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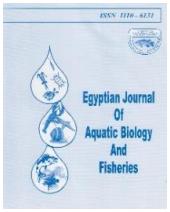

Selenium Nanoparticles Enhance Growth, Health, and Gene Expression in the Nile Tilapia

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ABSTRACT

The present study examined the impacts of two levels of selenium nanoparticles (SeNPs) on the Nile tilapia growth, survival, hematological and biochemical profiles, immunity, antioxidant activity, expression levels of growth-related genes, and histometric parameters of skeletal muscles. One hundred eighty monosex fingerlings (7.08± 0.08g) were randomly used to form three groups, with four replicates in each group. The first group was the control, which received a basal diet. The second group (treatment 1, T1) received 0.7mg/ kg diet SeNPs, while the third group (treatment 2, T2) was given 1.0mg/ kg diet SeNPs. The results indicated a growth enhancement in T2 ($P< 0.05$). In addition, a significant improvement in the feed conversion ratio was noticed in both T1 and T2 ($P< 0.05$). A longer mean survival time was demonstrated in T2 ($P< 0.05$). Significant improvements in the haematobiochemical parameters, immunological response, and antioxidant parameters in T2 fish were evident ($P< 0.05$). The expression of three growth-related genes, including growth hormone receptor, myostatin, and myogenin, were upregulated in T2. In contrast, the expression of myogenic factor 6 was downregulated in T2. Moreover, the histometric analysis revealed significantly larger muscle fibers in T2 than in the control and T1 ($P< 0.05$), indicating hypertrophy in T2. Additionally, the skeletal muscles from T1 had 19.38% more muscle fibers than T2 and 2.5% more muscle fibers than the control ($P= 0.001$). The current research not only indicated the positive impacts of SeNPs on the economic traits of the Nile tilapia but also investigated the underlying mechanisms responsible for these improvements on the cellular and molecular levels.

INTRODUCTION

Selenium is a vital trace element that enhances various physiological responses in fish, including the immune system, growth, production of hormones, and fertility. In

addition, it prevents cell damage by protecting the fish from free oxygen radicals **(Pecoraro *et al.*, 2022)**. Selenium can be present in chemical substances that are either organic or inorganic, but they have low water-solubility, permeability, and availability for biological and metabolic processes **(Ren *et al.*, 2021)**. Recently, the use of selenium nanoparticles (SeNPs) in aquatic diets has received more interest due to their rapid bioavailability, feed efficiency, and antibacterial potential **(Ghaniem *et al.*, 2022)**.

Previous studies indicated that beneficial effects of SeNPs in the Nile tilapia were achieved at concentrations ranging from 0.5- 1.5mg/ kg diet. Feeding the Nile tilapia (*Oreochromis niloticus*) diets enriched with SeNPs improves growth performance and feed utilization **(Sheikh *et al.*, 2023)**. Besides, SeNPs significantly enhance the immune response in the Nile tilapia. This was evidenced by the increased phagocytic activity, lysozyme activity, phagocytic index, and immunoglobulin M (IgM) **(Ghazi *et al.*, 2022)**. Additionally, SeNPs elevate the expression of immune-related genes, such as the tumor necrosis factor-alpha in the liver and spleen **(Al-Deriny *et al.*, 2020)** and interleukin-1 beta **(Iqbal *et al.*, 2020)**. Furthermore, SeNPs positively impacted biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, total serum protein, cholesterol, and triglycerides **(Rathore *et al.*, 2023)**. Similarly, SeNPs increase hematological parameters of the Nile tilapia, including hemoglobin (Hb) content, hematocrit values (HCT), count of red blood cells (RBCs), white blood cells (WBCs), and lymphocytes **(Joshna *et al.*, 2023)**. Finally, SeNPs enhance antioxidative responses by boosting the activity of several enzymes, while reducing lipid peroxidation through decreased malondialdehyde (MDA) levels **(Neamat-Allah *et al.*, 2019; Ni *et al.*, 2023)**.

Several factors regulate the growth and development of skeletal muscles. Among these molecules are the myogenic regulatory factors (MRFs), paired box proteins, insulin-like growth factor 1 (IGF-1), myostatin (MSTN), and growth hormone (GH). The MRFs include four transcription factors: Myogenic determination gene (*myod*), myogenin (*myog*), myogenic regulatory factor 4 (*mrf4*), and myogenic factor 5 (*myf5*). Each gene of the MRFs has evolved to control a particular stage of myogenesis. For example, *myod* and *myf5* are crucial for muscle cells since they regulate the growth and specialization of myogenic precursor cells into myoblasts **(Carrio *et al.*, 2015)**, whereas *myog* and *myf6* regulate myoblast differentiation into myotubes **(Zhao *et al.*, 2019)**.

MSTN acts as a negative regulator of myogenesis, preventing the division and differentiation of myoblast cells **(Khalil *et al.*, 2017)**. In the Nile tilapia, two versions of *mstn* genes are present, *mstna* and *mstnb* **(Wu *et al.*, 2023)**. Studies revealed that *mstnb* is expressed in muscles, whereas *mstna* is predominantly expressed in the brain of the Nile tilapia. Researchers utilized the CRISPR/Cas9 system to mutate the *mstnb* gene and successfully induced skeletal muscle hypertrophy in the Nile tilapia, resulting in increased body size and faster growth **(Wu *et al.*, 2023)**. Antisense RNA was introduced into the Nile tilapia eggs to knock down *mstn* expression. This resulted in a decrease in

MyoD and Myf5, leading to an imbalanced proliferation and differentiation of myoblasts, causing hyperplasia and increased adipogenesis in muscle tissue **(Wang *et al.*, 2023)**. GH promotes myogenic cell proliferation, hyperplasia, and hypertrophy **(Johnston *et al.*, 2009)**. GH binds to a growth hormone receptor (GHR) in the targeted tissue, triggering a signaling phosphorylation cascade **(Shimizu *et al.*, 2007)**. In the teleost fish, a positive correlation between the growth rate and hepatic *ghr*-1 mRNA levels was reported **(Ruan *et al.*, 2011)**.

Previous studies indicated that SeNPs could significantly improve the growth of the Nile tilapia; however, the relationship between SeNPs and myogenesis in the Nile tilapia remains largely unexplored. Only a few studies investigated the impact of SeNPs on the expression of myogenesis-related genes in fish, such as the *igf*-1 and *igf*-2 in seabream (*Sparus aurata*) **(AbdEl-Kader *et al.*, 2023)**. Information on the effect of SeNPs on myogenesis key genes in the Nile tilapia is limited. Additionally, the mechanism by which SeNPs enhance the growth rate in the Nile tilapia, whether hyperplasia or hypertrophy of muscle fibers, is not clearly comprehended. Therefore, the primary objective of the current study was to evaluate the effects of dietary supplementation of SeNPs on the Nile tilapia myogenesis and myogenesis-related gene expression, including *ghr*, *mstn*, *myog*, and *myf6*. The secondary objective was to assess the impact of SeNPs on the Nile tilapia growth, biochemical indicators, hematological profile, oxidative stress, disease resistance, and immune responses.

MATERIALS AND METHODS

1. Selenium nanoparticles

SeNPs were purchased from NaQaa Nanotechnology Network, Giza, Egypt. These nanoparticles were prepared by a chemical reduction method to achieve a particle size of 20nm, as verified by the transmission electron microscope evaluation. The precursor salt sodium selenite (99% Na_2SeO_3) was reduced by ascorbic acid, then the prepared SeNPs were coated with dextrin **(Malhotra *et al.*, 2014)**.

2. Experimental diet

Using a basal diet, three diets containing three SeNP dosages were prepared: 0.0, 0.7, and 1.0mg SeNPs/ kg diet. The ingredients were thoroughly mixed, pelleted with a laboratory pellet machine, air-dried at room temperature, and stored in plastic bags in a refrigerator till usage. The composition of the basal diet was 32% crude protein and 3,000 kcal digestible energy/ kg diet **(Abdelrazek *et al.*, 2017)**.

3. Experimental design

One hundred eighty monosex of the Nile tilapia fingerlings (7.08 ± 0.08 g) were used in the current study. The fish were acquired from the WorldFish Center, El-Abbassa,

Abou-Hammad, Sharkia, Egypt, and transported to the experimental aquaria in tanks provided with oxygen. Immediately after arrival, the fish were placed in glass aquaria filled with dechlorinated water and supplied with continuous aeration by a central air blower. The fish received the basal diet for three weeks to adapt to the new environment. At the beginning of the experiment, fish were randomly assigned to 12 glass aquaria (three groups, four replicates, 15 fish per replicate) (Fig. 1). Each glass aquarium (60 × 30 × 40cm) contained 60L of water. For six weeks, each group was fed its experimental diet twice a day (8.00 AM and 4.00 PM) till satiation, and daily feed consumption was recorded. Using aerated and dechlorinated water, one-third of each tank was replaced twice daily. The remaining feed and feces were siphoned with the changed water. The experimental parameters included water temperature of 26- 30°C, ammonia of less than 0.2mg/ L, a pH of 6.4, nitrite at 0mg/ L, total hardness of 144mg/ L, undetectable free chlorine, and total alkalinity of 120mg/ L. The lighting program was 12 hours of light and 12 hours of darkness. In the fourth week of the study, some fish in the control and T1 groups exhibited skin hemorrhages and fin and tail rot due to infection. Samples were taken from the affected groups and examined at the Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The appropriate antibiotic treatment (nalidixic acid/ciprofloxacin) was administered with feed at a rate of 5mg/ kg diet, in addition to a daily potassium permanganate bath for 2 minutes until mortality stopped. The antibiotic treatment and potassium permanganate bath were administered to all groups. Dead fish were collected and weighed, and the time of death was recorded.

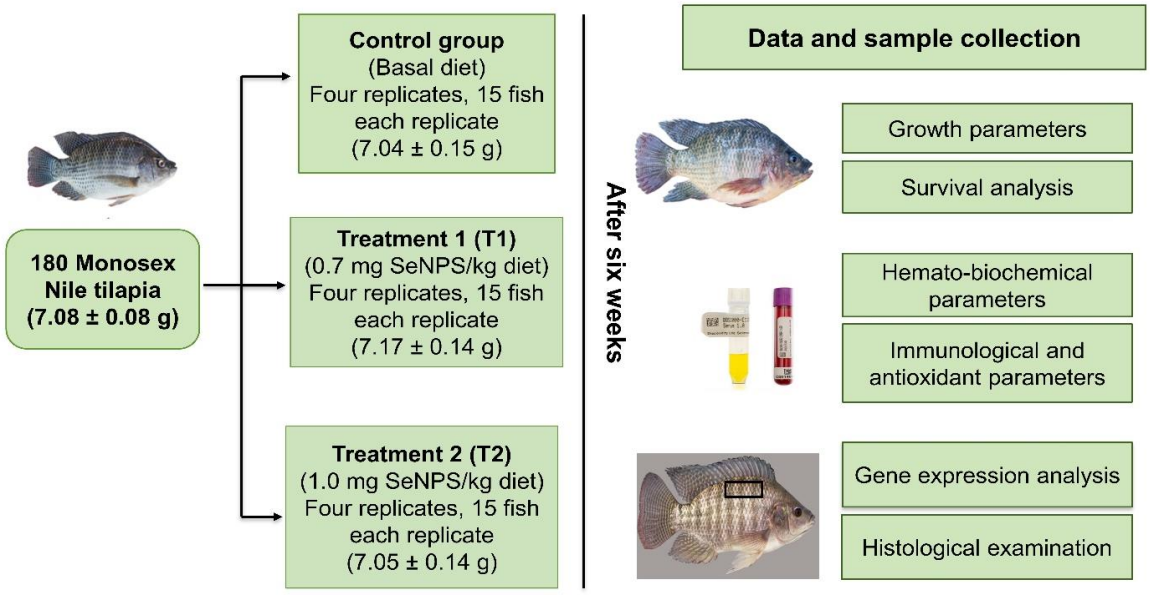

Fig. 1. Experimental design of the current study

4. Sample collection

After six weeks of feeding on experimental diets, fish were anesthetized using 25mg/ L tricaine methanesulfonate (MS-222). Then, the fish were weighed individually to obtain the final weight. For assays requiring whole blood, three fish per group had their caudal vein blood drawn onto EDTA. Blood was also collected in tubes without anticoagulant for serum preparation (three fish/group). Then, samples were centrifuged at 3000rpm/ 15min at 4°C to separate serum, which was then frozen at −20°C until use. For gene expression analysis, skeletal muscle samples from the anterior one-third of the dorsal musculature (Fig. 1) were collected from three fish per group on Cellixi-RNA Guard reagent (CelliXiza Biotechnology Company, Egypt) and frozen at -20°C until use. For histological analysis, skeletal muscle specimens were dissected from the same site (15 fish, five per group) and immediately fixed in a 10% neutral buffered formalin until analysis.

5. Growth parameters

As demonstrated below, the fish growth performance parameters were computed in accordance with **Abu-Elala *et al.* (2021)**:

$$\text{Body weight gain (BWG)} = \text{Final body weight (FBW)(g)} - \text{Initial body weight (IBW)(g)}$$

$$\text{Weight gain rate (WG\%)} = \frac{\text{FBW} - \text{IBW}}{\text{IBW}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{BWG (g)}}$$

$$\text{Specific growth rate (SGR\%/day)} = \frac{100\ (\ln \text{FBW} - \ln \text{IBW})}{\text{Number of days}}$$

6. Survival analysis

The analysis of survival rate and mean survival time was conducted according to the method of **Khalil *et al.* (2023)**. The survival rate was computed using the formula below:

$$\text{Survival rate} = \frac{\text{Total number of fish at the end of the experiment}}{\text{Total number of fish at the beginning of the experiment}} \times 100$$

We hypothesized that the fish that died soon after disease symptoms were less resistant than those that died after several days. All fish were included in the survival analysis. Dead fish were assigned a value representing the day of death, while alive fish were assigned a value of 42, representing the day when the experiment ended. Then, the Kaplan-Meier test was employed to calculate the mean survival time and evaluate the difference between the survival curves of the control and treatment groups. Afterward, the log-rank test was used for pairwise comparisons.

7. Hematological parameters

RBCs were counted microscopically using a hemocytometer after dilution with a normal saline solution **(Dacie & Lewis, 1980)**. Hb content was determined using a commercial kit from Bio-Lab Diagnostics Ltd. (Mumbai, India). Microhematocrit capillary tubes centrifuged at 14,800 ×g for three minutes were used to measure HCT **(Dacie & Lewis, 1980)**. Blood indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were determined using standard formulae of **Lee *et al.* (1998)**.

$$\mathrm{MCV} = (\mathrm{PCV}/\mathrm{RBC}) \times 10, \mathrm{MCH} = (\mathrm{Hb}/\mathrm{RBC}) \times 10, \text{and } \mathrm{MCHC} = (\mathrm{Hb}/\mathrm{PCV}\%) \times 100$$

The total leucocytic count was determined using a hemocytometer after dilution with 0.1% Leishman stain in 2% glacial acetic acid solution **(Dacie & Lewis, 1980)**. For the differential leucocyte count, thin blood films were spread on clean microscope slides and then stained with Leishman stain, and cells were counted according to **Houwen (2000)** using × 1000 oil-immersion light microscope to estimate the percentage of heterophils, lymphocytes, monocytes, eosinophils, and basophils. The total platelet count was determined using a 1% ammonium oxalate solution **(Dacie & Lewis, 1980)**.

8. Biochemical parameters

AST, ALT, serum glucose, triglycerides, cholesterol, and creatinine were assessed using kits from CliniChem Co. (Budapest, Hungary). Fish serum total proteins and albumins were measured following **Doumas *et al.* (1971)**, while globulin content was computed mathematically.

9. Immune and oxidative stress responses

IgM and immunoglobulin G (IgG) were determined using Fish IgM ELISA Kit and Fish IgG ELISA Kit, respectively (SunLong Biotech Co., LTD, China). Superoxide dismutase (SOD) level was measured in serum using kits from Cusabio Biotech Co. (Wuhan, Hubei Province, China) following the manufacturer's instructions. The activity of MDA was determined using the Fish MDA ELISA Kit (AFG Bioscience LLC, Northbrook, IL, USA).

10. Total RNA extraction, cDNA synthesis, and real-time PCR

Three skeletal muscle specimens per group were homogenized in TriQuick reagent (Beijing Solarbio Science & Technology Co., Ltd, China) using a tissue homogenizer. Then, total RNA was extracted according to the manufacturer's instructions. Gel electrophoresis was conducted to verify RNA integrity. The yield and purity of RNA were inspected by a UV1100 spectrophotometer (TechComp, Hong Kong). The RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Lithuania) was utilized for first-strand cDNA synthesis. Then, the cDNA was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Lithuania). Primers were designed using the primer-BLAST tool and synthesized by BIONEER Inc.

(Alameda, CA, USA) (Table 1). Real-time PCR was performed using Maxima SYBR Green qPCR Master Mix (2×), with ROX solution provided (Thermo Fisher Scientific, Lithuania) in a StepOnePlus real-time PCR machine. The PCR reaction included 8.5µL PCR-grade water, 12.5µL SYBR green, 1.5µL forward primer, 1.5µL reverse primer, and 1µL cDNA (≤ 500ng). The cycling conditions were initial denaturation for 8min at 95°C, 40 cycles of 95°C for 15s, 57°C for 30s for *ghr*, *mstn*, and *myog* genes, and 60°C for 30s for *myf6* gene, and 72°C for 30s. The product specificity was verified by assessing the melting curve (95°C for 15s, 60°C for 1min, and 95°C for 15s). Three technical replicates were performed for *mstn* and *myf6* genes, while two replicates were conducted for *ghr* and *myog*. Elongation factor 1a (*ef1a*) was used as a reference gene for data normalization **(Yang *et al.*, 2013)**. The real-time PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method **(Livak & Schmittgen, 2001)**.

Table 1. Gene-specific primers used in the current study

Gene*	Accession number	Primer sequence (5'-3')	Annealing temperature (°C)	Amplicon length (bp)
ghr	NM_001279455.1	F: ACATCAATCCTGGGCGGTC	57	165
		R: TAGTGGGGAGCAGTTAGAAGACA		
mstn	XM_003458832.5	F: AATGATGGCAACTGAACCTGAT	57	217
		R: CAAGGAGCGGATTCGTATGTG		
myog	NM_001279526.1	F: GAGGAGCACGCTGATGAACC	57	181
		R: TGACGACGACACTCTGGGC		
myf6	NM_001282891.1	F: TGGACGAGCAGGAGAAAACC	60	223
		R: CCTCACTGACTGCTGTCGTT		
ef1a	NM_001279647.1	F: CTTCAACGCTCAGGTCATCA	The same as the tested gene.	120
		R: ATCTTCTCAACCAGCTCGCT		

*Gene abbreviations: growth hormone receptor (*ghr*), myostatin (*mstn*), myogenin (*myog*), myogenic factor 6 (*myf6*), elongation factor 1a (*ef1a*), forward (F), and reverse (R).

11. Histological evaluation of skeletal myofibers

The skeletal muscle specimens (five/group) were processed and examined according to **Husseiny *et al.* (2021)**. Briefly, specimens were gradually dehydrated, cleared, embedded in paraffin, and cut into sections of 5- 7µm in thickness using a rotatory microtome. Two sections from each sample were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) stain, then inspected under a bright field microscope with a camera. Six photos of fixed cross-section areas were taken from different fields of each slide, and the numbers of cells were counted using the "Cell Counter" option of the ImageJ software. Additionally, the average size (µm²), total area (µm²), and percentage area of myofibers were also calculated using the same software program **(Schneider *et al.*, 2012)**.

12. Statistical analysis

The data were inspected for ANOVA assumptions, including the normality and homogeneity of variance. One-way ANOVA and duncan's multiple range test were employed for normally distributed data. For non-normally distributed data, the Kruskal-Wallis test with pairwise comparisons was used. The statistical analyses were conducted using SPSS version 25 software (IBM Corporation, Armonk, NY, USA). The significance was set at a probability value (*P*) of less than 0.05 and data were reported as the mean ± standard error (SE).

RESULTS

1. The effect of SeNPs on growth parameters

After the six-week rearing experiment, dietary SeNPs significantly improved the FBW, BWG, WG %, and SGR in T2 ($P< 0.05$) in contrast with T1 and the control. The FCR of the Nile tilapia was significantly improved ($P= 0.01$) in both T1 and T2 in comparison with the control (Table 2).

Table 2. The effect of SeNPs on growth performance and feed utilization in the Nile tilapia

Parameter	Control	T1	T2	*P*-value
Initial body weight (g)	7.04^{a} ±0.15	7.17^{a} ±0.14	7.05^{a} ±0.14	0.77
Final body weight (g)	32.91^{b}±1.27	34.98^{b}±1.49	38.62^{a}±0.92	0.005
Body weight gain (g)	25.88^{b} ± 1.28	27.77^{b}±1.53	31.58^{a}±0.94	0.005
Weight gain (%)	382.44^{b}±22.38	404.21^{ab}±26.00	464.59^{a}±18.46	0.03
Specific growth rate (%)	3.64^{b}±0.12	3.69^{b}±0.13	4.05^{a}±0.08	0.02
Feed conversion ratio	1.25^{a}±0.02	1.01^{b}±0.07	1.03^{b}±0.04	0.01

* In the same row, means with different superscript letters are significantly different (one-way ANOVA, $P< 0.05$). The control group received 0.0 SeNPs. T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet. Values are expressed as mean ± SE.

2. The effect of SeNPs on survival

The survival rate was higher in treatment groups than in the control, but the difference was not significant ($P = 0.605$, Table 3). The mean survival time was significantly longer in T2 in contrast with the control ($P = 0.005$, Fig. 2).

Table 3. The effect of SeNPs on survival rate and mean survival time of the Nile tilapia

Group	Total number	Dead fish		Alive fish		Survival rate Mean (%) ± SE	Survival time* Mean (Days) ± SE
		N	Percent	N	Percent		
Control	60	19	31.7%	41	68.3%	68.33±22.99	36.57^{b} ±1.09
T1	60	13	21.7%	47	78.3%	78.33±7.39	39.82^{ab}±0.59
T2	60	7	11.7%	53	88.3%	88.33±5.69	40.85^{a}±0.49

* In the same column, means with different superscript letters are significant, Kruskal-Wallis test, (P< 0.05). Data were expressed as the mean ± standard error. The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet.

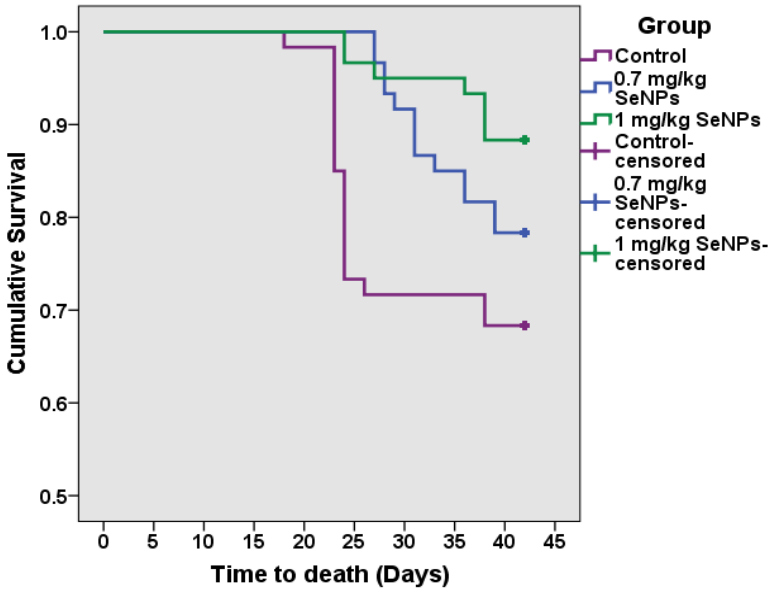

Fig. 2. Survival curves for the effect of SeNPs on survival time in the Nile tilapia. The control group (n=60) received 0.0 SeNPs, T1: treatment 1 (n=60) received 0.7mg SeNPs/ kg diet, and T2: treatment 2 (n=60) received 1.0mg SeNPs/ kg diet. Censored represents the alive fish at the end of the experiment

3. The effect of SeNPs on hematological profile

SeNPs resulted in significant differences in RBC count, Hb, HCT percent, and platelets count (P< 0.05) in comparison with the control, with the highest values observed in T2 (Table 4). The differences in MCV, MCH, and MCHC were not significant in the two treatments and control (P> 0.05).

Table 4. Effect of SeNPs on hematological parameters of the Nile tilapia

Parameter*	**Control**	**T1**	**T2**	***P*-value**
Red blood cell count ($\times10^{12}$/L)	$2.83^{c}\pm0.02$	$2.91^{b}\pm0.02$	$3.14^{a}\pm0.02$	< 0.001
Hemoglobin concentration (g/dL)	$8.47^{c}\pm0.05$	$8.74^{b}\pm0.04$	$9.41^{a}\pm0.06$	< 0.001
Hematocrit (%)	$25.44^{c}\pm0.14$	$26.25^{b}\pm0.11$	$28.23^{a}\pm0.17$	< 0.001
Mean corpuscular volume (fL)	89.79±0.07	90.49±0.27	89.91±0.09	0.08
Mean corpuscular hemoglobin (pg)	29.90±0.03	30.07±0.84	29.98±0.03	0.13
Mean corpuscular hemoglobin concentration (g/dL)	33.28±0.02	33.29±0.03	33.31±0.12	0.50
Platelets ($\times10^{9}$/L)	$45.35^{c}\pm0.38$	$46.71^{b}\pm0.24$	$50.52^{a}\pm0.43$	< 0.001

* In the same row, means with different superscript letters are significant. One-way ANOVA was used for RBCs, Hb, HCT, MCHC, and platelets. Kruskal-Wallis test was utilized for MCV and MCH. The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet.

4. The effect of SeNPs on WBCs and differential leucocytic count

Significantly higher WBCs and lymphocyte percentages were noticed in the two treatment groups, with the highest counts in T2 ($P< 0.05$). The neutrophil percentage was significantly higher in the control compared to the treated groups. Monocytes, basophils, and eosinophils were not significant among all groups ($P> 0.05$, Table 5).

Table 5. Effect of SeNPs on white blood cells (WBCs) and differential leucocytic count

Parameter*	**Control**	**T1**	**T2**	***P*-value**
WBCs ($\times10^{4}$/L)	$29.27^{c}\pm0.51$	$39.23^{b}\pm0.27$	$45.82^{a}\pm0.52$	< 0.001
Lymphocytes (%)	$71.67^{b}\pm0.88$	$74.33^{b}\pm0.67$	$80.33^{a}\pm0.88$	0.001
Neutrophils (%)	$20.33^{a}\pm0.88$	$17.33^{b}\pm0.33$	$10.33^{c}\pm0.67$	< 0.001
Monocytes (%)	4.33±0.33	5.33±0.33	5.33±0.33	0.14
Eosinophils (%)	3.33±0.67	3.00±0.58	3.00±0.00	0.87
Basophils (%)	0.33±0.33	0.00±0.00	1.00±0.00	0.06

*In the same row, means with different superscript letters are significant. One-way ANOVA was used for WBCs, lymphocytes (%), neutrophils (%), and eosinophils (%). For monocytes (%) and basophils (%), the Kruskal-Wallis test was used. The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet). Values were reported as the mean ± SE.

5. The effect of SeNPs on biochemical indicators

ALT, AST, cholesterol, triglycerides, total protein, albumin, and globulin were significantly elevated in T2 than in T1 and the control ($P< 0.05$). Glucose and creatinine were not significant between groups ($P> 0.05$, Table 6).

Table 6. The effect of SeNPs on biochemical indicators in the Nile tilapia

Parameter*	Control	T1	T2	*P*-value
Aspartate aminotransferase (U/L)	18.76^{b}±0.19	19.02^{ab}±0.11	19.57^{a}±0.21	0.04
Alanine aminotransferase (U/L)	6.72^{b}±0.12	6.89^{ab}±0.12	7.35^{a}±0.17	0.04
Total protein (g/dL)	3.82^{c}±0.04	4.06^{b}±0.05	4.39^{a}±0.06	0.001
Albumin (g/dL)	2.32^{c}±0.03	2.52^{b}±0.04	2.8^{a}±0.07	0.001
Globulin (g/dL)	1.51^{b}±0.01	1.53^{b}±0.02	1.59^{a}±0.17	0.02
Cholesterol (mg/dL)	68.93^{c}±0.38	73.26^{b}±0.69	80.86^{a}±1.17	< 0.001
Triglycerides (mg/dL)	81.73^{c}±0.36	87.07^{b}±0.79	95.05^{a}±1.55	< 0.001
Glucose (mg/dL)	58.91±0.19	58.87±0.16	59.27±0.25	0.36
Creatinine (mg/dL)	0.39±0.00	0.39±0.00	0.40±0.01	0.10

*In the same row, means with different superscript letters are significant. The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0 mg SeNPs/ kg diet. Values were presented as mean ± SE.

6. The effect of SeNPs on immunity and oxidative response of the Nile tilapia

IgG levels increased significantly in T1 and T2 than the control ($P< 0.05$, Table 7). No significant difference in IgM levels was detected between groups ($P> 0.05$). Diet supplementation with SeNPs significantly elevated SOD and reduced MDA levels than the control ($P< 0.05$). T2 had the highest SOD levels. The MDA activity of the treated groups was significantly lower than the control (Table 7).

Table 7. Effect of SeNPs on the immune and oxidative responses in the Nile tilapia

Parameter*	Control	T1	T2	*P*-value
Immunoglobulin G (ng/mL)	2.35^{c}±0.04	2.71^{b}±0.08	3.66^{a}±0.08	< 0.001
Immunoglobulin M (pg/mL)	622.80±2.01	622.07±1.99	623.70±0.70	0.80
Superoxide dismutase (ng/mL)	1.90^{c}±0.04	2.27^{b}±0.05	2.78^{a}±0.06	< 0.001
Malonaldehyde (pg/mL)	2.07^{a}±0.06	1.78^{b}±0.04	1.44^{c}±0.04	< 0.001

*In the same row, means with different superscript letters are significant (one-way ANOVA). The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet. Values were expressed as mean ± SE.

7. The effect of SeNPs on the expression of myogenesis-related genes

The expression level of *ghr* increased by 1.65 folds in T1 and 5.04 folds in T2. Additionally, the *mstn* expression increased by 11.50 folds in T1 and 20.66 folds in T2. Moreover, the *myog* gene expression level increased by 1.25 folds in T2 compared with no change in T1. On the contrary, the expression level of *myf6* decreased to 0.18 folds in T1 and 0.90 folds in T2 (Table 8).

Table 8. The effect of SeNPs on the fold change in gene expression of *ghr*, *mstn*, *myog*, and *myf6*

Gene*	Control	T1	T2
Ghr	1.00	1.65±1.10	5.04±2.54
Mstn	1.00	11.50±8.20	20.66±15.17
Myog	1.00	1.00±0.23	1.25±0.77
myf6	1.00	0.18±0.09	0.90±0.81

*The control group received 0.0 SeNPs, T1: treatment 1 received 0.7 mg SeNPs/ kg diet, and T2: treatment 2 received 1.0 mg SeNPs/ kg diet. Values were expressed as mean ± SE. *ghr*: growth hormone receptor, *mstn*: myostatin, *myog*: myogenin, *myf6*: myogenic factor 6.

8. Morphometrics of skeletal muscles

The skeletal muscles of the Nile tilapia from T1 had 19.38% more muscle fibers than T2 and 2.5% more muscle fibers than the control (P = 0.001). T2 exhibited 14.13% fewer muscle fibers than the control. A significant difference in the average size of muscle fibers was detected between all groups, with the largest size in T2 and the smallest in the control group (P< 0.001). The total area of muscle fibers increased significantly in T2 compared with the other two groups (P< 0.001). In addition, T1 had a larger total area of muscle fibers than the control group (P< 0.001). T2 had the highest percentage of muscle fiber area, followed by T1 and the control (Table 9, Fig. 3).

Table 9. The effect of SeNPs on the number, average size (µm²), total area (µm²), and percent area of muscle fibers from the skeletal muscles of the Nile tilapia

Parameter*	Control	T1	T2
Number	71.50^{a} ±1.99	73.30^{a}±2.30	61.40^{b}±1.90
Average size (µm²)	$2{,}965.06^{c}$±126.46	$4{,}147.89^{b}$±280.72	$5{,}894.99^{a}$±260.29
Total area (µm²)	$2{,}548{,}849.00^{c}$±8,740.20	$2{,}596{,}980.60^{b}$ ± 9,788.77	$2{,}659{,}703.40^{a}$ ± 12,375.08
% Area	55.31^{c}±1.32	60.67^{b}±1.09	66.07^{a}±0.76

*In the same row, means with different superscript letters are significant (P≤ 0.001; one-way ANOVA). The control group received 0.0 SeNPs, T1: treatment 1 received 0.7mg SeNPs/ kg diet, and T2: treatment 2 received 1.0mg SeNPs/ kg diet. Values are expressed as mean ± SE.

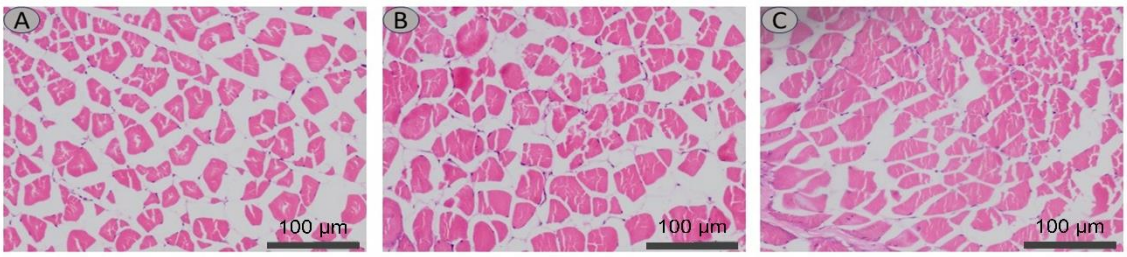

Fig. 3. Representative photomicrograph of H&E stained cross-section area from skeletal muscles from **(A)** the control fish, **(B)** T1, **(C)** T2. The control group received 0.0 SeNPs, T1 received 0.7mg SeNPs/ kg diet, and T2 received 1.0mg SeNPs/ kg diet. Scale bar = 100µm

DISCUSSION

The current study revealed that a 1.0mg SeNPs per kg diet enhanced the growth performance. Earlier studies documented the positive effect of SeNPs with a 1.0mg/ kg diet dose on growth **(Ghaniem *et al.,* 2022; Sheikh *et al.,* 2023)**. This improvement could be attributed to different reasons, such as the increased intestinal absorption and improved bioavailability of SeNPs **(Lee *et al.,* 2016)**. Selenium is a precursor for synthesizing selenoproteins that contribute to the high protein levels in the intestinal villi, improving the digestive enzyme activity, feed efficiency, metabolism, and growth performance **(Mehdi *et al.,* 2013)**. Additionally, selenium increases the intestine microbial population and activity as well as protease activity, which improves protein utilization and digestibility. **Ibrahim *et al.* (2021)** linked the growth-promoting effects of SeNPs to the increase in the mucosal length and width of the intestinal epithelium, which increases the surface area for nutrient absorption within the digestive tract.

In the current experiment, both treatment groups displayed about 21% improvement in FCR in comparison with the control fish, which aligns with the findings of **Abu-Elala *et al.* (2021)**. The FCR is a principal indicator in fish farming since it shows how well the fish can digest the feed. According to **Dunham and Elaswad (2018)**, the cost of aquaculture feeds surpasses half of the variable operating costs. Consequently, the observed improvement in FCR with SeNP supplementation highlights its potential economic benefits for tilapia aquaculture.

The present results revealed that dietary SeNPs at 1.0mg/ kg increased the survival time and decreased the mortality rate compared with control fish. Such findings agree with **Sheikh *et al.* (2023)**, who demonstrated an improvement in the Nile tilapia survival due to a SeNP dose of 1.0mg/ kg.

Blood parameters are beneficial indicators for the general health of animals, as they can be affected by factors such as nutrition, infection, and stressors. In this study, the T2 group had higher levels of Hb content, RBC count, HCT, platelets count, WBC count, and lymphocytes than T1. These results coincide with the previous research on the Nile tilapia fed similar SeNP dosage (1.0mg/ kg) **(Abu-Elala *et al.,* 2021; Rathore *et al.,* 2023)**. Additionally, higher RBC count, HCT, MCV, and MCH were noticed in the genetically improved farmed tilapia (GIFT) fed 0.75mg/ kg SeNPs **(Joshna *et al.,* 2023)**. Concurrently, **Ibrahim *et al.* (2021)** indicated that the Nile tilapia fed dietary SeNPs (0.8mg/ kg) had the highest value of HCT, Hb, RBCs, and WBCs than the tilapia fed either bulk-selenium or control diet. These findings are connected with the regulation of metabolic rate stimulated by SeNPs, as the higher the number of RBCs and Hb content, the more the availability of oxygen in body tissues **(Parrino *et al.,* 2018)**. The antioxidant properties of selenium protect RBCs and extend their lifespan by guarding them against reactive oxygen species (ROS), preventing membrane disruption, cell hemolysis, and degeneration, and maintaining the health and integrity of erythrocytes **(Ashouri *et al.,***

2015). Furthermore, the fish in the T2 group exhibited a higher WBC count and lymphocyte percentage. An increased WBC count indicates a stronger immune system.

Serum biochemical indices are crucial indicators of physiological responses. In the current study, the treatment fish showed the highest levels of AST and ALT, which is similar to **Ashouri *et al.* (2015)**, who stated that SeNPs (1- 2mg/ kg) elevated AST and ALT levels in common carp. The present study demonstrated significantly higher total serum protein, albumin, and globulin levels in the two SeNP treatments, with even higher levels in T2 than in the control fish. Similar findings were documented in the Nile tilapia **(Rathore *et al.*, 2021)**. **Eissa *et al.* (2023)** reported higher serum globulin levels in the Nile tilapia fed SeNPs. The current findings showed no significant difference in glucose levels between experimental groups, demonstrating the potential of selenium in alleviating stress and optimizing the physiological conditions of fish **(Kumar *et al.*, 2020)**.

This study revealed a higher level of IgG in T2, which was the only group not infected by the bacterial disease and had the longest mean survival time. This result is consistent with a study by **Ghazi *et al.* (2021)**, who reported a higher immune response, especially IgM activity and disease resistance, with SeNPs in the Nile tilapia. **Neamat-Allah *et al.* (2019)** concluded that SeNPs increased the resistance to *Aeromonas hydrophila* in the Nile tilapia, as well as the level of IgM. In the goldfish (*Carassius auratus*), selenium enhanced the immunity, lysozymes, and IgM **(Choi *et al.*, 2013)**. Selenium also protects B lymphocytes by stimulating GSH-Px **(Combs Jr & Combs, 1986)**. B-lymphocyte production plays a vital role in improving lysozyme activity and boosting immunity **(Fazio *et al.*, 2013)**. Here, the T2 group had more lymphocytes, which differentiate and produce immunoglobulins (IgM, IgG, and IgA), explaining why this group had a defense mechanism against disease.

Oxidative stress occurs in aquatic animals during stressful environmental conditions and pathogenic invasions, releasing harmful free radicals and ROS **(Martínez-Álvarez *et al.*, 2005)**. This can result in higher lipid peroxidation, imbalancing ROS production and removal, ultimately affecting the lipid content of cell membranes and damaging RNA **(Brewer, 2011)**. Fortunately, aquatic animals have natural enzymatic and non-enzymatic antioxidant defenses. Enzymes like SOD and MDA are biomarkers of oxidative stress **(Atencio *et al.*, 2009)**. SOD enzymes may be induced by ROS production to get rid of free radicals and reduce lipid peroxidation injury. MDA results from the breakdown of lipid peroxides, which can lead to body damage **(Talas *et al.*, 2008)**. The current findings displayed an improvement in the activity of SOD and a decrease in MDA, which are consistent with previous studies **(Eissa *et al.*, 2023)**.

In this study, SeNPs upregulated the expression of *ghr*, *myog*, and *mstn* and decreased the expression of *myf6*. In the teleost fish, a positive correlation between the growth rate and hepatic *ghr*-1 mRNA levels was reported **(Ruan *et al.*, 2011)**, supporting the use of *ghr* gene as an indicator of growth. The current results pointed out that SeNP

supplementation in T2 increased the expression level of *ghr* by 5.038 folds and T1 by 1.65 folds in contrast with control fish. Similarly, **Fasil *et al.* (2021)** found that SeNPs increased the expression of *gh* in the zebrafish muscles. **Abd El-Kader *et al.* (2020)** reported an upregulation of *gh* expression in the European sea bass fed on a SeNP diet. In the gilthead sea bream, *ghr2* expression in the liver and adipose tissue was lower than *ghr1*, but there was no difference in skeletal muscle expression **(Saera-Vila *et al.*, 2005)**. The opposite was detected in several black sea bream tissues, including the muscle, where *ghr2* expression surpassed *ghr1* **(Jiao *et al.*, 2006)**. These results may be due to utilizing minerals in their nano form, which may change their characteristics.

Fish skeletal muscle grows post-embryonically through hypertrophy or hyperplasia, as well as through the activity of undifferentiated myosatellite cells **(Johnston *et al.*, 2009)**. In myogenesis, myogenin regulates muscle cell differentiation and the growth of satellite cells **(Comai & Tajbakhsh, 2014)**. Satellite cells, when activated, undergo differentiation and cell division to produce new muscle fibers **(Berberoglu *et al.*, 2017)**, contributing to muscle hyperplasia and hypertrophy. Myogenin is necessary for myocyte fusion, which enables an increase in nuclear number and subsequently a development in the typical fiber size. Additionally, myogenin restricts the size of the myonuclear domain in both juvenile and adult muscles **(Ganassi *et al.*, 2018)**. The current study demonstrated that *myog* expression increased by 1.248-fold in T2 than in the control or T1 groups. A similar result was explained in the study of **Gao *et al.* (2018)**, where selenium increased the satellite cell proliferation and incorporation into muscle fiber cells, as well as the expression of *myod* and *myog*.

MYF6 controls the expression of genes specific to muscles, regulating myogenesis and muscle regeneration **(Lassar *et al.*, 1989)**. **Moretti *et al.* (2016)** reported that MYF6 negatively regulates adult skeletal muscles, as evidenced by the increase in muscle fiber size observed in adult skeletal muscle following the knockdown of *the myf6* gene. Higher expression of muscle-specific genes and protein synthesis explain the increase in muscle fiber. **Zou *et al.* (2015)** employed the single-stranded conformational polymorphism analysis to identify single-nucleotide polymorphism (SNP) within *myf6* in the Nile tilapia. SNPs were positively associated with body weight, depth, and length. In the current research, *myf6* expression levels decreased in T1 and T2 in contrast with the control group.

In the current study, *mstn* expression increased by 20.66-fold in T2 and 11.5-fold in T1 compared with control fish. This result conflicts with the study of **Ibrahim *et al.* (2021)**, who postulated that the *mstn* expression decreased by 0.2 and 0.3 fold in the Nile tilapia given selenium-loaded chitosan nanoparticles at concentrations of 0.5– 1mg/ kg. These differences may be due to the development stage, type of muscle, and state of nutrition **(Patruno *et al.*, 2008)**, which may affect the regulatory mechanisms of *mstn*. Elevated blood cortisol levels were correlated with higher *mstn* expression in fish **(Biga**

***et al.*, 2004)**, explaining why *mstn* expression increased in T2 due to higher cholesterol levels.

Quantitative analysis of skeletal muscle sections showed that the number of myofiber cells in T2 was smaller, while the average size, total area, and percentage of muscle fiber area of cells were larger than in T1 or the control group. This result indicates an induced skeletal muscle hypertrophy in fish fed SeNPs at a rate of 1.0mg/ kg diet. **Dal Pai-Silva *et al.* (2003)** concluded that hypertrophy is the primary process of muscle growth in the Nile tilapia. The average size, total area, and percentage of muscle fiber area of cells were larger in T1 than in the control, while the number of myofibers was not significant. The combination of large and small fiber diameters in white muscle forms a mosaic appearance. The predominance of the muscle fiber size (>50µm diameter) indicated active hypertrophic growth in several fish species **(Rowlerson & Veggetti, 2001)**, as in T2 in the present experiment. The hypertrophic growth of muscle fibers may be because a group of satellite cells supplies nuclei needed for hypertrophic growth **(Johnston, 1999)**. An increase in the total area of myofibers is equivalent to an increase in body weight. The current investigation shows a positive association between FBW and the total area of muscle fiber, as well as BWG and SGR. These results revealed that SeNPs promote muscle fiber hypertrophy, enhancing the growth rate. In rainbow trout, selenium led to the induction of hypertrophic muscle growth by boosting selenoprotein gene expression, specifically SelK and SelW, inhibiting protein breakdown mediated by the ubiquitin-proteasome pathway and the calpain system and encouraging myoblast fusion into preexisting muscle fibers **(Wang *et al.*, 2021)**

CONCLUSION

The current study assessed the influence of SeNPs on several parameters in the Nile tilapia. The addition of SeNPs enhanced several parameters in the Nile tilapia, including growth performance, FCR, survival, hematobiochemical parameters, oxidative capacity, immune response, myogenesis-related gene expression, and histometrics of skeletal muscles. The current research not only indicates the positive impacts of SeNPs on the economic traits of the Nile tilapia but also investigates the basis of such improvements on the cellular and molecular levels.

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