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Research article

Modulatory Potential of Selenium Supplementations on the Growth, Nutrients Profile and Regulation of Antioxidant Status of Striped Catfish (*Pangasius Hypophthalmus*)

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Abstract

This study is designed to evaluate the impact of varying selenium levels on growth potential, nutrient profile and antioxidant enzymes activity in striped catfish. Fish were randomly allocated into 15 circular indoor tanks, each with a capacity of 2000 L, (100 (H) x 200 cm (D)) dimension, and were raised for 12 weeks. Fifteen fish were allocated to each tank across four separate treatment groups, each replicated three times, following an entirely randomized design. Five iso-nitrogenous diets (30% CP) were prepared from basal diet in which one was control and other four were supplemented selenomethionine (Se-met) with levels of 0, 0.5, 1.0, 1.5 and 2.0 mg/kg. Upon completion of the feeding trial, a diet containing Se at 1.5 mg/kg resulted in a significant increase ($P<0.05$) in weight gain (229.60±3.00) and SGR (0.92±0.23), along with a lower FCR (2.18±0.32). While, proximate composition remains non-significant ($P > 0.05$) against dietary Se feed. Antioxidant enzymes activity in liver and muscle found higher in Se1.5 diet and Se2.0 mg/kg diet in comparison to lower dietary Se treatments. Moreover, dietary Se1.5 and Se2.0 treatments values remained non-significant. Overall, the results indicated that the incorporation of Se-met at 1.5 mg/kg in the diet can improve growth performance and antioxidant status in *P. hypophthalmus.*

Keywords: Striped catfish, growth, proximate composition, antioxidant enzymes

1. Introduction

Aquaculture production is increasing day by day to fulfill human demand for food and to reduce capture fisheries. Fish is an excellent source of vital fatty acids, high-quality protein, primarily long-chain polyunsaturated fatty acids, and various micronutrients (Se, phosphorus, and iron) [1]. However, capture production is higher than aquaculture, but in the last 20–30 years, aquaculture production has been progressively increasing, while capture production has almost steadied [2]. Production of high-quality fish is depending upon good quality feed and feeding methods to meet the dietary requirements of fish species. The demand for quality feed is increasing to ensure the species better production [3].

Trace elements are also essential for health, proper growth, and biochemical function. Se is a necessary element for animal and human nutrition. Se exists in both organic and inorganic forms, both of which have limited bioavailability, adherence properties, and solubility. Therefore, incorporating Se nanoparticles into aquafeed is strongly recommended to enhance the health and productivity of aquatic animals [4]. Se enhances metabolism and energy production, improving the physiological functions and overall development of fish. It is crucial for maintaining a healthy immune system, making fish more resistant to disease and environmental stress [5]. Se had enzymatic and structural functions, such as a catalyst for the production of active thyroid hormone and antioxidants [6]. Because Se helps in antioxidant defense and protects organelles and cellular membranes from peroxidative damage. Dietary Se has two main resources: organic and inorganic, which are both added to the commercial diet of fish [7]. Overall, organic minerals had a higher rate of retention and absorption in feed than the inorganic form due to higher bioavailability for striped bass and channel catfish [8]. Se has been used for studying the antioxidant and immune responses of fish. The study of Swain et al., (2019) suggested that Se significantly influences fish growth, making it crucial for sustainable aquaculture practices. Adequate Se intake leads to better overall growth in various fish species. The addition of Se in fish feed has been linked to enhance feed efficiency, weight gain, and growth rate in various studies [9]. Previous studies have revealed that the inclusion of Se in the diet of fish results in improvement of cell-mediated and humoral responses [10,11]. The observed increase in growth rates aligns with findings from Biller-Takahashi et al., who reported similar benefits of Se supplementation in pacu (*Piaractus mesopotamicus*) [12]. Different ratios of organic Se supplementation have been investigated for growth rate, muscle composition, and oxidative stress in farmed rainbow trout. It was recommended that 3 mg/kg Se supplementation is more effective for proper growth and hepatic antioxidant enzyme activity in rainbow trout [13].

Striped catfish is native to Thailand and Vietnam and most popular in inland fisheries in the world. Catfish is the best source of protein, vitamins, and mineral elements, which have enormous values due to their good impact on human health. These minerals are very essential because iron and calcium are necessary for the childhood growing stage and maintain optimal bone development [14]. Production cost of pangasius has increased due to high input cost, and farmers have incurred loss of 9% during the COVID Pandemic [15,16]. Farmed production of this species has been estimated to be 500,000 tonnes in 2021, with Vietnam being the biggest producer, representing 40% of the global farmed striped catfish. Although there is a growing interest in this fish species in countries such as Pakistan, functional feeds/feed additives are still needed for the optimization of fish health/welfare, growth, and feed efficiency [17].

Keeping in view the importance of the growing demand for striped catfish (*Pangasius hypophthalmus*) production and consumption worldwide and in Pakistan, there is a dire need to develop a nutritionally formulated diet to enhance its production performance in the country. The current study is therefore planned to determine the growth potential, nutrient profile, antioxidant enzymes response in striped catfish when provided with varying levels of dietary Se.

2. Materials and methods

2.1 Experimental setup

The pangasius fingerlings (n = 225; 100.78 g weight) were gained from fish ponds of the Department of Fisheries and Aquaculture, Research and Training Facilities (UVAS, Ravi Campus, Pattoki, District Kasur, Pakistan) and acclimatized into hatchery tanks for 2 weeks. Fish were randomly allocated into 15 circular cemented indoor tanks of 2000 L capacity (100 cm height x 200 cm diameter), and were raised for 12 weeks Forty-five fingerlings were allocated to each treatment group that were distributed into three replicates, following an entirely randomized design. The fish were fed twice a day (08:00-15:00) according to 3% of fish body weight. Water quality parameters were checked on a daily basis; the dissolved oxygen (6.0±0.09 mg/L), water temperature (17.5.0±0.20 °C), pH (7.2±0.05), and total dissolved solids (1280±0.03 mg/L) were noted. Water quality parameters were monitored with digital meter (HANNA) (HI-98194), and controlled throughout the study by exchanging 30% water from indoor tanks on daily basis.

2.2. Experimental diets formulation and preparation

Five iso-nitrogenous diets (30% CP) were prepared from a basal diet, of which one was the control and the other four were supplemented with graded levels of feed additive/oxidant such as selenomethionine (Se-met). The range of organic form of selenomethionine was used 0, 0.5, 1.0, 1.5, and 2.0 mg/kg (Control, Se0.5, Se1.0, Se1.5, and Se2.0). The supplemented Se level was used according to NRC (National Research Council) recommendations [18]. The European Union recommends a Se dose of 0.1-2.00 mg/kg in fish feed, as exceeding this can lead to negative consequences. Therefore, specific dosages of Se had been used in this study [19]. The composition of ingredients and nutrient profile of the diet are given in Table 1. The feed ingredients were sourced from Aqua Feeds Pvt Ltd., measured as per the formulation, and processed to 1.0 mm particle size. All feed ingredients were properly mixed with an electric mixer. The pellets (2.0 mm diameter) were made and dried. The resulting experimental diets were put in plastic zipper packets and kept in a -20 °C refrigerator before use.

2.3. Growth performance and feed utilization

Prior to stocking, the initial weight of all the fish were noted. Upon completion of the feeding trial, the fish were counted, and weighed for the following calculations.

$$\text{Survival rate} = \text{No. of survived fish/Initial no. of fish} \times 100$$

Where NGW indicates net weight gain

$$\text{NWG} = \text{Average final Wt (g)} - \text{Average intial Wt (g)}$$

$$\text{SGR} = \frac{\text{In (Final wet body weight} - \text{intial wet body weight)} \times 100}{\text{No. of days}}$$

Where SGR represent specific growth rate

$$\text{FCR} = \text{Feed given (g)/Wet weight gain (g)}$$

FCR indictats feed conversion ratio

2.4. Proximate composition

After 12-week of trial, both the formulated feed and the experimental fish underwent analysis for crude fat, moisture, crude protein, and ash. The valuation was conducted following the procedure by the Association of Official Analytical Chemists [20].

2.5. Antioxidant enzymes activity

At the completion of the investigation, two fish from each replicate were anesthetized using clove oil at a concentration of 50 ml/L. The fish were then dissected, and the tissues, *viz*., liver and muscles, were immediately removed.

Table 1. Diet formulation and proximate composition of the basal diet (%, dry weight).

Diet formulation	
Fish meal	30.00
Corn gluten	10.00
Soybean meal	15.00
Rice polish	13.00
Sunflower oil	6.00
Wheat flour	22.00
Vitamin premix	1.00
Mineral premix	1.00
Nutritional composition	
Moisture	9.00
Protein	29.22
Lipid	8.54
Ash	9.87.
Fibre	3.20
Energy, MJ/kg	20.82

These tissues were homogenized in 9 volumes of 20 mM phosphate buffer (pH 7.4), with EDTA (1 mM), and Triton X-100 (0.1%). The homogenates underwent centrifugation for 10 minutes (860 x g at 4 °C), after which the supernatant was preserved at -80 °C [21].

Catalase (CAT; EC 1.11.1.6) activity was assessed by measuring the reduction in concentration at 240 nm, following the method described by Chance and Mehaly (1977) [22]. Glutathione peroxidase activity (GSH-px EC 1.11.1.9) was assessed based on the procedure outlined by Flohe and Gunzler (1984) [23]. Superoxide dismutase activity (SOD; EC 1.15.1.1) was noted by computing its ability to avoid the photo reduction of nitroblue tetrazole (NBT) [24].

2.6. Statistical analysis

Data were first tested for normality and homogeneity of variance. Results were presented as mean ± standard error (n=3). One-way ANOVA was carried data to check the possible effects of the dietary Se, followed by Duncan's Multiple Range Test (SAS 9.1 Software package, Cary, North Carolina, U.S) [25].

3. Result

3.1. Growth performance

At the end of the 12-week feeding trial, the supplemented diets significantly enhanced growth rate and feed utilization as compared to control diet in striped catfish ($P<0.05$; Table 2). The significantly higher net weight gain (127.80 g), SGR (0.92 %) and lowest FCR (2.18) were observed in fish fed with Se1.5 diet ($P<0.05$). The survival rate remained non-significantly different ($P>0.05$).

3.2. Proximate body composition

The values of moisture content, crude protein, and crude fat were found non-significantly different in all tested diets fed to fish ($P>0.05$; Table 3). However, ash contents in control diet were found to be significantly lower (1.26 %) than other dietary groups range from 1.50 - 1.66% ($P<0.05$).

3.3. Antioxidant enzymes activity

Antioxidant enzymes activities were significantly improved with supplemented feed when compared to the control group in fish liver and muscle ($P<0.05$, Table 4). In fish liver samples, catalase (CAT) activity showed a significantly increasing trend in both the Se2.0 diet (76.43 U/mg) and Se1.5 diet (75.27 U/mg), then followed by Se1.0 (65.06 U/mg), Se0.5 (67.46 U/mg), and in the control group (56.52 U/mg) ($P<0.05$). The glutathione peroxidase activity (GPX) was significantly elevated in the Se1.5 diet (53.25 U/mg) as compared to control (34.67 U/mg) ($P<0.05$). While superoxide dismutase activity (SOD) was found significantly ($P<0.05$) higher in the Se1.5 diet (34.45 U/mg) and lower in the Se0.5 diet (29.29 U/mg) and control (20.10 U/mg).

Table 2. Growth performance and feed utilization of striped catfish fed with graded levels of Se (mg/kg) for 12-weeks.

	Test diets				
	Control	**Se0.5**	**Se1.0**	**Se1.5**	**Se2.0**
Initial mean weight; g/fish	101.86±0.15	101.13±0.24	101.22±0.01	101.80±2.18	101.87±1.20
Final mean weight; g/fish	202.56±1.41^d	209.67±2.62^c	213.23±2.60^b	229.60±3.00^a	212.67±3.45^b
Mean weight gain; g/fish	100.90±3.21^e	108.54±3.50^d	112.01±4.23^b	127.80±1.35^a	110.80±3.56^c
SGR; %	0.76±0.04^d	0.80±0.02^c	0.84±0.70^b	0.92± 0.23^a	0.89±0.07^a
FCR; g g^{-1}	2.42±0.12^d	2.34±0.07^c	2.35±0.10^c	2.18±0.32^a	2.27±0.09^b
Survival; %	93.40±0.03^a	93.40±0.06^a	95.00±0.60^a	95.00±0.70 ^{a}	95.00±0.50^a

Note: Mean ± SD, n=3 Results with distinct superscripts within the same row indicated as significant difference ($P<0.05$).

Table 3. Proximate composition of striped catfish fed with graded levels of Se (mg/kg) for 12-weeks.

	Test diets				
	Control	Se0.5	Se1.0	Se1.5	Se2.0
Moisture	74.16±0.25	74. 13±1.02	73. 87± 0.23	74.08±0.60	73.97±0.34
Crude protein	18.24±0.25	18.21±1.23	18.38±0.52	18.32±0.43	18.98±063
Crude fat	4.42±0.32	4. 23±0.22	4.39±0.32	4.06±0.21	4.56±1.34
Ash	1.26±0.43^{b}	1.59±0.05^{a}	1.66±0.05^{a}	1.50±0.60^{a}	1.65±0.45^{a}

Note: Mean±SD, n=3. Results with different superscripts on the same row indicated as significant difference (P<0.05).

Table 4. Antioxidant enzyme activity in liver and muscle of striped catfish fed with graded levels of Se (mg/kg) for 12-weeks.

	Test diets				
	Control	Se0.5	Se1.0	Se1.5	Se2.0
Liver; U/mg					
CAT	56.52±1.05^{c}	67.46±0.56^{b}	65.06±.21^{b}	75.27±1.37^{a}	76.43±1.23^{a}
GPx	34.67±0.47^{d}	43.60±2.07^{c}	49.08±1.41^{b}	53.25±1.25^{a}	46.32±0.89^{b}
SOD	20.10±0.31^{c}	29.29±0.83^{b}	30.71±0.43^{b}	34.45±0.32^{a}	33.78±0.87^{a}
Muscle; U/mg					
CAT[1]	35.01±2.15^{d}	39.32±0.65^{c}	43.47±0.10^{b}	49.66±2.29^{a}	49.87±1.34^{a}
GPx[2]	27.87±4.44^{e}	28.81±3.65^{d}	34.77±1.97^{c}	38.18±2.20^{b}	40.32±1.23^{a}
SOD[3]	18.73±1.40^{d}	19.28±0.83^{c}	25.45±0.23^{b}	28.02±0.26^{a}	25.98±1.76^{b}

Note: Mean±SD, n=3. Values with different superscripts on the same row indicated as significant difference (P<0.05).

In fish muscles, CAT activity was found higher in both Se2.0 and Se1.5 dietary groups. The maximum GPx enzyme activity was observed in Se2.0 (40.32 U/mg) then Se1.5 diet (38.18 U/mg) over control treatment (27.87 U/mg). In addition, SOD activity was greater in Se1.5 (28.02 U/mg) folloed by Se2.0 (28.02 U/mg) diet fed to fish than control (18.73 U/mg).

4. Discussion

Trace elements played an important role in many biological systems, such as growth, survival rate, and physiological events in organisms. The mineral determination in fish seems to be difficult because both waterborne and dietary minerals have to be considered during estimating mineral budgets. When fish are reared in cages and indoor systems, they cannot move freely to be fed on natural feed (e.g., algae, aquatic invertebrates, and aquatic plants) [26]. In contrast, the addition of supplements in diets helps to support natural food available in the ponds and raceways. Although a supplemented diet does not provide full complements of minerals, they help to fortify available diets with extra carbohydrates, proteins, and lipids. Minerals aid in bone formation, regulate osmotic balance, and are also components of hormones and enzymes in the fish body (e.g., Se and Zn) [27].

Various feed components are sources of Se, which are commonly included in feed stuff for cultured fish. Growth performance and feed utilization, such as weight gain, specific growth rate, and feed conversion ratio, can be used as parameters to evaluate the optimal range of dietary elements [28]. According to the present study, a higher growth rate of pangasius was noticed in the Se1.5 dietary group; increasing dietary Se content did not influence the growth rate. Studies reveled that feeding rainbow trout with dietary selenomethionine (4.6 mg/kg) for 12-weeks significantly decreased the weight gain and length [29]. This trend is similar to the present work because dietary Se increased growth up to the optimum range. In contradict to the present study, the effect of dietary Se levels on black seabream showed that weight gain, FCR, SGR, and FE were found to be not significantly different among treatments [30].

The physical and chemical properties of feed are increased by adding supplementation, which ultimately results in increased fish production [26]. In the present study, proximate composition remains non-significant, as a similar study of feeding Se supplementation did not affect the proximate composition of zebrafish [31].

Fish fed with excess Se levels also cause oxidative stress; therefore, it's imperative to determine the dietary Se requirements in catfish. Miller et al., (1993) reported that liver antioxidant enzymes like CAT, GPx, and SOD are biomarkers of oxidative damage [32]. According to Portner (2002), increased levels of antioxidant enzymes result in reducing oxidative stress [33]. While Se is essential for GPx activity, aiding in the protection against oxidative damage and facilitating the catalytic oxidation of GSH to oxidized glutathione [34]. Se concertation improved the antioxidant enzyme activity of tissues samples in juvenile black seabream fed to 1.29 mg Se/kg; beyond increasing its level, it caused toxic effects (12.3 mg/kg) [30]. Knowledge about Se content in fish feed can boost profitability for the feed industry by enhancing farmer's confidence and demand for fish feed. The feed industry can mitigate disease outbreaks and enhance fish welfare by offering Se-enriched feeds. Se can enhance fish health and ensure secure food production, despite several countries implementing restrictions on Se concentration in fish feed. Several studies showed that Se concentration greater than 2.0 kg had negative consequences on fish health [35]. To ascertain the juvenile gibel carp (*Carassius auratus gibelio*) requirement for Se (Se), six diets were prepared and supplemented with Selenomethionine (0, 0.1, 0.25, 0.5, 1.0, 2.5, and 5 mg Se/kg). The results indicated that the dietary Se requirement for gibel carp is 1.18 mg Se/kg diet concluded on the improved weight gain, SOD, and GPx activities. In relating to the present result, higher antioxidant activity was noticed at a 1.5 mg/kg diet of SeMet [36].

The ideal amount of Se for fish development is 1.5 mg/kg. This will help aquaculture practitioners to prevent excessive costs as well as any possible side effects from over supplementation. The dosage encourages cost-effective feeding techniques that support fish health and growth by maintaining a balance between cost and performance.

5. Conclusion

Se is the important nutritional component that facilitates in improved fish health. Among five experimental treatments, Se 1.5 mg/kg diet exhibited best growth and improved antioxidative capacity. Further increasing its level did not influence striped catfish growth and health. This study reveals that Se supplementation has ideal characteristics and may be a dietary source of a natural antioxidant agent in fish feed. The aquaculture practitioners can use Se as a feed ingredient and the results of the study indicate that fish development and health can be enhanced by incorporating 1.5 mg/kg of Se in fish feed, without requiring higher dosages. The ideal dosage facilitates the development of economical feeding strategies that promote fish health and growth.

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Data Availability statement

The data supporting the results of this study can be obtained from the corresponding author upon request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors Contribution

Sheeza Bano conducted the research, collected, and analyzed the data, and wrote the manuscript. Noor Khan, supervised, edited, and reviewed. Sadia Nazir, Moazama Batool formal analysis, edited and reviewed. Dilawar Hussain, Ayesha

Tanveer, Maryam Tahir, writing, editing, and review. Simon J. Davies, reviewer, writing and editing.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

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