 76.09 ± 0.01 ^{e,f} | 76.47 ± 1.06 ^{e,f} |
| Glucose (mg/dl) | 6.59 ± 0.01 | 6.23 ± 0.01 | 6.47 ± 0.01 | 5.63 ± 0.03 | 6.56 ± 0.02 | 6.61 ± 0.06 | 6.02 ± 0.02 | 6.53 ± 1.19 |

¹Treatments=Basal diet without supplementation (Control), supplementation with selenium (S), supplementation with vitamin C (C), supplementation with vitamin E (E), supplementation with both selenium and vitamin C (SC), supplementation with both selenium and vitamin E (SE), supplementation with both vitamin E and C (EC), and supplementation with selenium, vitamin C, and vitamin E (SCE). The supplementation levels for selenium, vitamin C, and vitamin E were 0.9 mg/kg, 300 mg/kg, and 100 mg/kg of the basal diet, respectively.

^{a-f} superscripts within the same row show a significant difference between means and standard error of means presented in the row's cells at $p < 0.05$ while, cells within the same row containing shared subscript have no statistically significant difference ($p > 0.05$).

muscle of the fish. However, changes in liver catalase activity were not statistically significant ($p > 0.05$) among the dietary treatments.

3.4 Meat chemical composition

The differences in means and standard errors of the means for carcass composition parameters, including dry matter, fat, ash, and protein contents, were not statistically significant ($p > 0.05$) among the treatments. These findings indicate that dietary supplementation with selenium (Se), vitamin C, and vitamin E did not affect the composition of fish meat (Table 5).

3.5 Discussion

In recent years, researchers have explored various nutritional strategies to enhance the growth performance of intensively reared aquaculture species, including fish, without compromising their health or meat quality. Among these strategies, the supplementation of selenium (Se), vitamin C, and vitamin E has garnered significant attention. This discussion delves into the potential effects of these supplements on fish growth performance, their mechanisms of action, and associated health benefits. Previous studies have demonstrated that optimizing dietary Se is crucial for improving growth performance and enhancing the antioxidant system in fish species (Hoffmann and Berry, 2008; Schrauzer and Surai, 2009). Similarly, enriching diets with vitamins C and E has been shown to result in better weight gain and overall health in fish (Harsij et al., 2020). Dietary Se increases its bioaccumulation in various tissues, facilitating the formation of selenocysteine, which plays a vital role in mitigating chronic stress conditions.

In the current experiment, the results indicate that the SCE-supplemented diet significantly improved growth performance

parameters such as body weight gain, specific growth rate, and feed conversion ratio in juvenile *Hypophthalmichthys molitrix*. These findings align with previous research on rainbow trout (Harsij et al., 2020) and tilapia (Dornelles Mello et al., 2013), which also reported enhanced weight gain and feed efficiency with diets supplemented with Se, vitamin C, and vitamin E. The superior growth performance observed in the SCE-fed group may be attributed to the additive or synergistic effects of these nutrients. Studies have revealed that simultaneous supplementation of selenium with either vitamin C or vitamin E yields synergistic benefits, enhancing growth and health outcomes (Ghazi Harsini et al., 2012; Shakeri et al., 2020). These effects can be explained by the complementary roles of these nutrients in the redox system, including the regeneration of antioxidant substances, acting as cofactors for redox enzymatic processes, reducing oxidative stress, neutralizing ROS, and protecting cellular structures to maintain integrity (Pisoschi et al., 2021).

Hematological health is influenced by various nutrients, hormones, diseases, and genetic factors (Onasanya et al., 2015). A balanced diet with diverse nutrients is essential for optimal hematological function (Pohlenz and Gatlin, 2014). In this study, hematological parameters such as WBC, RBC, HGB, HCT, and MCHC were significantly improved by individual supplementation and dietary combinations of selenium, vitamin C, and vitamin E. Research has shown that these nutrients, individually or in combination, enhance hematological parameters (Lu et al., 2016; Khan et al., 2017; Zehra and Khan, 2021). Selenium supports hematopoiesis, aiding the production and function of erythrocytes and leukocytes. It also facilitates heme synthesis, a crucial component of hemoglobin (Liao et al., 2018; Oliveira et al., 2018). Vitamin C enhances iron absorption, supporting red blood cell production (Bhadra and Deb, 2020; Godswill et al., 2020), while vitamin E prevents oxidative damage to red blood cell membranes, maintaining their structural integrity and reducing premature cell

TABLE 4 TBARS and antioxidant enzymes in different tissues of juvenile *Hypophthalmichthys molitrix* fed on diets supplemented with methionine selenium, Vitamin C, and E.

| Parameters | Dietary Treatments ¹ | | | | | | | |
|----------------------------|---------------------------------|-------------------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|-----------------------------|
| | Control | S | C | E | SC | SE | EC | SCE |
| TBARS Muscle (µg/mg) | 7.37 ± 0.12 ^b | 6.30 ± 0.2 ^a | 6.33 ± 0.06 ^a | 6.40 ± 0.1 ^a | 6.43 ± 0.06 ^a | 6.30 ± 0.17 ^a | 6.43 ± 0.12 ^a | 6.43 ± 0.06 ^a |
| TBARS Serum (µg/mg) | 5.33 ± 0.12 ^b | 3.37 ± 0.06 ^a | 3.27 ± 0.15 ^a | 3.40 ± 0.17 ^a | 3.20 ± 0.17 ^a | 3.20 ± 0.17 ^a | 3.47 ± 0.15 ^a | 3.17 ± 0.12 ^a |
| SOD Muscle (µ/mg) | 6.30 ± 0.35 ^a | 6.62 ± 0.20 ^b | 6.69 ± 0.01 ^{b,c} | 6.82 ± 0.00 ^c | 6.85 ± 0.2 ^{b,d} | 6.73 ± 0.06 ^{b,c} | 6.87 ± 0.00 ^{c,f} | 6.92 ± 0.00 ^{c,f} |
| SOD fish whole body (µ/mg) | 6.73 ± 0.26 ^a | 6.81 ± 0.02 ^b | 6.82 ± 0.02 ^{b,c} | 6.80 ± 0.14 ^{b,d} | 6.83 ± 0.16 ^{b,d} | 6.87 ± 0.12 ^c | 6.93 ± 0.12 ^{f,e} | 6.96 ± 0.05 ^f |
| SOD Liver (µ/mg) | 6.52 ± 0.02 | 6.67 ± 0.21 | 6.67 ± 0.00 | 6.67 ± 0.16 | 6.73 ± 0.26 | 6.63 ± 0.12 | 6.70 ± 0.10 | 6.67 ± 0.06 |
| CAT Muscle (µ/mg) | 70.78 ± 0.96 ^a | 72.71 ± 0.97 ^{c,b} | 72.07 ± 0.51 ^{c,b} | 71.76 ± 0.45 ^{a,c} | 71.13 ± 0.84 ^{a,c} | 72.96 ± 0.68 ^{c,b} | 74.87 ± 0.99 ^d | 78.13 ± 0.50 ^{d,f} |
| CAT Liver (µ/mg) | 76.71 ± 0.51 | 76.48 ± 0.12 | 76.54 ± 0.89 | 76.82 ± 1.06 | 75.88 ± 1.33 | 76.13 ± 0.54 | 75.55 ± 0.92 | 76.09 ± 0.66 |
| CAT fish body (µ/mg) | 62.93 ± 0.41 ^a | 63.10 ± 0.13 ^{a,b} | 67.75 ± 0.99 ^c | 69.84 ± 0.5 ^{c,d} | 68.38 ± 0.43 ^{c,d} | 68.90 ± 1.17 ^{c,d} | 72.95 ± 0.9 ^e | 75.57 ± 0.93 ^f |
| Per Oxidase Muscle (µ/mg) | 273.57 ± 36.95 ^a | 281.43 ± 106.48 ^b | 369.13 ± 45.02 ^c | 373.47 ± 17.64 ^{c,d} | 372.03 ± 15.17 ^{c,d} | 373.30 ± 71.0 ^{c,d} | 382.03 ± 83.82 ^c | 394.47 ± 95.69 ^f |
| Per Oxidase Liver (µ/mg) | 260.10 ± 30.68 ^a | 262.23 ± 73.22 ^{a,b} | 312.97 ± 63.72 ^c | 342.57 ± 93.7 ^d | 338.73 ± 71.52 ^{d,e} | 339.93 ± 55.86 ^{d,e} | 373.77 ± 101.89 ^f | 384.50 ± 45.20 ^f |

¹Treatments=Basal diet without supplementation (Control), supplementation with selenium (S), supplementation with vitamin C (C), supplementation with vitamin E (E), supplementation with both selenium and vitamin C (SC), supplementation with both selenium and vitamin E (SE), supplementation with both vitamin E and C (EC), and supplementation with selenium, vitamin C, and vitamin E (SCE). The supplementation levels for selenium, vitamin C, and vitamin E were 0.9 mg/kg, 300 mg/kg, and 100 mg/kg of the basal diet, respectively.

^{a-f} superscripts within the same row show a significant difference between means and stander error of means presented in the row's cells at *p*<.05 while, cells within the same row containing shared subscript have no statistically significant difference (*p* > 0.05).

lysis (Jena et al., 2023). Thus, these supplements promote erythropoiesis and minimize cellular breakdown (Khan et al., 2017).

Liver enzyme activities, including AST, ALT, and ALP, are key biomarkers for assessing liver health. In this experiment, these enzyme activities significantly decreased with increased dietary supplementation of selenium, vitamin C, and vitamin E. These findings align with previous studies (Li et al., 2014), which reported reduced enzyme activities with antioxidant supplementation. The decrease in ALT, AST, and ALP levels can be attributed to selenium's ability to restore proper enzyme function and reduce oxidative stress (Chen et al., 2022). Deficiencies in selenium or vitamins C and E are associated with elevated liver enzyme levels, indicating compromised liver health (Coz-Rakovac et al., 2005). Vitamins C and E, as potent antioxidants, protect liver cells from oxidative damage, normalize enzyme activities, and provide

hepatoprotective effects in certain liver conditions (Uboh et al., 2012). Thiobarbituric acid reactive substances are markers of lipid peroxidation, often indicative of oxidative stress (Niki, 2021). Elevated TBARS levels in the control group in this experiment suggest oxidative stress due to overcrowding. Antioxidant supplementation with selenium, vitamins C, and vitamin E reduced TBARS levels (Chan et al., 2021), demonstrating their efficacy in mitigating oxidative stress. The reduced activities of SOD, CAT, and GPx in the whole body and muscle tissues of fish fed the SCE diet can be explained by the neutralization of free radicals facilitated by these supplements. Selenium and vitamins C and E are essential micronutrients that act as antioxidants, contributing to cellular stability and protection against ROS (Rodrigo et al., 2005). Lastly, dietary supplementation with selenium, vitamin C, and vitamin E did not alter the body

TABLE 5 Meat chemical composition of juvenile *Hypophthalmichthys molitrix* fed on diets supplemented with methionine selenium, Vitamin C, and E.

| Parameters | Dietary Treatments ¹ | | | | | | | |
|-------------------|---------------------------------|----------------------------|---------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | Control | S | C | E | SC | SE | EC | SCE |
| Dry Matter (%) | 63.32 ± 7.22 ^a | 61.04 ± 10.23 ^a | 62.32 ± 7.23 ^a | 60.26 ± 12.66 ^a | 63.65 ± 5.46 ^a | 62.61 ± 5.61 ^a | 61.65 ± 6.46 ^a | 63.84 ± 6.64 ^a |
| Crude protein (%) | 17.24 ± 1.35 ^a | 16.45 ± 1.65 ^a | 16.22 ± 1.84 ^a | 17.21 ± 1.64 ^a | 17.41 ± 1.89 ^a | 16.47 ± 1.67 ^a | 17.28 ± 1.96 ^a | 16.87 ± 1.92 ^a |
| Fat (%) | 6.53 ± 1.99 ^a | 6.29 ± 1.64 ^a | 6.59 ± 1.96 ^a | 7.64 ± 1.62 ^a | 5.86 ± 1.64 ^a | 7.44 ± 1.82 ^a | 7.35 ± 1.61 ^a | 7.67 ± 1.35 ^a |
| Ash (%) | 6.63 ± 1.23 ^a | 6.23 ± 1.48 ^a | 6.28 ± 1.56 ^a | 5.64 ± 1.94 ^a | 5.58 ± 1.31 ^a | 5.91 ± 1.68 ^a | 5.94 ± 1.35 ^a | 5.43 ± 1.69 ^a |

¹Treatments=Basal diet without supplementation (Control), supplementation with selenium (S), supplementation with vitamin C (C), supplementation with vitamin E (E), supplementation with both selenium and vitamin C (SC), supplementation with both selenium and vitamin E (SE), supplementation with both vitamin E and C (EC), and supplementation with selenium, vitamin C, and vitamin E (SCE). The supplementation levels for selenium, vitamin C, and vitamin E were 0.9 mg/kg, 300 mg/kg, and 100 mg/kg of the basal diet, respectively.

^{a-f} superscripts within the same row show a significant difference between means and stander error of means presented in the row's cells at *p*<.05 while, cells within the same row containing shared subscript have no statistically significant difference (*p* > 0.05).

chemical composition of juvenile *Hypophthalmichthys molitrix*. These results are consistent with previous studies on other fish species (Harsij et al., 2020; Mushtaq et al., 2022).

3.6 Conclusion

This study demonstrates the significant benefits of dietary supplementation with selenium (Se), vitamin C, and vitamin E on the growth performance, hematological parameters, antioxidant status, and liver health of juvenile *Hypophthalmichthys molitrix*. Combined supplementation improved body weight gain, specific growth rate, and feed conversion ratio without altering body composition. Enhanced antioxidant enzyme activities (SOD, CAT, GPx) and reduced TBARS levels highlight their role in mitigating oxidative stress, while improved hematological indices and liver enzyme activities (AST, ALT, ALP) indicate overall health benefits. These findings support the strategic inclusion of Se, vitamin C, and vitamin E in aquafeeds to promote growth, health, and antioxidant defenses in aquaculture species, with further research needed to optimize dosages and evaluate long-term effects.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal studies were approved by the Animal Use and Care Committee of the University of Veterinary and Animal Science, Lahore, before the research trial (DR/175, dated 05-04-2022). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

HY: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Formal analysis, Funding acquisition. MM: Conceptualization, Investigation, Methodology, Visualization,

Writing – original draft, Project administration. SR: Investigation, Methodology, Writing – original draft, Formal analysis, Resources, Validation. UA: Formal analysis, Data curation, Visualization, Writing – review & editing. MK: Data curation, Visualization, Writing – review & editing, Conceptualization, Investigation, Methodology, Resources, Writing – original draft. AR: Conceptualization, Formal analysis, Resources, Supervision, Validation, Writing – review & editing, Writing – original draft. GQ: Software, Supervision, Validation, Writing – review & editing. MF: Conceptualization, Project administration, Resources, Supervision, Visualization, Writing – review & editing. SN: Conceptualization, Project administration, Resources, Supervision, Writing – review & editing. MZ: Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. MA: Conceptualization, Resources, Validation, Writing – original draft, Writing – review & editing.

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