

Research Article

The Assessment of Different Dietary Selenium Resources on Reproductive Performance, Spawning Indicators, and Larval Production of Red Tilapia (*Oreochromis mossambicus* × *O. niloticus*) Broodfish

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This trial aimed to investigate whether dietary selenium form influenced the reproductive performance of red tilapia broodfish. Four experimental broodstock diets were prepared employing two types of selenium. The first diet was free of additives and acted as the control diet. While the other three formulated diets were supplemented with conventional selenium sources (sodium selenite, Na₂SeO₃; 1 mg/kg), selenium nanoparticles (NPSe, 1 mg/kg), or a combination of them (0.5 mg Na₂SeO₃/kg + 0.5 mg NPSe/kg), respectively. Twelve cement ponds (each 24 m²) were subjected to fish brooder experimental groups. Each pond received six prespawning females (mean initial weight, 60.9 ± 0.4 g) and two males (mean weight 80.3 ± 0.8 g) of red tilapia. Each formulated diet was supplied to three broodfish cement ponds, and the reproductive traits of 18 adult female fish were monitored over 25 weeks. The findings showed that female fish fed NPSe-enriched diets had significantly higher viscera, liver, and gonad weight than other experimental groups. At the same time, the highest levels of LH, progesterone, and estradiol-17β, as well as the lowest levels of FSH, were detected in fish fed the NPSe diet, followed by those on the Na₂SeO₃ + NPSe and Na₂SeO₃ diets, respectively. Furthermore, the diameter, weight, and volume of eggs, as well as the number and weight of larvae in red tilapia brooder fish fed the various dietary selenium forms, increased markedly ($P < 0.001$). Female red tilapia broodfish given selenium-based diets enhanced all spawning performance indicators (particularly total spawned egg per pond or fish and initial spawning interval) when compared to a control group fed an unsupplemented diet. Besides, as compared to other treatment groups, the spawning frequency of each female fish fed NPSe-supplemented diets (alone or in combination with Na₂SeO₃) was considerably ($P < 0.001$) promoted. The fish group fed NPSe alone or mixed with Na₂SeO₃ had a well-developed stroma structure, many mature vitellogenic and postvitellogenic oocytes, and a remarkable intensity of mature spermatozoa in the testis. In conclusion, incorporating NPSe into red tilapia broodstock diets might be a safe and efficient way to enhance reproductive function and fry production.

1. Introduction

The seafood industry significantly contributes to the global food source while supplying an essential animal protein resource [1]. According to FAO [2] statistics, overall fish supply would rise

from 154 million tonnes in 2011 to 186 million tonnes in 2030, with aquaculture accounting for most of the increment. The highest aquaculture increase is anticipated for tilapia and shrimp [3, 4], with the most extensive expansion in India, Latin America, the Caribbean, and Southeast Asia [5]. This fast

increase in global tilapia production is due, in part, to the increasing intensification of farming practices, which has led to a critical requirement for massive quantities of fingerlings for aquaculture farms [6]. Therefore, the world's rapidly increasing seafood consumption poses a significant challenge for global fisheries and aquaculture to enhance management and develop a sustainable seafood industry [7]. Compared to Nile tilapia (*O. niloticus*), red tilapia (*Oreochromis mossambicus* × *O. niloticus*) have a higher market price, can survive salty water above 10 ppt, and can tolerate handling and transportation stress throughout the harvest process via net [8]. However, major drawbacks of red tilapia production include the difficulties of spawning certain strains of red tilapia and the poor survivability of red tilapia eggs and fry [9, 10]. Thus, new management approaches are necessary to boost fish brooder reproductive performance and larvae quality [8, 11].

Feed additive supplementation has been a popular choice in recent decades since it is a low-cost option for the fish feed industry [12]. It may be utilized to promote fish reproductive performance [13], as well as to boost fish growth performance, stimulate immunological responses, and improve the efficiency of consumed feed [14, 15]. Several functional feed additives may perform these biological functions, each having a unique chemical composition and source that can be classified into distinct categories [16, 17]. The desirable effects, contribution price, and commercial availability should all be considered when selecting suitable feed additives [18].

Selenium (Se) is a trace element required for entire cellular mechanisms and is essential for aquatic animals' performance, antioxidant defense mechanisms, and reproduction [19]. In males, Se is a component of selenoproteins, which protect spermatozoa from oxidative damage during the sperm maturation process [20]. A possible function in oocyte development and fertilization in females has been proposed, but no extensive controlled studies have been conducted [21]. Several studies have shown that selenium deficiency reduces muscle tone, depresses growth, impairs redox status, induces oxidative damage, and leads to high mortality rates, as well as reproductive failure, tissue degradation, and induced teratogenic organ malformations during the early larval developmental phase [22]. From the information mentioned above, it is evident that Se level is critical for the reproductive performance of various fish brooders and should be closely adhered to avoid Se deficiency. Thus, there are critical demands for conducting more *in vivo* studies to estimate the recommended dose and suitable selenium forum to promote the reproduction performance of various marine fish species.

In the past, sodium selenite (Na_2SeO_3) was the most prevalent and conventional inorganic source of Se supplied into animal diets, including aquafeeds [23, 24]. Many reports have proven that inorganic forms of Se are less digestible and more hazardous than organic forms in various aquatic species [25, 26]. Hence, the application of sodium selenite alone is recently being discussed. Therefore, innovative approaches for transporting selenium elements into target tissue, increasing bioavailability, and allowing for controlled release throughout the body are critical [27, 28]. Nanoparticle oral administration is

considered the most suitable and cost-effective procedure of supplementation [29]. Moreover, it has been proven that selenium nanoparticles (NPSe) absorption from the intestinal lumen is more remarkable than sodium selenite [30].

Furthermore, compared to sodium selenite, enriched fish diets with NPSe resulted in greater selenium concentrations in fish tissues [31]. Recent reevaluations of dietary Se supplementation in tilapia (*Oreochromis niloticus*) diets indicate limited or highly variable Se bioavailability [14]. Hence, assessing the combination of low doses from various selenium sources would be a profitable technique for preventing hazards that might be induced by applying inorganic forms of Se alone into fish feeds for a long period [32]. There is limited evidence on how different Se sources incorporated into brooder diet impact reproductive performance, larval production, and reproductive hormone activity in red tilapia fish. Thus, the current experiment sought to evaluate the influence of different types of dietary Se on red tilapia reproductive performance, body indices, spawning performance, larval production, reproductive hormone activity, and gonad histological structure.

2. Materials and Methods

2.1. Synthesis and Characterization of Selenium Nanoparticles (NPSe). Sigma-Aldrich provided the selenium (5 mm, >99.99%). The nanoselenium was generated through dry milling employing the high energy ball milling technique, as described by Abd El-Naby et al. [33]. The XRD and TEM examinations were applied to characterize the NPSe, and a UV spectrum analysis identified the size range and distribution of the formed nanoparticle size in solution. The TEM image showed a virtually spherical and stable form with no aggregates (Figure 1(a)), while the produced size calculated by Scherrer's equation was around 24 nm NPSe, which confirmed the results of the UV spectrum analysis (Figure 1(c)).

2.2. Prepared Experimental Broodstock Diets. Four experimental broodstock diets were prepared using various selenium types (Table 1). The formulated diets were iso-nitrogenous (35%) and iso-lipidic (10%). Except for the additional selenium sources, the total component content of all examined diets was similar. Casein and gelatin were the primary protein sources, accounting for 27% of dietary crude protein. At the same time, fish and soybean meals were included in fish diets to enhance palatability and supply the remaining 8% of crude protein. Fish oil and crude palm oil were included in broodstock diets as lipid sources. All oils offered were bought from a local market. Finally, the prepared rations were formulated to fulfill the NRC [34] standards for the essential nutritional requirements of tilapia fish. In a Hobart N50 5-Quart commercial stand mixer, the dry components were combined with oil and water to manufacture the formulated diets. The wet dough was screw-pressed through a 3-mm die, and the produced feed pellets were subdivided into 2–5-mm long pellets, fan dried, and stored in a refrigerator at -20°C until further usage. The diets for fish brooders were divided into four different categories. The first diet was free of supplements and acted as the control diet. Whereas, the other three

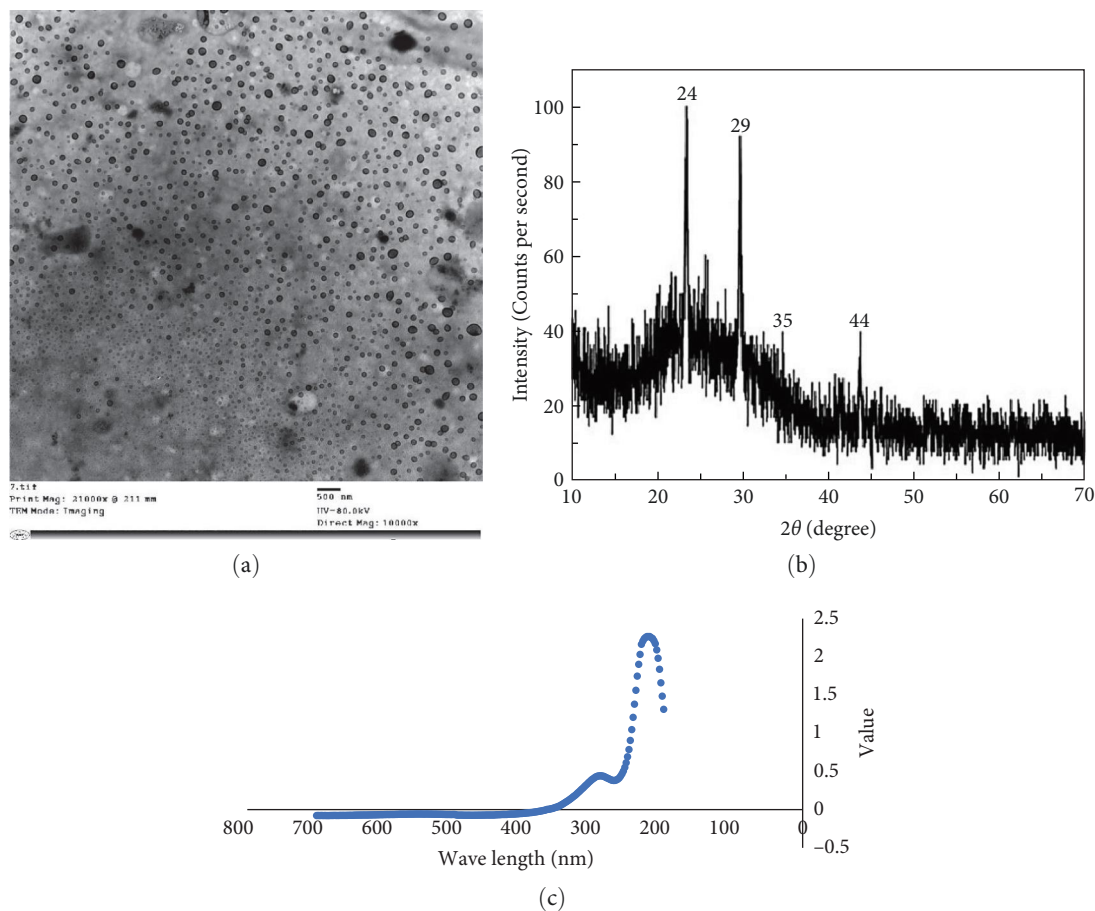


FIGURE 1: Physicochemical assessment of generated NPSe in solution, with illustrative images of (a) TEM, (b) XRD, and (c) UV spectrum.

formulated diets were supplemented with sodium selenite (1 mg $\text{Na}_2\text{SeO}_3/\text{kg}$), selenium nanoparticles NPSe (1 mg NPSe/kg), or a combination of them (0.5 mg $\text{Na}_2\text{SeO}_3/\text{kg}$ + 0.5 mg NPSe/kg). The Na_2SeO_3 and NPSe dosages were determined based on the findings of Wangkahart et al. [14] and Dawit Moges et al. [35], respectively.

2.3. Prepared Diets Chemical Analysis. The chemical composition from prepared diet samples (all analyses performed in five replicates) has been estimated and total moisture, dry matter, protein, crude fat, and ash content were computed using the Association of Official Agricultural Chemists [36] standard technique. The nitrogen-free extract (NFE) was estimated statistically by applying the following formula:

$$\text{NFE (g/kg)} = 100 - (\text{crude protein} + \text{crude lipids} + \text{ash} + \text{crude fiber}). \quad (1)$$

The chemical compositions of the formulated diets are shown in Table 1. However, the analyzed Se level in formulated diets was estimated using an atomic absorption spectrometer procedure via a VGA-77 hydride generation unit (Agilent Technologies, Inc., Santa Clara, CA, USA).

2.4. Brooders' Welfare and Management. The trial was carried out employing prespawning red tilapia brooders, *Oreochromis aureus* × *Oreochromis mossambicus*, which were earlier obtained from the marine hatchery Kilo 21 at Alexandria Government and acclimatized to our laboratory conditions. All fish brooders used in this trial were obtained from the same parental breeding pairs to avoid any possible genetic impacts on later reproductive performance. All brooders were individually color labeled before the trial began. A needle with vinyl thread was employed to penetrate the body dorsally between both the fourth and fifth spines of each fish. To mark each broodfish, different colored plastic disc tags were connected to both ends of the vinyl strand. All male red tilapia had their upper lip bone trimmed to avoid any induced injury to the female fish during mating habits. Under greenhouse conditions, 12 cement ponds were subjected to fish brooder experimental groups. Each treatment group contains three cement ponds, each 24 m² in size (4 m width × 6 m length × 1 m depth). Each pond received six prespawning female (mean initial weight, 60.9 ± 0.4 g) and two male (mean weight 80.3 ± 0.8 g) red tilapia, respectively. Six rectangular plastic pots were placed at the bottom of each cement pond to establish breeding areas for female red tilapia. Flow-through water and continuous aeration were employed

TABLE 1: Ingredient and proximate composition (g/1,000 g dry diet) of the red tilapia brooders diets.

Ingredient (g/kg)	CTR	Na ₂ SeO ₃ [*]	NPSe ⁺	Na ₂ SeO ₃ + NPSe
Soybean meal	102.4	102.4	102.4	102.4
Fish meal	38.6	38.6	38.6	38.6
Casein	226.1	226.1	226.1	226.1
Gelatin	60	60	60	60
Fish oil	45.2	45.2	45.2	45.2
Crude palm oil	46.3	46.3	46.3	46.3
Corn starch	247.6	247.6	247.6	247.6
Vitamin mix ^a	30	30	30	30
Ascorbic acid	5	5	5	5
Mineral mix ^b	20	20	20	20
Di calcium phosphate	10	10	10	10
Carboxyl methylcellulose	20	20	20	20
α -Cellulose	148.8	148.8	148.8	148.8
Total	1,000	1,000	1,000	1,000
Se supplementation				
Na ₂ SeO ₃	0.0	0.001	0.0	0.0005
NPSe	0.0	0.0	0.001	0.0005
Proximate composition				
Moisture	12.5	12.5	12.5	12.5
Crude protein	35.91	35.91	35.91	35.91
Crude lipids	10.2	10.2	10.2	10.2
Ash	4	4	4	4
Fiber	6.4	6.4	6.4	6.4
NFE ^c	43.49	43.49	43.49	43.49
Analyzed dietary Se levels (mg Se/kg)	0.59	1.38	1.49	1.44

^{*}Na₂SeO₃, sodium selenite; ⁺NPSe, selenium nanoparticles. ^aFormulation (g/kg): α -tocopheryl acetate, 2; inositol, 5; choline bitartrate, 136.06; niacin, 4.5; riboflavin, 1; pyridoxine-HCl, 1; thiamin-HCl 0.92; D calcium pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione 1.67; D-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; and cellulose, 834.167. ^bFormulation (g/kg): calcium phosphate monobasic, 135.5; calcium L-lactate hydrate, 327.0; ferric citrate, 29.7; magnesium sulfate·7H₂O, 132.0; potassium phosphate dibasic, 239.8; sodium phosphate monobasic·H₂O, 87.2; sodium chloride, 43.5; potassium iodide, 0.15; cuprous chloride, 0.2; manganous sulfate·H₂O, 0.8; cobalt chloride·6H₂O, 1.0; zinc sulfate·7H₂O, 3.0; and sodium selenite, 0.011. ^cNitrogen-free extract (NFE) = 100 – (protein + lipid + ash + fiber).

in the brooder ponds, which were kept in an open shed with a roof and natural light.

2.5. Water Quality Measurements. Throughout the study period, water quality measures were assessed once each week. The water dissolved oxygen (DO), pH, and temperature were taken in each pond using a multiparameter probe meter (HI9829-03042-HANNA[®] instruments, <https://www.hannainst.com>). Whereas, a portable colorimeter (Martini MI 405) was applied to estimate total ammonia nitrogen, ammonia (NH₃), and nitrite (NO₂) levels. Salinity was determined using a refractometer (Jelly-Tech S.R.O., BENEVOV, Czech Republic). Water quality metrics were within acceptable ranges for red tilapia hatching in all examined groups and throughout the study period [37], as follows: salinity 2.5 ppt, temperature 27 ± 0.5, DO 6.8 mg/L, pH 7.6, NH₃ 0.01 mg/L, NH₄ 0.4 mg/L, and NO₂ 0.042 mg/L.

2.6. Reproductive Traits Assessments. Over 25 weeks, the reproductive traits of each female tilapia brooder fed a different diet were observed. Every female broodfish was monitored daily for spawning activities. The produced eggs were carefully retrieved and counted from the buccal cavity of

brooding fish. Before returning the brooder fish to their separate ponds, the fish's live body weight and spawning date were reported. During the first through fourth spawning periods, a subsample of eggs was weighed, and 10 eggs were randomly selected and preserved in 10% formalin for further egg biometric measurements. Because tilapia eggs are oval, the long- and short-axis lengths were estimated under a calibrated microscope to the closest 0.001 mm to estimate the accurate egg diameter, mean egg wet weight, and mean egg volume. During spawning, the collected eggs were counted and hatched in specialized plastic bottles at a controlled water temperature (28 ± 0.5°C) in an indoor hatching system. To simulate the incubation process in the female's fish mouth, a constant water flow was supplied through the plastic bottles to keep the eggs gently floating in water. Also, the average number of fries per female fish and the weight of the fry were reported. After 25 weeks, the viscera, gonad, and liver were gently dissected and weighed to calculate the viscera-somatic index (VSI), gonado-somatic index (GSI), and hepato-somatic index (HSI). These body morphometric indices were determined as a percentage of organ weight to total fish body weight.

TABLE 2: Body-organ indices of adult female red tilapia fish fed diets supplemented with various selenium sources over 25 weeks.

Measurements	Experimental groups				P Value
	CTR	Na ₂ SeO ₃	NPSe	Na ₂ SeO ₃ + NPSe	
HSI (%)	1.87 ± 0.06 ^{b,c}	1.67 ± 0.21 ^c	2.33 ± 0.31 ^a	2.17 ± 0.29 ^a	0.008
VSI (%)	14.33 ± 0.29 ^c	14.73 ± 0.21 ^b	15.33 ± 0.12 ^a	15.0 ± 0.10 ^{a,b}	<0.001
GSI (%)	3.63 ± 0.23 ^c	4.07 ± 0.51 ^b	5.27 ± 0.25 ^a	4.87 ± 0.35 ^{a,b}	<0.001

CTR, control group fed unsupplemented diet; Na₂SeO₃, fish group administered diet supplemented with sodium selenite (1 mg Se/kg); NPSe, fish group administered diet supplemented with selenium nanoparticles (1 mg NPSe/kg); Na₂SeO₃ + NPSe, fish group administered diet supplemented with combination of sodium selenite (0.5 mg Se) and selenium nanoparticles (0.5 mg NPSe) per kg. HSI, hepato-somatic index; VSI, viscera-somatic index; GSI, gonado-somatic index. The presented data were analyzed by applying one-way ANOVA. Data are the mean ± SD. The significant between represented values was identified at $P < 0.05$. ^{a,b,c}Different lowercase superscript letters in the same row indicate that the difference between groups is significant.

2.7. Reproductive Hormone Analysis. Blood samples were taken after 25 weeks to assess the influence of dietary selenite and/or selenium nanoparticles on blood hormone levels. Blood samples were taken (six fish brooder per treatment) from the caudal vein using a 3-mL sterile syringe and afterward collected in the Eppendorf tube without any anticoagulant and separated by centrifugation of clotted whole blood (8.45 g for 15 min). The supernatant serum was isolated and maintained at -40°C in new plastic Eppendorf tubes until it was required for hormonal analysis. Hormonal concentrations in blood, including β -HCG, FSH, and LH, were measured using enzyme-linked immunosorbent assay (ELISA kits; DRG instrument GmbH), as reported by Aizen et al. [38]. Meanwhile, the steroid hormone concentrations (estradiol 17- β and progesterone) were measured using suitable FRANSA radio-immunoassay kits (Cat Nos. Estradiol 17- β : SB-ESTR; Progesterone: CM-PROG provided by FRANSA), as ascribed by Cornish [39]. All radioactivity values were collected using a Beckman Gamma 8500 Microprocessor Counter.

2.8. Gonad Histological Examination. At the end of the trial, gonad samples (six fish per group) were obtained from experimental brooder fish. The testes or ovaries were preserved in 10% neutral formalin solution, dehydrated in alcohol, cleaned in xylene, embedded in paraffin, and then dissected into 5 μm sections. The serial sections had been stained with hematoxylin and eosin as indicated by Naiel et al.'s [40] procedure. According to Tope-Jegade et al.'s [41] investigation, the gonad histological development and structure were identified.

2.9. Statistical Procedure. All results reported were conducted a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to identify whether the estimated measurements (reproductive traits, hormonal activity, and gonadal development) were significantly influenced by different dietary selenium sources (selenite or selenium nanoparticles) in fish brooder diets. Differences in means were declared significant at the 0.05 level. SPSS program v24.0.1 (SPSS Inc., Chicago, IL, USA) was applied to analyze all data.

3. Results

3.1. Body Organ Indices. The impact of two different selenium sources on organ body indices of adult female red

tilapia fish revealed significant ($P < 0.05$ or 0.001) differences between experimental groups (Table 2). The dietary administration of selenium nanoparticles (NPSe) alone or in combination with sodium selenite (Na₂SeO₃) significantly improved the HSI ($P < 0.05$), VSI values ($P < 0.001$), and the GSI ($P < 0.001$). Specifically, the fish group that received NPSe-supplemented diets showed higher HSI, VSI, and GSI values followed by fish fed a combination of NPSe and Na₂SeO₃ than other treatment groups.

3.2. Reproductive Hormone Activity. Female red tilapia administered selenium-based diets had higher corpus luteum (LH) and estradiol (E₂) levels than broodfish fed basal diets (Table 3). The maximum LH and E₂ levels were reported in adult female fish provided the NPSe diet, followed by those on the Na₂SeO₃ + NPSe and Na₂SeO₃ diets, respectively. Also, NPSe supplementation alone or in combination with Na₂SeO₃ significantly affected blood progesterone levels ($P < 0.001$). The highest progesterone levels were found in the NPSe fish group, followed by the Na₂SeO₃ + NPSe group, with the lowest in the Na₂SeO₃ and control groups, respectively. The FSH levels were significantly higher ($P = 0.001$) in fish fed Na₂SeO₃-supplemented diets alone or in combination with NPSe than in broodfish fed NPSe alone or control diets. While β -HCG levels did not differ significantly between all broodfish groups fed treatment diets.

3.3. Egg Biometric and Larval Production. All eggs appear normal in form, color, and size based on all reported subsequent spawning. In addition, the diameter, weight, and volume of eggs produced by red tilapia brooder fish received the different dietary selenium forms improved significantly ($P < 0.001$) (Table 4). Despite spawning more frequently, tilapia broodfish fed selenium-rich diets produced no morphologically aberrant eggs. Furthermore, the mean fry weight and number improved significantly ($P < 0.001$) in all selenium-based diets, with the highest values obtained in eggs from broodfish administered the NPSe diet (Table 4).

3.4. Spawning Performance. Fed female red tilapia brooder on selenium-based diets had improved spawning performance ($P < 0.001$), especially total spawned egg per pond or fish and first spawning interval, as compared to broodfish fed a basal diet (Table 5). The highest number of spawned eggs per pond or fish and the shortest first spawning time were observed in fish groups receiving NPSe-supplemented

TABLE 3: Serum reproductive hormone levels of adult female red tilapia fish fed diets supplemented with various selenium sources over 25 weeks.

Measurements (IU/mL)	Experimental groups				P Value
	CTR	Na ₂ SeO ₃	NPSe	Na ₂ SeO ₃ + NPSe	
β -HCG	2.73 ± 0.21	2.67 ± 0.23	2.87 ± 0.06	2.76 ± 0.12	0.416
FSH	2.37 ± 0.40 ^{b,c}	2.43 ± 0.06 ^{b,c}	2.20 ± 0.20 ^d	2.96 ± 0.06 ^a	0.001
LH	13.17 ± 0.29 ^d	14.37 ± 0.32 ^c	19.23 ± 0.25 ^a	15.80 ± 0.10 ^b	<0.001
Estradiol-17 β	3083.3 ± 76.37 ^d	3543.3 ± 40.41 ^c	3946.6 ± 45.09 ^a	3880 ± 75.50 ^b	<0.001
Progesterone	9.85 ± 0.30 ^{c,d}	10.23 ± 0.23 ^c	13.3 ± 0.26 ^a	12.36 ± 0.06 ^b	<0.001

CTR, control group fed unsupplemented diet; Na₂SeO₃, fish group administered diet supplemented with sodium selenite (1 mg Se/kg); NPSe, fish group administered diet supplemented with selenium nanoparticles (1 mg NPSe/kg); Na₂SeO₃+NPSe, fish group administered diet supplemented with combination of sodium selenite (0.5 mg Se) and selenium nanoparticles (0.5 mg NPSe) per kg. β -HCG, human chorionic gonadotropin beta; FSH, follicle-stimulating hormone; LH, corpus luteum hormone. The presented data were analyzed by applying one-way ANOVA. Data are the mean ± SD. The significant between represented values was identified at $P < 0.05$. ^{a,b,c,d}Different lowercase superscript letters in the same row indicate that the difference between groups is significant.

TABLE 4: Egg biometric indices and fry measurements of adult female red tilapia fish fed diets supplemented with various selenium sources over 25 weeks.

Measurements	Experimental groups				P Value
	CTR	Na ₂ SeO ₃	NPSe	Na ₂ SeO ₃ + NPSe	
Egg diameter ^a (μ m)	20.76 ± 0.68 ^c	23.9 ± 0.36 ^b	26.23 ± 0.25 ^a	25.8 ± 0.10 ^a	<0.001
Mean egg volume ^b (mm ³)	5.47 ± 0.41 ^d	6.30 ± 0.75 ^c	6.92 ± 0.68 ^a	6.80 ± 0.29 ^{a,b}	<0.001
Mean egg wet weight (mm)	4.85 ± 0.11 ^c	5.59 ± 0.38 ^{b,c}	6.14 ± 0.24 ^a	6.03 ± 0.47 ^{a,b}	<0.001
Mean fry number	792.6 ± 11.02 ^d	1016 ± 29.46 ^c	1,324.3 ± 21.36 ^a	1,256.3 ± 51.82 ^b	<0.001
Mean fry weight (g)	10.83 ± 0.15 ^d	13.93 ± 0.06 ^c	16.8 ± 0.26 ^a	15.6 ± 0.66 ^b	<0.001

CTR, control group fed unsupplemented diet; Na₂SeO₃, fish group administered diet supplemented with sodium selenite (1 mg Se/kg); NPSe, fish group administered diet supplemented with selenium nanoparticles (1 mg NPSe/kg); Na₂SeO₃ + NPSe, fish group administered diet supplemented with combination of sodium selenite (0.5 mg Se) and selenium nanoparticles (0.5 mg NPSe) per kg. ^aMean egg diameter (μ m) = (long-axis length + short-axis length)/2. ^bMean egg volume (mm³) = $\pi/6 \times$ long axis \times short axis². The presented data were analyzed by applying one-way ANOVA. Data are the mean ± SD. The significant between represented values was identified at $P < 0.05$. ^{a,b,c,d}Different lowercase superscript letters in the same row indicate that the difference between groups is significant.

TABLE 5: Spawning performance of adult female red tilapia fish fed diets supplemented with various selenium sources over 25 weeks.

Measurements	Experimental groups				P Value
	CTR	Na ₂ SeO ₃	NPSe	Na ₂ SeO ₃ + NPSe	
TSE/pond	16,092.3 ± 85.22 ^d	20,707 ± 67.61 ^c	22,572.7 ± 53.51 ^a	21,732.6 ± 24.43 ^b	<0.001
TSE/fish	2,641 ± 62.96 ^c	3,952.7 ± 61.06 ^a	3,878.3 ± 72.29 ^b	3,848.7 ± 79.05 ^b	<0.001
Relative fecundity ^a	7.0 ± 0.01	6.9 ± 0.02	7.0 ± 0.03	7.0 ± 0.01	0.926
Absolute fecundity ^b	815.3 ± 4.09	765.7 ± 15.01	787 ± 17.06	779.7 ± 25.03	0.060
Spawning frequency per female	3.77 ± 0.48 ^b	4.0 ± 0.85 ^b	4.95 ± 0.96 ^a	4.93 ± 0.17 ^a	<0.001
First spawning interval (day)	44.93 ± 1.01 ^a	31.15 ± 0.46 ^c	31.71 ± 0.77 ^c	41.67 ± 0.21 ^b	<0.001

CTR, control group fed unsupplemented diet; Na₂SeO₃, fish group administered diet supplemented with sodium selenite (1 mg Se/kg); NPSe, fish group administered diet supplemented with selenium nanoparticles (1 mg NPSe/kg); Na₂SeO₃ + NPSe, fish group administered diet supplemented with combination of sodium selenite (0.5 mg Se) and selenium nanoparticles (0.5 mg NPSe) per kg. TSE/pond, total spawned egg per pond; TSE/fish, total spawned egg per fish. ^aMean number of eggs at each spawning per gram body weight. ^bMean number of eggs at each spawning per fish. The presented data were analyzed by applying one-way ANOVA. Data are the mean ± SD. The significant between represented values was identified at $P < 0.05$. ^{a,b,c,d}Different lowercase superscript letters in the same row indicate that the difference between groups is significant.

diets. Whereas, the spawning frequency of each female fish provided NPSe-supplemented diets (alone or in combination with Na₂SeO₃) was significantly ($P < 0.001$) enhanced when compared to other treatment groups.

3.5. Gonad Histological Structure. All experimental dietary regimens had a relatively similar distribution of gonad development stages (Figure 2). Intertreatment microscopic examination revealed no remarkable variations in ova developmental stage across experimental groups. While all female brooders

administered selenium-supplemented diets showed a slight improvement compared with the control group fed unsupplemented diets. Specifically, the experimental group, which received diets supplemented with NPSe alone or in combination with selenite, had a well-developed stroma structure, a high number of mature vitellogenic oocytes, a remarkable number of well-developed postvitellogenic oocytes, and no empty follicles.

Histological sections of the testis from male red tilapia brooders fed a supplemented or unsupplemented diet revealed

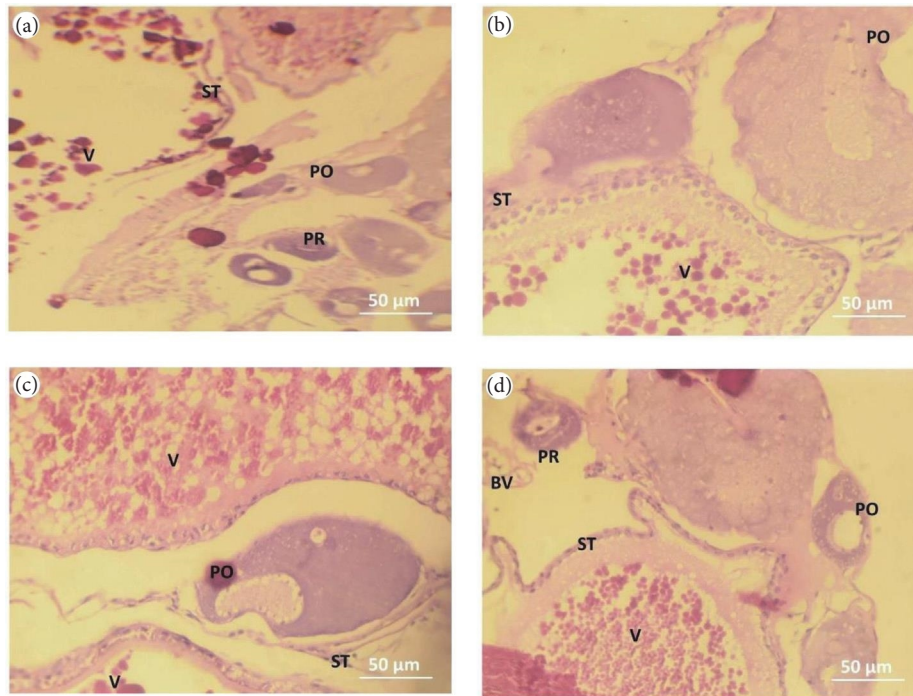


FIGURE 2: Cross-sections of tissues micrographic of ovary from female brooder fishes revealed four treated groups: (a) (CTR) control group fed basal diet, (b) (Na_2SeO_3) fish group fed selenite supplemented diets, (c) (NPSe) fish group received nanoselenium supplemented diet, and (d) ($\text{Na}_2\text{SeO}_3 + \text{NPSe}$) fish group fed a mixture of selenite and nanoselenium group. The vitellogenic oocyte (V), stroma (ST), previtellogenic oocyte phase (PR), postvitellogenic oocyte phase (PO), and empty follicle (E) (H&E and scale bar: 50 μm).

normal structure for all phases of spermatogonia, primary and secondary spermatocytes, spermatozoa, sperms, and interstitial tissues (Figure 3). There was a remarkable intensity of mature spermatozoa in the male fish group received NPSe-supplemented diet, followed by the fish group administered combination of conventional selenium source and NPSe compared to sodium selenite and control groups.

4. Discussion

During the maturation and spawning phase, most nutrients are directed into the maturing gonads, producing eggs, and ovulation activities of fish brooders [42]. In the current experiment, red tilapia brooder fed the NPSe-supplemented diets remarkably increased the VSI, HSI, and GSI. It is widely documented that the GSI parallels gonadal maturation peaking at the ripe stage and subsequently declining following spawning and spermination, particularly in females [43]. Moreover, higher HSI values indicate greater hepatic involvement in the vitellogenesis process [44]. Also, determining the VSI values might be a very clear indication of substrate mobilization during vitellogenesis process [45]. Similarly, Saffari et al. [46] reported that increasing dietary nano-Se levels raised selenium retention in blood, liver, ovary, eggs, and 3-day larvae of female Arabian yellowfin sea bream (*Acanthopagrus arabicus*). Moreover, the findings demonstrated that NPSe supplementation may stimulate female lipid stores within the abdomen region, which are used as a source of energy for ovarian expansion during the reproduction phase of Adult Nile Tilapia (*Oreochromis niloticus*) fish [47].

Despite that, the reproduction phase significantly differs from earlier life stages since energy is metabolized to the reproductive organs rather than utilized for growth, notably in *Danio rerio* females where nutrients are stored in the ovarian oocytes during vitellogenesis [48]. The effects of selenium nanoforms on improving gonad weight and activity might be attributed to their ability to stimulate the expression of both growth hormone (GH) and insulin-like growth factor (IGF-1) genes, which is associated with the improvement of performance, development, reproduction, and differentiation of zebrafish (*Danio rerio*) [49].

Similar to other vertebrates, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) induce gonadal growth and development in teleost fishes [50]. For example, during spermatogenesis and vitellogenesis, FSH plasma levels predominate, while LH levels rise at spermatogenesis in fish male brooders and final oocyte maturation and ovulation in female fish brooders of salmon (*Salmo salar*) [51]. Moreover, plasma levels of FSH in the sea urchin (*Lytechinus variegatus*) male fish brooders are linked with levels of testosterone and androgens, while circulating levels of LH in both sexes correlate with estradiol and progesterone levels [52]. According to our findings, female fish brooders fed an NPSe-supplemented diet had higher blood LH, FSH, progesterone, and E_2 levels. In the same context, according to the gonad developmental histological analysis, female fish groups fed NPSe-supplemented diets had a well-developed stroma structure, a high number of mature vitellogenic oocytes, a remarkable number of well-developed postvitellogenic oocytes, and no empty follicles. Moreover,

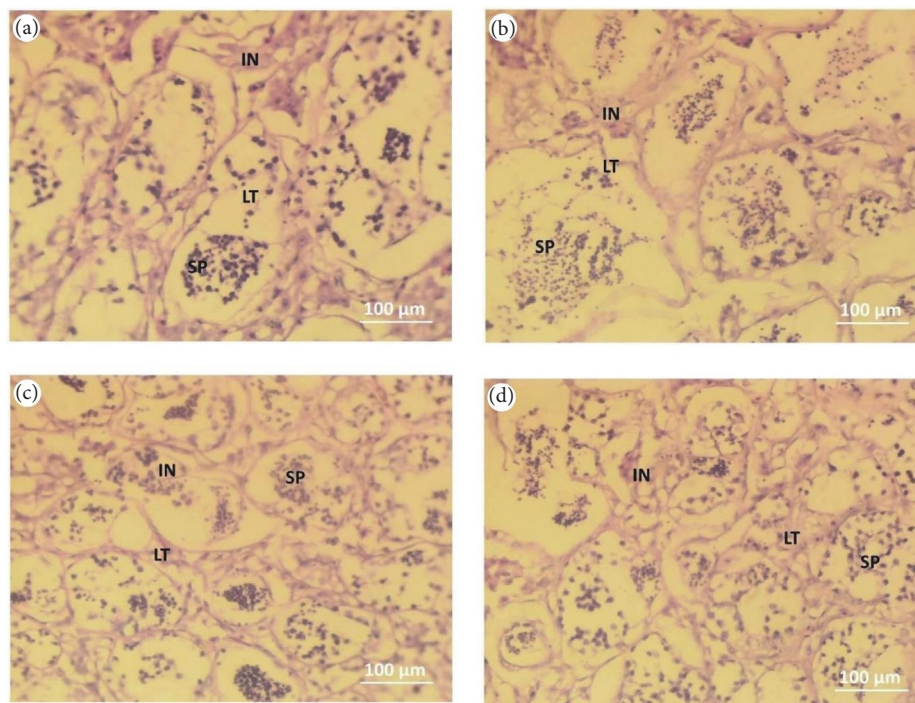


FIGURE 3: Cross-sections of tissues micrographic of testis from male brooder fishes revealed four treated groups: (a) (CTR) control group fed basal diet, (b) (Na_2SeO_3) fish group fed selenite supplemented diets, (c) (NPSe) fish group received nanoselenium supplemented diet, and (d) ($\text{Na}_2\text{SeO}_3 + \text{NPSe}$) fish group fed a mixture of selenite and nanoselenium group. Lobule of testicles (LT), spermatozoa (SP), and interstitial tissues (I) (H&E and scale bar: $100\ \mu\text{m}$).

compared to the Na_2SeO_3 and control groups, the male fish groups receiving NPSe-supplemented diets had a notable intensity of mature spermatozoa. The high levels of gonadotropic hormones associated with reproduction in red tilapia brooders indicated the capability of NPSe to upregulate the gonadotropin-releasing hormone receptor (GnRHR) in the pituitary gland [53]. Specifically, NPSe seems to enhance the release of LH from the pituitary gland as well as the activation of LH (LHR) and follicle-stimulating hormone (FSHR) receptors in the ovaries. Besides, Se is necessary for synthesizing sex hormones such as testosterone, estradiol (E_2), and progesterone [35].

It is well-established that supplementing the rainbow trout brooders' diet with Se increases the number of spawning females while influencing the quantity of Se delivered into their offspring [48]. The present trial found that NPSe-supplemented diets significantly improved the spawning performance of female red tilapia brooders. The acquired results were comparable to the findings of Saffari et al. [54] that dietary administration of 2–4 mg NPSe/kg in a plant protein-based diet had desirable influences on the reproduction performance of Arabian yellowfin seabream female fish (*Acanthopagrus arabicus*). Moreover, Khalafalla et al. [55] demonstrated that supplementing the Nile tilapia (*Oreochromis niloticus*) diet with 0.3 ppm NPSe significantly improved spawning and reproductive performance. The variations in spawning characteristics between our experimental groups that received various forms of dietary Se supplementation could be linked to altered vitellogenesis and blood steroid levels, which were previously reported to be influenced in

rainbow trout (*Oncorhynchus mykiss*) females fed higher level of selenomethionine (4.54 Se mg/kg) [48]. Nevertheless, any direct comparisons with previous data from other research should take into account the variations in tilapia strains, brooders age, and experimental settings employed.

According to Dziewulska et al. [56], the optimum Se level in the female fish brooder body positively influenced egg survival and hatching rate. Our findings showed no morphologically abnormal eggs in red tilapia brooder-fish administered selenium-based diets. Additionally, the mean fry weight and number increased significantly in all fish groups that received NPSe-supplemented diets. Similarly, Saffari et al. [54] demonstrated that eggs from *Acanthopagrus arabicus* females administered the control or 2 mg NPSe per kg diets exhibited better hatchability percentage and survived post larvae. The improvement in all egg biometric indicators might be attributed to NPSe's antioxidant properties against free radicals and the protection of biological membranes from lipid oxidation [46].

Selenium toxicity may be associated with lower reproductive success in several fish species. For example, it has been found that high selenium levels in Belevs Lake reduce fish populations and are connected to fish reproductive failure [19]. In addition, many necrotic, degenerating ovarian follicles were detected in redear sunfish (*Lepomis microlophus*) and green sunfish (*Lepomis cyanellus*) female fish raised in selenium-contaminated areas of Martin and Belevs Lakes, suggesting that selenium harms the ovaries of this species [57]. Our gonad histological examination findings reveal that the ovary and testis of fish brooder groups that

receive any form of selenium have normal structures. Also, the findings of our investigation demonstrated that the applied levels of both selenium forms are safe for fish brooders and have no adverse effects on gonad structure. Furthermore, the acquired results were consistent with Lemly's [58] findings that dietary concentrations of selenium in dry feed above 3 mg per kg over a longer period could be hazardous to rainbow trout. Therefore, depending on the fish species, feeding trial period, and experimental regimes, the incorporation of Se nanoparticles into fish brooder feed diets ranges from 0.15 to 4 mg/kg [59].

5. Conclusion

Finally, dietary selenium-supplemented diets may improve red tilapia brooder reproduction and spawning performance due to its forms. Specifically, dietary NPSe resulted in larger gonad sizes, earlier first spawning activity, shorter inter-spawning intervals, a longer period of broodfish fertility, higher overall egg biometric indices, high activities of gonadotrophic hormones, and no incidence of egg deformities, when compared to broodfish fed a sodium selenite-supplemented or unsupplemented diets. Thus, including NPSe (1 mg NPSe/kg) in red tilapia broodstock diets might be a safe and efficient strategy to improve reproductive function and fry production.

Data Availability

The authors confirm that the data supporting the findings of this study are available upon reasonable request from the corresponding author.

Ethical Approval

The in vivo trial used fish subjected to the general protocol standards for the Care and Use of Laboratory Animals and approved by Zagazig University's Institutional Animal Care and Use Committee (no ZU-IACUC/2/F/159/2023).

Conflicts of Interest

The authors declare that they have no conflicts of interest

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