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Growth, Feed Efficiency, Hemato-Biochemical Indices, and Flesh Quality of Adult Nile Tilapia, *Oreochromis niloticus*, Fed a Diet Supplemented with Nano-Selenium

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ABSTRACT

This study was designed to evaluate the effects of graduated levels of nano-selenium (Nano-Se) in fish diet at 0, 0.5, and 1mg/ kg on growth performance, feed utilization, organ indices, hematological, serum biochemical, and muscular chemical composition, and flesh quality parameters in the adult Nile tilapia, *Oreochromis niloticus* (female and male) for 10 weeks. Two hundred and forty adult Nile tilapia, male (n = 120) and female (n = 120), with an average initial body weight of 94.43±3.77 g and 103.22±2.77 (mean ± SD), respectively were subject to study. In adult males, a significant increase was recorded in final weight with decreases in red blood cells, hemoglobin, hematocrit, AST, total protein, albumin, and globulin, compared to females. The addition of Nano-Se resulted in significant reductions in all males and females growth performance parameters, while the feed conversion ratio increased. When compared to other levels, the dietary addition of 1mg of Nano-Se/ kg resulted in a significant increase in hemoglobin and white blood cells. Dry matter and fat content significantly increased as Nano-Se levels increased, while protein content significantly decreased. In terms of growth performance, feed efficiency, body indices, hematological, serum liver function enzymes, and flesh quality parameters, no significant interaction was detected between sex and different levels of Nano-Se. Finally, the results showed that the addition of Nano-Se had a negative impact on Nile tilapia growth performance, feed utilization, serum liver function enzymes, and flesh quality measurements. Thus, it was deduced that, the tested levels of Nano-Se may be higher than the appropriate concentrations for both adult male and female of the Nile tilapia.

INTRODUCTION

To increase fish productivity, it is important to provide a balanced diet containing macronutrients such as protein, lipids, and carbohydrates in addition to micronutrients viz. vitamins and minerals **(Teves & Ragaza, 2016; Van Doan *et al.*, 2020)**. Fish need very small amounts of microelements that play a role in several metabolic, physiological, and functional processes in different fish organs **(Watanabe *et al.*, 1997)**. Selenium (Se) is an indispensable trace element for fish, which plays a vital role in immune function and

several physiological functions **(Watanabe *et al.*, 1997; Khan *et al.*, 2017)**. Moreover, Se is essential for cytokine production, thyroid hormone synthesis, fecundity and DNA synthesis **(Curran *et al.*, 2005; Papp *et al.*, 2007)**. Several factors influence fish requirements for Se, including species, age, source/form of Se and environmental stress factors **(Khan *et al.*, 2017)**. According to the **National Food Safety Standard (2010)**, the maximum allowed level of Se is 0.5mg/ kg. **NRC (2011)** also reported a dietary Se requirement varying from 0.15 to 0.7mg/ kg for different fish species. The requirement for Se varies significantly among different fish species depending on the physiological state of the organism **(Schriever *et al.*, 2009)**. The boundary between the requirements of fish for Se and its toxicity is very narrow **(Domínguez *et al.*, 2020)**. **Khan *et al.* (2017)** reported that, abnormal conditions increase requirements for Se, which are toxic under normal conditions.

Many studies have reported that adding Se to fish feed improved growth performance, feed utilization and health status **(Raza, 2012; Khan *et al.*, 2016)**. In recent years, using nanoparticles in fish feed, especially nanoparticles of Se (Nano-Se), has led to increased efficiency through higher chemical consistency, safety, bioactivity, and higher ability to trigger Se after digestion, compared to other conventional types of Se **(Abd El-Kader *et al.*, 2020; Al-Deriny *et al.*, 2020; Dawood, 2021)**. The addition of Nano-Se improves growth as well as physiological and immune responses in many fish species, such as common carp, *Cyprinus carpio*, the African catfish, *Clarias gariepinus*, the Nile tilapia, *Oreochromis niloticus*, and rohu, *Labeo rohita* **(Ashouri *et al.*, 2015; Saffari *et al.*, 2017; Chris *et al.*, 2018; Swain *et al.*, 2019; Dawood *et al.*, 2020; Ibrahim *et al.*, 2022)**. Furthermore, adding Nano-Se at a dose of 0.8mg/ kg significantly improved the Nile tilapia's performance, nutritional utilization, digestive enzyme activity, intestinal absorption surface, haemato-biochemical and oxidative stress responses **(Ibrahim *et al.*, 2022)**. **Rathore *et al.* (2021)** postulated that, the Nile tilapia fed a 1mg/ kg diet showed the highest weight gain (WG), specific growth rate (SGR), average growth rate (ADG), and protein efficiency ratio (PER), and ameliorated disease resistance exposed to *Aeromonas hydrophila* and *Streptococcus iniae* infections **(Neamat-Allah *et al.*, 2019)**. Additionally, nano-Se alleviated the pathological disorders of Caspian roach (*Rutilus caspicus*) induced by Cd toxicity **(Abu-Elala *et al.*, 2020)** and had positive effects on hematological and biochemical indices of the Nile tilapia exposed to malathion **(Zahmatkesh *et al.*, 2020)**. **Moustafa *et al.* (2021)** noted that, dietary nano-Se supplemented with a dietary regime feeding the fish for one day followed by a one-day starvation showed an enhancement of growth efficiency, physiological and immunity responses and decreased the feeding cost. Despite the positive results achieved by the addition of nano-selenium in fish diets; however, most of the research used small fish at the stage of fingerlings **(Mechlaoui *et al.*, 2019; Abu-Elala *et al.*, 2020; Zahmatkesh *et al.*, 2020; Moustafa *et al.*, 2021; Rathore *et al.*, 2021; Ibrahim *et al.*, 2022)**. The effect of Nano-Se on adult tilapia nutrition is scarce. Therefore, this study was designed to

evaluate the effects of graduated levels of Nano-Se at 0, 0.5, and 1mg/ kg diet on growth performance, feed utilization, organs indices, hematological, serum biochemical, chemical composition, and flesh quality of the adult Nile tilapia, *O. niloticus*, female and male for 10 weeks.

MATERIALS AND METHODS

1. Experimental management

Two hundred and forty adult Nile tilapia, *O. niloticus*, male (*n* = 120) and female (*n* = 120), with an average initial body weight (mean±SD) of 94.43±3.77 g and 103.22±2.77 g, respectively, were obtained from the Fish Research Unit, Faculty of Agriculture, Mansoura University, Egypt. Fish were adapted for fifteen days. During adaptation, fish were fed a basal diet (30.93% crude protein). Fish were randomly distributed into six experimental treatments (two replicates for each treatment). Fish were stocked at 20 fish per one plastic tank (1 m^3 in volume) and aerated by an electric compressor. Every day, fresh underground water replaced 20% of the total water volume in each tank.

The water temperature (T, °C), dissolved oxygen (DO, mg/L), total ammonia, and pH values were determined. Throughout the experiment, water temperature was adjusted at 24.17 2.77°C using a heater thermostat, which included temperature (via a thermometer). Water pH-value (using Jenway Ltd., Model 350-pH-meter, Staffordshire ST15 0SA, UK) and DO (using Jenway Ltd., Model 970-DO meter, Staffordshire ST15 0SA, UK) were measured twice per week, which were 8.16±0.203 and 7.56±0.31 mg/L, respectively. The ammonia concentration of water was tested by direct Nesslerization methods, using CHEMETS® test kits (CHEMETRICS, INC, USA) according to **APHA (1992)**.

2. Synthesis of selenium nanoparticles

The eco-friendly synthesis of Nano-Se was carried out using the method of **Pattanayak and Nayak (2013)**, with slight modifications of **El-Refai *et al.* (2018)**. For both solutions, aqueous solutions of Se dioxide for synthesis (SeO_2, Merck Schuchardt OHG, Germany) and ascorbic acid were prepared using deionized water in concentration of 200ppm). Ascorbic acid (20mL) was applied to the same metal salt solution concentration (20mL) for reducing very carefully to stop a rapid reaction causing greater particle size and the formation of precipitate at room temperature under stirring for two hours. The resulting nanoparticles were synthesized according to an equimolar ratio (1:1), according to **Ibrahim *et al.* (2019)**. Then, the mixture was lyophilized to a fine powder.

3. Experimental diet and feeding

The basal diet was bought from Aller Aqua for Fish Feed Manufacture, Plot 59 and 60 Street 86, the 6th of October City—Giza, Cairo, Egypt. The proximate chemical analysis was conducted according to **AOAC (2016)** and displayed in Table (1). The Nano-Se was added to the basal diet at levels of 0, 0.5 and 1mg/ kg diet, and then denoted to treatments T_1, T_2 and T_3, respectively, for females and T_4, T_5 and T_6 treatments for

males. All diets were pressed using a manufacturing machine (pellets size 1mm). The experimental diets were introduced two times at 10 a.m. and 14 p.m. by hand. Fish were fed at a rate of 3- 2% of their biomass and weighed every fifteen days to adjust their daily feed intake.

Table 1. Nutrient composition (% on dry matter basis) of the experimental diet

Nutrient composition	%
Dry matter (%)	87.04
Crude protein (CP, %)	30.92
Crude fat (CF, %)	5.34
Ash (%)	8.10
Total carbohydrates (%)	55.64
* Gross energy (GE, KJ / Kg DM)	1919.7
** Protein/energy ratio (P/E, mg CP / KJ GE)	16.10

* Gross energy (GE, MJ/100 g DM) = (CF × 39.54) + (CP × 23.64) + (total carbohydrate × 17.57) calculated according to **NRC (2011)**

** P/E ratio (mg crude protein/MJ gross energy) = (CP /GE) × 1000

4. Measurements criteria

4.1. Structure characterization of the selenium nanoparticles

4.1.1. **Transmission electron microscope (TEM).** The physical properties and chemical structure, i.e., particle's size, shape, surface nature, crystal structure, and morphological data of the prepared nanoparticles were identified as conveyed by **Otunola *et al.* (2017)**, using TEM (JEOL TEM-2100, Tokyo, Japan) at the Electron Microscope Unit, Mansoura University, Egypt. The analysis was run with a 100nm magnification value.

4.1.2. **Nanoparticles characteristic via zeta potential.** The surface charge of the prepared Nano-Se in the suspension was characterized by applying Zeta potential technique, using Malvern Instruments Ltd. Zeta Potential Ver. 2.3 (Kassel, Germany) according to **Bhattacharjee (2016)** at the Electron Microscope Unit, Mansoura University, Egypt. The process is significant for studying the surface nature of nanoparticles, and the stability of these particles can be expected to last for long-term periods **(Honary & Zahir, 2013)**.

4.2. Growth performance and feed utilization parameters

At the end of the experiment, fish in each earthen pond were weighed to calculate the growth performance parameters according to **Lovell (2001)**, with the following equations:

- Total weight gain (TWG, g) = FW (g)/ IW (g)
- Average daily gain (ADG, g/fish/day) = TWG (g) / T
- Relative growth rate (RGR, %) = (TWG (g) / IW (g)) × 100

- Specific growth rate (SGR, %/day) = [(Ln FW – Ln IW) / T] × 100

Where,

FW: Final weight (g); IW: Initial weight (g); T: The experimental period (day).

Feed intake (FI, g) was recorded, then feed utilization parameters were calculated according to the following equations;

- Feed conversion ratio (FCR) = FI / TWG
- Feed efficency (%) = (TWG / FI) × 100
- Protein efficiency ratio (PER) = live weight gain (g) / protein intake (g)
- Protein productive value (PPV, %) = [retained protein (g)/protein intake (g)] × 100
- Energy utilization (EU %) = [retained energy (Kcal)/energy intake (Kcal)] × 100

4.3. Somatic indices

Fish weight and length (*n* = 10/treatment) were recorded to calculate the condition factor (CF). Then, fish were dissected and obtained by weight from different organs such as viscera, liver, spleen, and intestine to calculate the somatic index according to the succeeding equations:

- CF (%) = [fish weight/(fish length)^3] × 100
- Viscera somatic index (VSI, %) = (viscera weight / fish weight) × 100
- Hepato-somatic index (HSI, %) = (liver weight / fish weight) × 100
- Spleen-somatic index (SSI, %) = (spleen weight / fish weight) × 100
- Intestine-somatic index (ISI, %) = (intestine weight / fish weight) × 100

4.4. Blood sampling and analytical methods

At the end of the experiment, fish (*n* = 12/treatment) were randomly taken and anesthetized by adding commercial clove oil extract (three drops in 10L water) to get the blood samples. Blood samples (n = 6/treatment) were collected from the fish caudal peduncles. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hematological parameters. Blood haematology was measured using an Auto Counter (920 EO+ manufactured by Swelab, Switzerland) to determine the total count of red blood cells (RBCs), hemoglobin (Hb), haematocrit (Hct), the total count of white blood cells (WBC_S), and lymphocytes **(Decie & Lewis, 2006)**.

Other blood samples (n = 6 per treatment) were collected in dried plastic tubes and transferred by centrifugation for 15 minutes at 3500rpm to obtain blood serum. Serum samples were kept in the deep freezer for biochemical analysis. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercial test kits (Humalyzer 3000, manufactured by Human, Germany). Then, the determination of glucose **(McGowan *et al.*, 1983)**, total protein **(Tietz, 1990)**, and albumin **(Wotton and Freeman, 1982)** concentrations were analyzed in addition to the calculation of globulin levels by subtracting albumin from total protein.

4.5. Muscular chemical composition

After collecting blood, six fish (*n* = 6/treatment) were taken to determine the chemical composition of the muscles and flesh quality. For the chemical composition,

muscular samples were taken at the end of the experiment (n = 5/treatment) and kept frozen at –20°C until the chemical analysis was done according to **AOAC (2016)**.

4.6. Flesh quality

Flesh quality parameters included water holding capacity (WHC), stored loss (SL), drip loss (DL) and frozen leakage rate (FLR). WHC was estimated by weighing a part of the dorsal muscle and placing it between two filter papers, then placing a weight of 3.5kg on it for 15 minutes. The WHC is estimated as a percentage by dividing the difference between the two weights by the fresh weight. Each group received five fish fillets (average weight, 5 0.5g) to determine the DL of fish muscle. Fillets were placed in plastic bags and stored at 4°C for 72 hours to determine drip loss. Then, DL was calculated according to **Bosworth *et al.* (2004)** by the following equation:

$$DL\ (\%) = [(W_0 - W_1)/ W_0] \times 100$$

Where,

W_0 is the weight of fillet sample before storage, and W_1 is the weight after storage (three days).

The SL and the FLR were determined according to **Lingqiao *et al.* (2014)**. Ten sample fillets were weighed with an average of 5±0.5 g and packed in plastic bags before being divided into two sets (each set containing five samples). Samples were then stored at 4 and 20°C for 24 hours to determine SL and FLR, respectively. SL and FLR were calculated as the percentage of original weight lost.

4.7. Statistical analysis

The data were statistically analyzed using SAS® 9.4 **(SAS, 2016)** for the user's guide, with a factorial design (2×3) and a two-way ANOVA to test the effects of sex and Nano-Se levels. All ratios and percentages were arcsine-transformed before statistical analyses. The differences between the means of treatments were compared using *Tukey's post hoc* significant test, and differences were considered statistically significant at $P \leq 0.05$.

RESULTS

1. Characterization of the prepared nanoparticles

1.1. Transmission electron microscope (TEM)

The produced Nano-Se, Se distributions and TEM images are shown in Fig. (1). They appear to be spherical particles only. Fig. (1) demonstrates the formation of the spherical and tetragonal shapes of Nano-Se, supported by the emission diffraction of the chosen area. The Nano-Se particles range in size from 38.45 - 66.78nm.

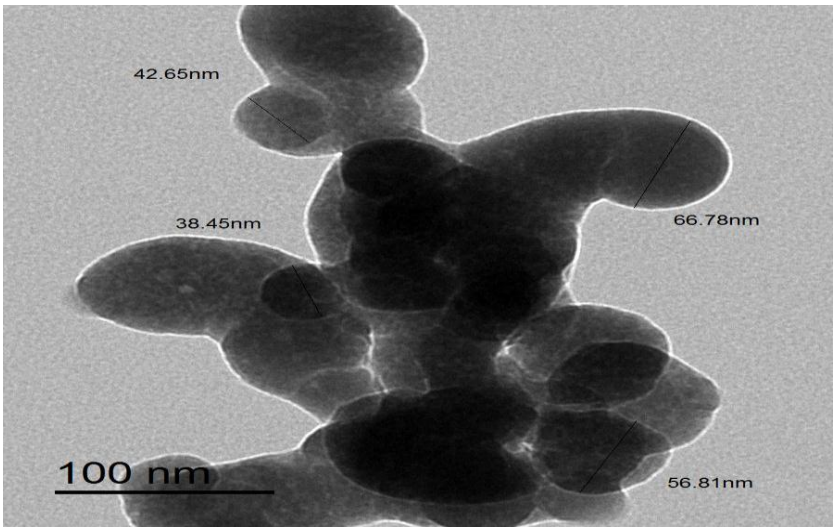

Fig. 1. TEM micrographs and size distributions for selenium nanoparticles synthesized by ascorbic acid at a 100nm magnification value

1.2. Zeta potential analysis

Fig. (2) shows the zeta potential analysis for the Nano-Se produced. Results showed a double layer of ions present in the nanoparticles, and Se particles produced with ascorbic acid have zeta potential values of 5.61 and 8.78 mV, which were very stable.

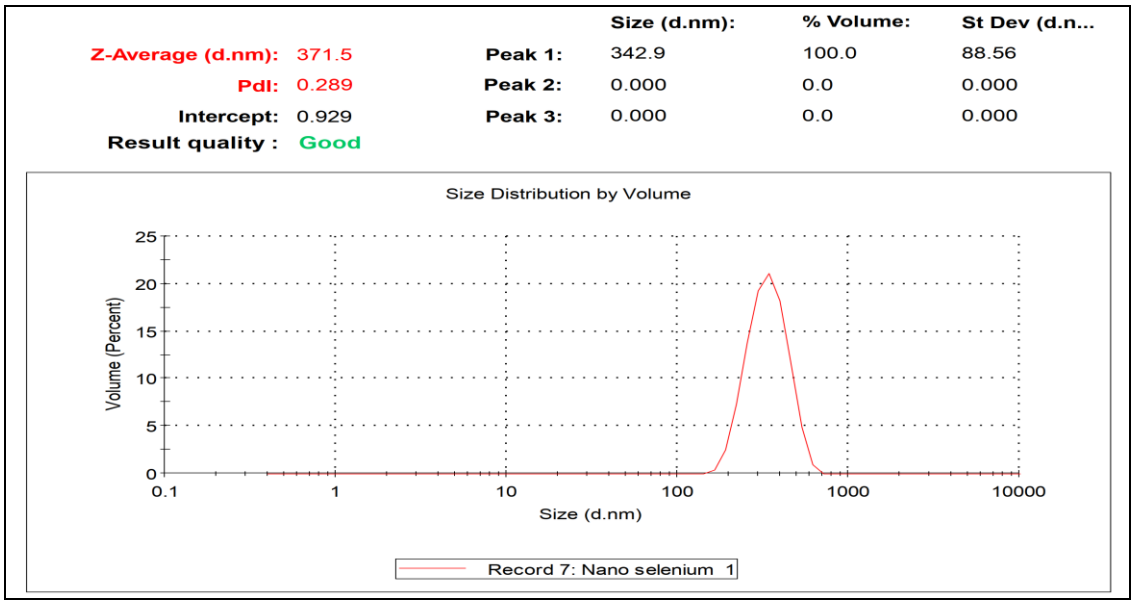

Fig. 2. Zeta potential charts of the prepared selenium nanoparticles synthesized by ascorbic acid

2. Growth performance

Table (2) presents the growth performance of male and female adult of the Nile tilapia fed different levels of Nano-Se, adding to their interactions. Results showed that the FW of males was the highest significant compared to females ($P \leq 0.05$). On the other hand, no significant difference was detected between males and females in TWG, ADG,

RGR and SGR ($P > 0.05$). All growth parameters, whether male or female, were significantly reduced by the addition of Nano-Se ($P \leq 0.05$). Simultaneously, no significant interaction was defined between sex and different levels of Nano-Se in growth performance parameters ($P > 0.05$).

Table 2. Effect of sex (female and male) and different levels of Nano-Se on growth performance parameters of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	FW (g)	TWG (g)	ADG (g/fish/day)	RGR (%)	SGR (%/day)
Female		159.3±2.06^{b}	64.84±2.06	0.93±0.03	68.67±2.18	4.14±0.12
Male		170.9±3.00^{a}	67.72±3.00	0.97±0.04	65.61±2.90	4.16±0.16
	0	173.1±3.23^{a}	74.27±1.58^{a}	1.06±0.02^{a}	75.20±1.25^{a}	4.59±0.07^{a}
	0.5	161.0±3.27^{b}	62.19±2.71^{b}	0.89±0.04^{b}	63.07±3.03^{b}	3.92±0.16^{b}
	1	161.2±3.45^{b}	62.39±2.00^{b}	0.89±0.03^{b}	63.15±1.68^{b}	3.93±0.10^{b}
Female	0	166.4±0.36	71.95±0.36	1.03±0.01	76.20±0.38	4.54±0.02
	0.5	156.9±3.25	62.50±3.24	0.89±0.05	66.19±3.44	4.01±0.18
	1	154.5±0.95	60.08±0.95	0.86±0.01	63.62±1.01	3.87±0.05
Male	0	179.8±2.66	76.58±2.66	1.09±0.04	74.20±2.57	4.64±0.14
	0.5	165.1±5.11	61.89±5.11	0.88±0.07	59.96±4.95	3.84±0.29
	1	167.9±3.70	64.69±3.70	0.92±0.05	62.67±3.58	3.99±0.20
Two-way ANOVA (*P*-value)						
Sex		0.0006	0.2814	0.2824	0.2475	0.9145
Nano-Se		0.0030	0.0030	0.0028	0.0025	0.0030
Sex*Nano-Se		0.6371	0.6371	0.6612	0.6720	0.6513

Means in the same column having different small letters are significantly different ($P \leq 0.05$). FW: Final weight; TWG: Total weight gain; ADG: Average daily gain; RGR: Relative growth rate; SGR: Specific growth rate.

3. Feed efficiency parameters

Feed efficiency parameters of the Nile tilapia (male and female) fed different levels of Nano-Se are illustrated in Table (3). No significant differences were observed between males and females in FCR, PER, PPV and EU ($P > 0.05$). With increasing the levels of Nano-Se, FI, PER, PPV, and EU were significantly decreased, while FCR was increased compared to those fed the free-Se diet ($P \leq 0.05$). In terms of feed efficiency parameters, no discernible interaction was observed between sex and different levels of Nano-Se ($P > 0.05$).

Table 3. Effect of sex (female and male) and different levels of Nano-Se on feed utilization parameters of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	FI (g)	FCR	PER	PPV (%)	EU (%)
Female		156.9±17.4^{b}	2.43±0.0	1.33±0.0	21.95±0.66	19.21±0.36
Male		165.3±18.3^{a}	2.48±0.1	1.32±0.0	23.25±1.55	19.36±0.64
	0	164.8±27.47^{a}	2.22±0.04^{b}	1.45±0.03^{a}	25.86±1.15^{a}	20.24±0.37^{a}
	0.5	159.8±26.62^{b}	2.59±0.12^{a}	1.26±0.05^{b}	19.03±0.87^{b}	17.72±0.57^{b}
	1	158.6±26.44^{b}	2.55±0.06^{a}	1.26±0.03^{b}	22.9±0.49^{ab}	19.89±0.31^{b}
Female	0	161.1±53.71	2.24±0.01	1.44±0.00	23.49±0.06	19.73±0.03
	0.5	154.8±51.60	2.49±0.10	1.30±0.05	19.60±0.94	17.94±0.60
	1	154.6±51.55	2.57±0.02	1.25±0.01	22.78±0.20	19.96±0.11
Male	0	168.5±56.16	2.21±0.08	1.46±0.05	28.24±0.99	20.75±0.64
	0.5	164.7±54.90	2.70±0.22	1.21±0.10	18.45±1.61	17.49±1.11
	1	162.6±54.19	2.52±0.12	1.28±0.06	23.05±1.07	19.83±0.68
Two-way ANOVA (*P*-value)						
Sex		<.0001	0.6689	0.7956	0.1284	0.7857
Nano-Se		0.0003	0.0143	0.0073	<.0001	0.0039
Sex*Nano-Se		0.5182	0.4797	0.5111	0.0256	0.5025

Means in the same column having different small letters are significantly different ($P \leq 0.05$). FI: Feed intake; FCR: Feed conversion ratio; FE: Feed efficiency; PER: Protein efficiency ratio; PPV: Protein productive value; EU: Energy utilization.

4. Somatic indices

The effects of different levels of Nano-Se on body indices of the Nile tilapia (male and female) are summarized in Table (4). Results showed that, females gave the highest values of HSI, VSI, ISI, and GSI and the lowest SSI compared to males. No significant differences were found in HSI and VSI among different levels of Nano-Se. However, the addition of Nano-Se at the level of 1mg/ kg led to a significant decrease in SSI and a significant increase in ISI and GSI. There is no evident relationship between sex and different levels of Nano-Se in terms of all body indices ($P > 0.05$).

5. Hematological parameters

The effects of different levels of Nano-Se on haematological parameters of the Nile tilapia (male and female) are shown in Table (5). Results revealed that, females recorded the highest values of RBCs, Hb, Hct, and lymphocytes compared to males ($P \leq 0.05$). No significant differences were detected in WBCs between males and females nor in Hct values between Nano-Se levels ($P > 0.05$). There was a significant increase of RBCs, Hb, Hct, WBCs, and lymphocytes with increasing the levels of Nano-Se ($P \leq 0.05$). There is no evident relationship between sex and different levels of Nano-Se of Hct and WBCs ($P > 0.05$). Nevertheless, the interaction between sex and different levels of Nano-Se in RBCs, Hb and lymphocytes was significant.

Table 4. Effect of sex (female and male) and different levels of Nano-Se on body indices of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	HSI (%)	VSI (%)	SSI (%)	ISI (%)	GSI (%)
Female		3.33±0.12[a]	12.65±0.28[a]	0.145±0.01[b]	4.08±0.15[a]	3.31±0.27[a]
Male		2.88±0.10[b]	9.14±0.24[b]	0.167±0.01[a]	3.55±0.14[b]	0.667±0.06[b]
	0	3.05±0.15	10.85±0.54	0.174±0.01[a]	3.83±0.14[b]	1.78±0.42[b]
	0.5	3.16±0.13	10.39±0.55	0.163±0.01[a]	3.29±0.18[b]	1.86±0.35[b]
	1	3.10±0.16	11.44±0.41	0.131±0.01[b]	4.34±0.17[a]	2.34±0.38[a]
Female	0	3.25±0.25	12.82±0.55	0.163±0.01	4.01±0.25	3.06±0.62
	0.5	3.46±0.11	12.41±0.52	0.135±0.01	3.77±0.25	3.11±0.42
	1	3.28±0.26	12.71±0.41	0.137±0.01	4.46±0.28	3.78±0.35
Male	0	2.85±0.16	8.88±0.28	0.185±0.01	3.65±0.10	0.493±0.04
	0.5	2.86±0.19	8.38±0.32	0.192±0.01	2.80±0.16	0.607±0.09
	1	2.92±0.1	10.17±0.44	0.126±0.01	4.21±0.20	0.901±0.13
Two-way ANOVA (*P*-value)						
Sex		0.033	<0.001	0.005	0.016	<0.0001
Nano-Se		0.995	0.056	0.001	0.002	0.0219
Sex*Nano-Se		0.774	0.901	0.222	0.286	0.844

Means in the same column having different small letters are significantly different ($P \leq 0.05$). HSI: Hepatosomatic index; VSI: Visceral somatic index; SSI: Spleen somatic index; ISI: Intestine somatic index; GSI: Gonadosomatic index.

6. Serum biochemical parameters

Data in Table (6) show the serum biochemical parameters of adult Nile tilapia, male and female, fed different levels of Nano-Se and their interactions. Results showed that the AST, TP, ALB, and GLB of females had the highest significant values and the lowest values of ALT and GLU compared to males ($P \leq 0.05$). Fish fed Nano-Se at 0.5mg/ kg diet showed a significant increase in AST and ALT. However, fish fed Nano-Se at 1mg/ kg diet showed a significant increase in GLU, compared to other levels. On the other hand, no significant differences were recorded in TP, ALB, and GLB among different levels of Nano-Se ($P > 0.05$). Simultaneously, no significant interaction was depicted between sex and different levels of Nano-Se in TP and GLB ($P > 0.05$). Nevertheless, the interaction between sex and different levels of Nano-Se in AST, ALT, ALB, and GLU was significant ($P \leq 0.05$).

Table 5. Effect of sex (female and male) and different levels of Nano-Se on hematological parameters of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	RBCs ($\times 10^6$/mm^3)	Hb (g/dL)	Hct (%)	WBCs ($\times 10^3$/mm^3)	Lymphocytes (%)
Female		1.87±0.06^{a}	10.43±0.19^{a}	24.39±0.84^{a}	47.72±1.31	91.32±0.78^{a}
Male		1.51±0.05^{b}	8.71±0.19^{b}	21.81±0.79^{b}	46.99±1.62	88.15±0.82^{b}
	0	1.61±0.06^{b}	9.20±0.38^{b}	23.37±0.67	45.42±0.90^{b}	89.40±1.87^{b}
	0.5	1.70±0.12^{a}	9.58±0.58^{ab}	21.63±1.41	44.94±1.72^{b}	88.70±0.36^{b}
	1	1.71±0.07^{a}	9.93±0.30^{a}	24.30±1.02	51.71±1.00^{a}	91.10±0.58^{a}
Female	0	1.74±0.05	9.93±0.34	24.43±0.84	45.04±1.70	93.30±0.76
	0.5	1.95±0.14	10.85±0.03	23.93±1.80	47.58±2.53	88.75±0.66
	1	1.91±0.13	10.50±0.29	24.80±2.10	50.55±1.91	91.90±0.95
Male	0	1.48±0.04	8.47±0.24	22.30±0.65	45.79±1.02	85.50±1.33
	0.5	1.46±0.07	8.30±0.17	19.33±1.19	42.30±1.16	88.65±0.43
	1	1.65±0.04	9.35±0.20	23.80±0.69	52.87±0.00	90.30±0.35
Two-way ANOVA (*P*-value)						
Sex		<0.0001	<0.0001	0.035	0.581	0.005
Nano-Se		0.0015	0.0291	0.170	0.001	0.033
Sex*Nano-Se		0.0271	0.0272	0.413	0.079	0.001

Means in the same column having different small letters are significantly different ($P \leq 0.05$). RBCs: Red blood cells; Hb: Hemoglobin; Hct: Haematocrit; WBCs: White blood cells.

7. Flesh quality

7.1. Muscular chemical composition

The muscular chemical composition of the Nile tilapia (male and female) fed different levels of Nano-Se is illustrated in Table (7). The Nile tilapia females showed a significant increase in DM, fat, and ash contents and a significant decrease in protein content, compared to males ($P \leq 0.05$). Similar trends were observed with increasing levels of Nano-Se for DM, fat, and protein contents. However, fish fed 0.5mg Nano-Se/ kg diet gave the highest values of ash content, compared to other levels ($P \leq 0.05$). The interaction between sex and different levels of Nano-Se was significant ($P \leq 0.05$).

Table 6. Effect of sex (female and male) and different levels of Nano-Se on serum biochemical parameters of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	AST (U/L)	ALT (U/L)	TP (g/dL)	ALB (g/dL)	GLB (g/dL)	GLU (mg/dL)
Female		443.2±22.26[a]	130.1±6.80[b]	4.12±0.06[a]	0.90±0.02[a]	3.22±0.05[a]	51.86±5.03[b]
Male		277.7±42.20[b]	243.8±6.40[a]	3.42±0.07[b]	0.63±0.02[b]	2.80±0.06[b]	70.39±4.46[a]
	0	324.8±91.46[b]	168.9±29.1[b]	3.81±0.19	0.74±0.07	3.06±0.12	60.73±6.87[ab]
	0.5	394.8±10.91[a]	195.5±27.1[a]	3.74±0.19	0.75±0.08	2.99±0.13	57.90±9.88[b]
	1	361.8±24.44[ab]	196.4±21.7[a]	3.78±0.14	0.80±0.04	2.98±0.10	64.73±3.23[a]
Female	0	529.0±1.15	105.33.30	4.19±0.08	0.89±0.04	3.29±0.04	46.17±4.65
	0.5	415.0±11.55	136.0±1.2	4.13±0.16	0.93±0.03	3.21±0.16	39.00±3.23
	1	385.5±8.37	149.0±5.80	4.05±0.05	0.88±0.05	3.17±0.04	70.40±1.10
Male	0	120.5±10.10	232.5±13.10	3.43±0.16	0.59±0.01	2.83±0.15	75.30±1.50
	0.5	374.5±7.22	255.0±11.0	3.34±0.09	0.57±0.01	2.78±0.08	76.80±10.97
	1	338.0±48.50	243.9±8.61	3.50±0.12	0.72±0.03	2.79±0.10	59.07±4.36
Two-way ANOVA (*P*-value)							
Sex		<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0012
Nano-Se		0.0209	0.0209	0.8441	0.2096	0.6963	0.4682
Sex*Nano-Se		<0.0001	<0.0001	0.5319	0.0228	0.9359	0.0015

Means in the same column having different small letters are significantly different ($P \leq 0.05$). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; GLB: Globulin: GLU: Glucose.

7.2. WHC, stored loss, drip loss and frozen leakage rate

Data in Table (8) show the differences in WHC, SL, DL, and FLR under the effect of sex (female and male) and different levels of Nano-Se. The Nile tilapia females demonstrated a significant increase in WHC and a significant decrease in DL and FLR during different times, compared to males ($P \leq 0.05$). There was no significant difference in SL between females and males. WHC, DL, and FRL at time 1h showed no significant difference among different levels of Nano-Se. The SL and FLR at times 0h and 2h were significantly increased for fish fed 0.5mg of Nano-Se/ kg diet level, followed by those fed 1mg of Nano-Se/ kg diet. The interaction between sex and different levels of Nano-Se was insignificant for all the afore- mentioned flesh quality parameters.

Table 7. Effect of sex (female and male) and different levels of Nano-Se on muscular chemical composition of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	Dry matter (%)	On dry matter basis (%)		
			Protein	Fat	Ash
Female		32.57±0.38[a]	87.93±0.35[b]	5.35±0.03[a]	6.72±0.36[a]
Male		29.81±0.31[b]	93.07±0.07[a]	4.83±0.11[b]	2.10±0.17[b]
	0	28.70±0.32[c]	92.02±0.05[a]	4.49±0.16[b]	3.49±0.12[c]
	0.5	31.76±0.30[b]	89.08±0.82[c]	5.36±0.05[a]	5.56±0.86[a]
	1	33.10±0.73[a]	90.41±0.54[b]	5.42±0.05[a]	4.18±0.52[b]
Female	0	30.42±0.11	91.77±0.00	5.37±0.03	2.86±0.03
	0.5	30.19±0.30	84.59±0.00	5.15±0.05	10.26±0.05
	1	37.10±0.05	87.44±0.12	5.52±0.11	7.04±0.01
Male	0	26.98±0.16	92.27±0.09	3.60±0.09	4.12±0.02
	0.5	33.33±0.08	93.57±0.07	5.56±0.11	0.86±0.05
	1	29.11±0.01	93.37±0.00	5.32±0.10	1.31±0.10
Two-way ANOVA (*P*-value)					
Sex		<0.0001		<0.0001	<0.0001
Nano-Se		<0.0001		<0.0001	<0.0001
Sex*Nano-Se		<0.0001		<0.0001	<0.0001

Means in the same column having different small letters are significantly different ($P \leq 0.05$).

Table 8. Effect of sex (female and male) and different levels of Nano-Se on WHC, stored loss, drip loss and frozen leakage rate of adult Nile tilapia

Sex	Nano-Se level (mg/ kg diet)	WHC (%)	SL (%)	DL (%)	Frozen leakage rate (%)		
					0 h	1 h	2 h
Female		2.43±0.15[a]	0.94±0.07	2.12±0.10[b]	0.93±0.08[b]	1.32±0.12[b]	1.86±0.14[b]
Male		1.98±0.14[b]	0.97±0.06	2.95±0.10[a]	1.08±0.13[a]	1.49±0.11[a]	2.13±0.13[a]
	0	2.29±0.21	0.81±0.06[b]	2.39±0.18	0.83±0.08[b]	1.20±0.10	1.85±0.15[b]
	0.5	2.20±0.25	1.01±0.08[a]	2.54±0.13	1.16±0.10[a]	1.71±0.15	2.34±0.18[a]
	1	2.11±0.10	1.04±0.08[a]	2.67±0.23	1.02±0.19[a]	1.31±0.12	1.81±0.12[b]
Female	0	2.45±0.25	0.74±0.06	1.95±0.20	0.78±0.10	1.18±0.10	1.80±0.18
	0.5	2.69±0.37	1.02±0.07	2.35±0.17	1.27±0.10	1.67±0.24	2.18±0.29
	1	2.13±0.10	1.05±0.15	2.04±0.08	0.75±0.09	1.12±0.18	1.61±0.20
Male	0	2.14±0.35	0.88±0.09	2.84±0.09	0.89±0.13	1.22±0.19	1.89±0.26
	0.5	1.71±0.13	0.99±0.15	2.74±0.17	1.05±0.18	1.75±0.20	2.50±0.22
	1	2.08±0.19	1.03±0.08	3.29±0.18	1.30±0.34	1.50±0.10	2.00±0.07
Two-way ANOVA (*P*-value)							
Sex		0.039	0.747	<0.0001	0.0326	0.0261	0.0140
Nano-Se		0.765	0.084	0.223	0.212	0.020	0.038
Sex*Nano-Se		0.184	0.713	0.032	0.112	0.563	0.771

Means in the same column having different small letters are significantly different ($P \leq 0.05$). WHC: Water holding capacity; SL: Stored loss; DL: Drip loss.

DISCUSSION

Fish growth and feed utilization are the most important metrics used to judge environmental conditions, culture systems, and food additives, as well as indicating fish productivity. In the present study, the Nile tilapia males gave the highest values of FW, TWG, and ADG. In this respect, **Fairbair (1997)** reported that, in most tilapia species, males grow bigger and faster than females. Several previous studies recorded the positive effect of Nano-Se on growth performance, physiological responses, and disease resistance of tilapia **(Moustafa *et al.*, 2021; Rathore *et al.*, 2021; Ibrahim *et al.*, 2022)**. Nevertheless, in the current study, results showed harmful effects of the addition of Nano-Se on the growth performance and feed utilization of the Nile tilapia males and females. This effect may be related to the age of the tilapia (adult) consuming the highest levels of Nano-Se, compared to tilapia fry and fingerlings used in other studies **(Rathore *et al.*, 2021; Ibrahim *et al.*, 2022)**. Furthermore, Nano-Se is distinguished by its ability to be rapidly absorbed through the cells of the body, suggesting that fish get a greater amount than those of the permissible concentrations for tilapia fish, which is reflected in negatively affecting growth performance and nutritional efficiency. Thus, **Dawood *et al.* (2020)** observed that, Se in a nano form was easy and had higher absorption in fluids and tissues. The harmful effects regarding dietary addition of Nano-Se, on the other hand, may be related to the fact that, fish of this size work on directing nutrients to the formation of the gonads, which negatively impacted growth performance and feed utilization of the adult Nile tilapia male and female. This confirmed the positive effects of GSI in the current study. In addition, **Zakeri *et al.* (2009)** showed that, the adult fish use a large portion of their food energy for reproduction rather than growth. This assumption is confirmed by the improvement in the health status of males and females as observed in the hematological indicators, as well as the fact that we did not notice any abnormal symptoms or mortality in fish. Similarly, supplementation of Nano-Se had no significant effect on FW of rainbow trout brood stock (**Wischhusen *et al.*, 2019)** and the female Arabian yellowfin sea bream, *Acanthopagrus arabicus* (**Saffari *et al.*, 2021)** .

FCR reduction is one of the most important indicators of diet quality. Many fish species fed diets supplemented with Nano-Se showed low values or were not significantly different from those in the control group **(Zhou *et al.*, 2009; Ashouri *et al.*, 2015; Saffari *et al.*, 2017; Neamat-Allah *et al.*, 2019; Abd El-Kader *et al.*, 2020; Dawood *et al.*, 2020)**. In contrast, the current results showed an increase in FCR of fish-fed diet supplemented with different levels of Nano-Se, compared to the control diet. This may be attributed to the mechanism of adult fish transforming part of the food to the formation of gonads and reproductive activity, which increased FCR, and consequently led to depressing effects on growth rates. The same trend was observed for other parameters such as PER, PPV and EU. **Coward and Bromage (2000)** explained the findings pointing to the fact that, in order to maintain their reproductive capacities tilapia species sacrifice growth, resulting in poor growth and FCR.

HSI are directly affected by fish nutritional status. **Saffari *et al.* (2021)** found that Arabian yellowfin sea bream fed 2mg of Nano-Se/ kg diet significantly increased HSI and VSI. Similar results were observed in the present study, where Nile tilapia females gave the highest values of HSI and VSI compared to males, indicating a metabolic strategy in females to meet the metabolic requirements during vitellogenesis **(Lubzens *et al.*, 2010)**. In other fish species, HSI modifications have been observed during reproduction **(Rinchard & Kestemont, 2003; Nunes *et al.*, 2011)**. The liver is the primary organ for storage, nutrients metabolism, detoxifying processes and the synthesis of vitellogenin **(Lubzens *et al.*, 2010)**. In the current study, compared to males, the VSI increased in females via increasing the level of dietary Nano-Se. These results showed that Nano-Se supplementation can increase the lipid reserves in the abdominal area of females, which are utilized as a source of nutrients for ovarian growth during the reproduction period **(Lal & Singh, 1987)**. These results supported others such as the increased fat content of body fish in the present study. Additionally, results in the current study demonstrated a significant increase in GSI in females and males with increasing Nano-Se levels, which may indicate an enhancement of gonad development and fertility. In fish, the GSI is an important metric to monitor nutritional change, fertility, spawning, gonad development and gonad quality **(Adams *et al.*, 1996; Yeldan & Avsar, 2000)**.

The current findings showed significantly increased RBCs, Hb and Hct in tilapia females compared to males. Furthermore, increasing the levels of Nano-Se caused positive effects on hematological parameters, such as RBCs, Hb, Hct, and WBCs, indicating an enhancement of the health status of fish fed supplemented diets with Nano-Se. This effect could be related to the effect of Nano-Se protecting RBCs membranes from the harmful effects of free radicals and oxygen, as well as reducing the hemolysis and degeneration **(Ashouri *et al.*, 2015; Khan *et al.*, 2016)**. In this manner, **Dawood *et al.* (2019)** postulated that, Nano-Se works on increasing hematology indices by increasing oxygen availability in the cell's tissues, and subsequently regulating the metabolic rate of nutrients. The improvement of hematological parameters of fish fed Nano-Se was also observed in many fish species such as yellowtail **(Le and Fotedar, 2014)**, common carp **(Saffari *et al.*, 2017)**, the European seabass **(Dawood *et al.*, 2019)** and the Nile tilapia **(Rathore *et al.*, 2021; Ibrahim *et al.*, 2022)**. Based on the present results, the total count of WBCs was increased, which indicates that higher immunity was provided to fight pathogens and invaders **(Fiúza *et al.*, 2015)**. Similar results were recorded in the study of **Rathore *et al.* (2021)** on the Nile tilapia pre- and post- exposure to *A. hydrophila* and fed 1mg of Nano-Se/kg. On the contrary, **Owolabi and Barbarinsa (2020)** found that, WBCs values decreased significantly in the African sharptooth catfish fed Nano-Se.

AST and ALT play an important role in nitrogen metabolism in cells, transferring amino acids to liver cells and monitoring toxic effects **(Abdel-Tawwab, 2016)**. In the current study, the activity of liver function enzymes in females is higher than in males, and the rate increases with increasing the levels of Nano-Se. The increase of the activity

of liver enzymes in females compared to males may be related to reproductive activity, where the liver plays an important role in egg vitellogenesis **(Patiño & Sullivan, 2002)**. On the other hand, no significant differences were found in ALT and AST activities for the European seabass (*D. labrax*) fed different levels of Nano-Se **(Abd El-Kader *et al.*, 2020)**. On the contrary, the addition of Nano-Se led to a decrease in the activities of AST and ALT of the African catfish **(Abdel-Tawwab *et al.*, 2007)**, loach **(Hao *et al.*, 2014)** and the Nile tilapia **(Rathore *et al.*, 2021; Ibrahim *et al.*, 2022)**. Serum proteins content plays several functions in cells, such as nutrient metabolism, enzymes, and hormone secretion **(Shi *et al.*, 2006)**. In the present study, serum total protein levels significantly increased in females compared to males, but were not affected by the addition of Nano-Se. The increased serum total protein with increasing the levels of Nano-Se were also noted in many fish species, including the African catfish **(Abdel-Tawwab *et al.*, 2007)**, common carp **(Ashouri *et al.*, 2015)**, sea bass **(Dawood *et al.*, 2019)** and the Nile tilapia **(Dawood *et al.*, 2020)**. It was noted that, the current findings differed from the previous studies, and this may be due to the difference in the experimental conditions, fish size, age, and the physiological status of the fish.

Proximate compositions have been used as indicators of the efficiency of feed additives and the nutritional condition of fish **(Torrecillas *et al.*, 2007; Sang *et al.*, 2015)**. The current results showed significant differences in the chemical composition of the females and males of the Nile tilapia. The dry matter and fat contents increased, while the protein content decreased in females compared to males. These differences are due to the difference in reproductive activity in adult fish. The same trend was observed with increasing the levels of Nano-Se. Similarly, **Ibrahim *et al.* (2021)** noticed that, the chemical composition and flesh quality of the Nile tilapia were significantly affected by dietary Nano-Se. In rainbow trout (*Oncorhynchus mykiss*), **Knight *et al.* (2016)** reported that, extra levels of Se in the diet significantly increased the expression of genes related to long-chain fatty acids, and lipid transport. These results are in harmony with other results on growth performance, feed utilization, and flash quality obtained in the present study. On the other hand, dietary Se forms do not affect the proximate body composition of Nile tilapia, as reported in several studies **(Rathore *et al.*, 2021; Dawit Moges *et al.*, 2022; Ghaniem *et al.*, 2022)**. Inversely, the chemical composition of other fish species was not affected by the dietary addition of Nano-Se, as reported for crucian carp **(Wang *et al.*, 2007; Zhou *et al.*, 2009)**, yellowtail kingfish **(Le and Fotedar, 2014)**, common carp **(Ashouri *et al.*, 2015)**, and rainbow trout **(Naderi *et al.*, 2019)**. **Mechlaoui *et al.* (2019)** also found that there were no significant differences in the moisture, protein, and ash levels of the liver and muscle of seabream juveniles fed by *Sparus aurata* on different sources of Se.

Fish fillet quality was classified into two categories: nutritional quality based on chemical composition, and technological and sensory quality based on interactions between chemical composition and metabolic properties (**Lawrie and Ledward, 2006**).

Ibrahim *et al.* (2021) observed that dietary bulk-Se or Nano-Se significantly affects the chemical composition and the flesh quality of Nile tilapia. More recently, **Dawit Moges *et al.* (2022)** also found that Nano-Se supplementation in Nile tilapia diets enhanced the growth performance and nutrition quality. In the present study, addition of Nano-Se to adult Nile tilapia diets resulted in an increase in the percentage of SL and FLR at times 0 and 2 hours, while WHC% and DL% were unaffected. This change may result in increased fat content and decreased protein content in muscle. In this respect, **Kristoffersen *et al.* (2006)** and **Hernández *et al.* (2009)** found that changes in muscle proteins and the endogenous proteolysis enzymes could cause low WHC. Similarly, **Lin (2014)** demonstrated that dietary Se supplementation enhanced meat quality, including the cutting force, gel strength, and WHC of juvenile grouper *Epinephelus malabaricus*.

CONCLUSION

Generally, the results in the current study exhibited that addition of Nano-Se at levels of 0.5 and 1 mg/kg diet led to harmful impacts on growth performance, feed utilization, liver functions enzymes, and flesh quality of adult Nile tilapia males and females. Despite this, enhanced health status is observed regarding the hematological parameters. Therefore, it could be concluded that the tested Nano-Se levels are not suitable for both adult Nile tilapia males and females. Thus, further studies are needed to determine the suitable dietary level of Nano-Se for both adult Nile tilapia (males and females), and/or broodstock, as well as to evaluate the expected effects of Nano-Se on reproductive efficiency parameters of both fish males and females.

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