Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 3979203, 17 pages
https://doi.org/10.1155/2022/3979203

Research Article

Oxidative Stress-Related Semen Quality and Fertility in the Male Arabian Yellowfin Sea Bream (Acanthopagrus arabicus) Fed a Selenium Nanoparticle-Supplemented Plant Protein-Rich Diet

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Received 16 May 2022; Revised 3 July 2022; Accepted 8 July 2022; Published 19 July 2022

Academic Editor: Liqiao Chen

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A three-month research was conducted to evaluate the influence of supplementing a plant protein- (PP-) rich diet (60% of fish meal (FM) was replaced with a mixture of alternative PP sources) with selenium nanoparticles (Se-N) on sperm kinetics, fertilization capacity, and antioxidative responses in Arabian yellowfin sea bream (Acanthopagrus arabicus) males. In this point, graded levels of Se-N at 0, 0.5, 1, 2, and 4 mg/kg were added to the PP-rich diet. Also, a FM-based diet was used as a positive control. Two hundred and eighty-eight brooders (males: 195.3 ± 10.8 g and females: 237.5 ± 8.1 g; mean ± standard error) were randomly distributed into eighteen 10 m^3 rectangular concrete tanks (8 males: 8 females in each tank), and every three tanks were fed with one of the experimental feeds for three months. The seawater temperature and salinity were 18-22°C and 40-41‰, respectively, during the husbandry period. Selenium retention increased in the liver, testes, and semen of males with increasing dietary Se-N level ($P < 0.05$). Sperm density in fish fed with 2 mg Se-N/kg PP-rich diet (42.1×10^9/mL) was higher than those fed control, 0, 0.5, and 1 mg Se-N/kg PP-rich diets. The percentages of sperm motility in 60 s and 300 s after activation, sperms with straight movement, the longevity of sperm motility, and fertilization rate increased with increasing Se-N level in the PP-rich diets ($P < 0.05$). The highest and lowest semen superoxide dismutase activities were in fish fed 4 and 2 mg Se-N/kg PP-rich diets, respectively. The highest glutathione peroxidase activity in semen was in fish fed 4 mg Se-N/kg PP-rich diet, and the lowest values were in fish fed 0 and 0.5 mg Se-N/kg PP-rich diets. Total antioxidant capacity values in semen of fish fed 1 and 4 mg Se-N/kg PP-rich diets were higher than the other treatments. Fish fed with PP-rich diet without any Se-N supplementation had higher malondialdehyde level in the semen compared to the other groups. Results showed that supplementing PP-rich diet with 2-4 mg Se-N/kg can improve sperm kinetics and enhance fertility potential in A. arabicus males.

1. Introduction

Fishmeal (FM) is well known as the gold standard, nutrient-dense, and unique protein source in feed formulation for most of the farmed aquatic species, mainly due to its high palatability, digestibility, balanced amino acid profile, and the considerable amounts of long-chain polyunsaturated fatty acids (LC-PUFA) and phospholipids in its residual fat [1–4]. It is also a valuable source for trace elements, including selenium (Se), that are nutritional requirements for fish growth [5]. However, the use of marine-derived ingredients including FM and fish oil (FO) in aquafeeds cannot be sustainable in long term due to the environmental and ecological concerns such as heavy fishing pressure on fish stocks

[6]. In 2018, about 19% of the world capture fishery production was processed to FM and FO, and over 70% of these ingredients were directed to aquafeed industry, especially pelleted feeds for carnivorous fish species (The Marine Ingredients Organization). Therefore, introducing economic and eco-friendly alternative protein sources are the major concerns for sustainability of aquaculture industry. During last two decades, terrestrial protein sources, particularly plant proteins (PPs), have been considered as inexpensive and nutritious feedstuffs allowing for FM sparing and reducing "Fish In: Fish Out" ratio for major fed farmed aquatic species [7, 8]. However, PPs display several restricting factors such as the presence of diverse antinutritional factors, lower protein content, deficiencies or imbalances in some essential amino acids, lesser palatability and digestibility, and scarcity or low bioavailability of trace elements compared to FM [1, 9, 10]. In addition, with the ongoing FM-sparing strategy by application of PP sources in aquafeeds, generally the amounts of trace elements such as Se may not meet its nutritional requirement in various farmed aquatic species. Thus, supplementing aquafeeds with Se is required in PP-rich diets [11].

Selenium is an important trace element that exerts its biological functions including structural, catalytic, and antioxidative roles through the biosynthesis and the activation of selenoenzymes and selenoproteins (e.g., glutathione peroxidase and thioredoxin reductases) [11, 12]. Furthermore, Se has vital role in cell signaling, metabolism of thyroid hormones, and immunocompetence of organisms [13–17]. Up to 41 selenoproteins have been so far identified in teleosts [18]. The Se requirements in fish are ranged between 0.15 and 0.70 mg/kg of feed, depending on species, life stage, physiological state, feed formulation, Se form in diet, water quality, and the culture conditions [19–21]. A meta-analysis study revealed that species and glutathione peroxidase (GPx) activity in the liver are the most reliable criteria for determining dietary Se requirement in fish [19]. In this context, it has been shown that selenoproteins are important to maintain the cellular redox, antioxidant capacity, and neutralizing reactive oxygen species (ROS) [22], thus play critical role in male gamete quality [23] as oxidative stress reduces sperm quality [24]. It has been also observed that Se increases the spermatozoa viability by enhancing semen antioxidative capacity resulting in improvement of sperm production and male fertility [22, 25]. In fact, the low amounts of cytoplasm, aerobic metabolism, and the presence of immature and/or abnormal spermatozoa and leucocytes, which are the main sources of ROS in semen, increase spermatozoa susceptibility to oxidative stress [26]. Furthermore, high LC-PUFA levels in the cytoplasm of spermatozoa increases their vulnerability to ROS, lipid peroxidation, protein, and DNA oxidation. Thus, disturbance of redox balance in spermatozoa leads to the impairment of their motility as a consequence of reduction of its plasma membrane fluidity, inactivation of vital enzymes, induction of DNA fragmentation, apoptosis, and chromatin abnormalities [27–29]. Selenoproteins (e.g., selenoprotein P and phospholipid hydroperoxide glutathione peroxidase) transport Se to the testes, and its absorption is regulated by apolipoprotein E receptor 2 in sertoli cells [29–33]. The optimum amount of Se is required for normal spermatogenesis in the form of GPx4, and its uptake increases in late spermatids suggesting the vital role of Se in protecting spermatozoa against ROS [34]. During spermatogenesis, GPx4 provides the integrity to the midpiece of spermatozoon by becoming a structural component of mitochondrial sheath circumventing the flagellum, which is a crucial cell part for stability and motility [22]. In this context, it has been confirmed that the inclusion of glutathione in an extender solution increased sperm quality in African sharptooth catfish (Clarias gariepinus) after cryopreservation; it is also provided protection against mercury (Hg) toxicity on the male reproductive system [23]. Moreover, supplementing PP-based diet with organic Se (Se-yeast) positively affected Se-dependent GPx activity and upregulated the gene expression of selenoprotein P in rainbow trout (Oncorhynchus mykiss) fry [35]. Furthermore, Se supplementation in a PP-based diet for rainbow trout brooders could improve reproductive performance, antioxidant capacity, and stress resistance in their progeny [36–38]. In zebrafish, sex-specific expression patterns for selenoproteins were found and Se supplementation was shown to increase its reproductive success [39]. These studies show that Se could improve sperm quality, although optimal level of Se might be species specific [24].

The Se products exist in different forms on the market including organic, inorganic, and nanoselenium (Se-N), and its form profoundly affects its metabolism and efficiency. A plethora of studies demonstrated that organic Se (e.g., Se-enriched yeast or hydroxyselenomethionine) has more bioavailability and higher beneficial effects on health and performance of fish compared to inorganic forms (i.e., Na_2SeO_2) [12]. On the other hand, it has been confirmed that dietary Se-N has more bioavailability, higher chemical stability and biocompatibility, lower toxicity, and great capacity to gradually release Se after ingestion in fish than the other forms. What means Se-N can be utilized more efficiently for selenoprotein synthesis [11, 40]. A recent study reported that the supplementation of Se-N (0.3 mg/kg body weight) markedly enhanced the level of Se in the testes of male boar goats; what resulted in increased GPx and ATPase activities in the sperm and consequently increased the protection of membrane integrity, as well as the conservation of the mitochondria in spermatozoa compared to those fed control diet (0.06 mg/kg body weight) [41].

During reproduction, the energy and nutritional requirements and metabolism markedly increase and may compromise antioxidant defense regulation and consequently lead to oxidative stress [42]. Thus, the present study is aimed at evaluating the effects of supplementing Se-N in a PP-rich diet on sperm quality, fertilization rate, and antioxidant capacity in Arabian yellowfin sea bream (Acanthopagrus arabicus, Sparidae) which is a great carnivorous species to develop marine aquaculture in the South coasts of Iran [43]. The Arabian yellowfin sea bream possesses many advantages as the brooders spawn without hormonal manipulation in captivity and is also characterized by high fecundity, high survival rate during larval stage, ease of larviculture, appropriate growth, and high tolerance to

diverse environmental conditions [43, 44]. Former studies have reported nutritional requirements of this species regarding macronutrients [45], but the information regarding its dietary mineral requirements is still scarce. The present study provides very valuable information to better understand the biological effects of Se in a PP-rich diet on sperm quality to develop methods for artificial reproduction and seed production in fish farms.

2. Materials and Methods

2.1. Ethical Statement. All experiments and samplings were done based on the ethical recommendations in the guide for the care, protection, and use of laboratory animals approved by the Institutional Animal Care and Use Committee of the Iranian Fisheries Science Research Institute [46].

2.2. Diets. Basal feeds including a FM-based diet as a positive control and a PP-rich diet in which 60% of FM was replaced with a mixture of PP sources were designed (Table 1). Bovine serum albumin-loaded-Se-N (30–45 nm particle size, spherical in shape, 99.95% purity, 3.89 g cm^3 true density, Iranian Nanomaterials Pioneers, Iran) was added into the PP-rich diet at 0, 0.5, 1, 2, and 4 mg/kg to produce experimental feeds. Selenium nanoparticles were synthetized by inclusion of bovine serum albumin to the redox system of selenite and glutathione [47]. Each milliliter of the bovine serum albumin-loaded-Se-N contained 1 mg of Se-N. To prepare the diets, all dry feedstuffs were blended for 20 min, and oils were then poured on them and mixed for 10 min. Finally, the prescribed Se-N dosages were dissolved in distilled water, then added to the mixture, and blended for 10 min to form a soft dough, which subsequently were pelleted with a meat grinder equipped with a 6 mm die stainless steel grinder plate. The pellets were dried at room temperature with an oscillating table air circulation. After preparation of the experimental diets, they were kept in a freezer -20°C until use.

2.3. Animals and Experimental Groups. A. arabicus brooders were wild caught and stocked into three 10 m^3 circular tanks (10 fish/m^3) and acclimated to the captive condition. Tanks were supplied with filtered and disinfected (calcium chloride 10 mg/L and neutralized with 5 mg/L sodium thiosulfate) seawater (40.2‰, 18-20°C) in a flow-through system (10 L/min). During acclimation period, brooders were fed with a commercial pelleted feed containing 42% crude protein, 15% crude lipid, and 20 MJ/kg gross energy (BFM21, Shiraz, Iran).

The sex of brooders was identified by mild pressure on the abdomen of males with running milt and using cannulation method for the females with oocyte stages III (350-400 μm) and IV (400-500 μm). Then, the weights of 288 brooders (males: 195.3 ± 23.1 g and females: 237.5 ± 28.6 g; mean ± SD) were individually determined, and they were stocked into eighteen 10 m^3 rectangular concrete tanks (16 fish in each tank, 8 males: 8 females) with running seawater and 50% water exchange rate daily. Brooders were fed with the six experimental feeds every day at noon for 90 d during prespawning (60 d) and spawning (30 d) periods. The water quality parameters during the experiment were as follows: temperature: 18-22°C, salinity: 40-41‰, pH: 7.5-8.2, and dissolved oxygen: 7.2-7.8 mg/L. The photoperiod was 8 h light (L) and 16 h darkness (D) during prespawning period; meanwhile, the photoperiod during spawning of brooders was 11L : 13D.

Table 1: Ingredients and composition of the fishmeal- (FM-) and a plant protein- (PP-) based diet (FM) used in the present study.

Ingredients (g kg^{-1})[a]	FM-based diet	PP-rich diet
Fish meal	750	300
Soybean meal	-	100
Wheat gluten meal	-	200
Corn gluten meal	-	200
Beef gelatin	25	25
Wheat middling	120	30
Fish oil	20	31
Canola oil	20	31
Soy lecithin	20	20
DL-methionine	-	3
L-Lysine	-	5
Se-free premix[b]	40	40
Butyric acid	2.5	2.5
Vitamin C	2.5	2.5
Di-calcium phosphate	-	10
Proximate composition (g/kg)		
Crude protein	500.6	502.9
Crude lipid	163.5	150.4
Ash	112.5	106
Moisture	77	64

[a]Composition of ingredients (fish meal (620 g kg^{-1} crude protein and 150 g kg^{-1} crude lipid), soybean meal (410 g kg^{-1} crude protein and 42 g kg^{-1} crude lipid), wheat gluten meal (500 g kg^{-1} crude protein and 40 g kg^{-1} crude lipid), corn gluten meal (714 g kg^{-1} crude protein and 41 g kg^{-1} crude lipid), beef gelatin (850 g kg^{-1} crude protein and 42 g kg^{-1} crude lipid), and wheat middling (120 g kg^{-1} crude protein and 30 g kg^{-1} crude lipid)). [b]Included are as follows: (as mg kg^{-1} of premix) vitamin A, 50000 (IU kg^{-1}); vitamin D3, 10,000 (IU kg^{-1}); vitamin E, 30; vitamin B_1, 20; vitamin B_2, 10; vitamin B_6, 3; vitamin K_3, 15; nicotinamide, 150; calcium pantothenate, 40; copper (Cu^{++}), 30; iron (Fe^{++}), 100; zinc (Zn^{++}), 150; manganese (Mn^{++}), 200.

2.4. Semen Collection and Artificial Fertilization. Under physiological condition, the A. arabicus broodfish naturally spawn in captivity without any need to employ hormonal manipulation. Sperm quality was evaluated throughout the spawning season at the beginning (day 1), middle (day 15), and at the end of the spawning season (day 30). In this regard, 4 males from each tank were individually anaesthetized with 300 mg/L 2-phenoxyethanol [48], semen was then collected by mild pressure on the abdomen into a 2 mL sterile microtube, and its volume also recorded. Particular consideration was taken to avoid semen contamination with water and urine, and the genital pore was wiped by a tissue paper. The collected semen was aliquoted to three 200 μL portions. Two aliquots were transferred into a liquid nitrogen for determining Se concentration and antioxidant parameters. The last portion was kept on ice to determine sperm quality parameters.

To collect the eggs, the inlet water was closed and reopened at 05 : 00 pm and 07 : 00 am, respectively. Then, the floating eggs were collected by a funnel net (300 μm), washed with seawater, and transferred into a 1 L graduated cylinder to separate buoyant (alive) eggs. For incubation, the buoyant eggs from each experimental tank were distributed into three 20 L plastic buckets (10 mL eggs in each bucket) filled with disinfected seawater (20–21°C) and mild aeration. To evaluate fertilization rate, three samples of 100 buoyant eggs of each bucket were examined by a microscope (40x magnification) to count eggs with cell divisions. Fertilization rate was assessed as total number of eggs with cell division × 100/total number of incubated eggs. To evaluate hatching rate, three samples of 100 fertilized eggs were transferred into three 1 L plastic beakers filled with seawater under mild aeration. Hatching rate was assessed as total number of hatched eggs × 100/total number of incubated eggs. The fertilized eggs were hatched within 20 to 24 h. These sampling methods were done for three days (days 1, 15, and 30 of the spawning period) for each replicate (3 days × 3 tanks × 3 buckets = 27 samples for each group).

2.5. Circulation, Tissue and Semen Antioxidant Capacity, and Se Concentration. Antioxidant capacity parameters and Se concentration were measured in the serum, tissues (the liver and testes), and semen. To collect the blood, three anaesthetized males from each replicate were bled with a syringe from their caudal vein. The collected blood was centrifuged (5000 g, 10 min), and serum was separated, aliquoted to three 100 μL portions, transferred into liquid nitrogen, and kept at -80°C until analyses of antioxidant capacity parameters and Se concentration ($n = 9$ samples per treatment). Three males of each replicate were sacrificed (overdose of 2-phenoxyethanol, 1000 mg/L) to collect the liver and testes in cryotubes and frozen in liquid nitrogen for determining antioxidant capacity parameter concentration ($n = 9$ samples per treatment) and Se concentration ($n = 3$ samples per treatment). Also, total mass of the liver and testes were determined.

2.6. Determination of Se Concentration. The AOAC [49] standard methods were used for proximate analyses of the experimental feeds. To measure Se concentration in the experimental diets, tissues, and semen, samples were first digested using concentrated nitric acid and hydrogen peroxide. The digesta was diluted up to reduce nitric acid concentration to 2%, and Se concentration was measured by application of inductively coupled plasma mass spectrometry (Agilent 7500; Yokogawa Analytical Systems, Japan) [35].

2.7. Spermatozoa Quality Assessment. After semen collection, the biomass of semen samples was measured by using a microbalance (Sartorius, TE214S, Germany, precision: 1 μg). The percentage of spermatozoa motility and its longevity were visually observed and evaluated using a light microscope (Motic BA210, China) at magnification of 100x. To activate spermatozoa motility, a drop of fresh semen was mixed with seawater at ratio 1 : 1000-1 : 2000 on a glass slide. The percentage of motility was determined by evaluating the motility of 50 randomly chosen spermatozoa on a glass slide that were tracked for each treatment [50]. The longevity of spermatozoa motility was assessed by using a stopwatch, just after mixing of semen with the seawater till almost all spermatozoa became immotile in the visual field. In addition, the spermatozoa motility was also recorded after 60 s and 300 s. These sampling methods were done for three days (days 1, 15, and 30 of the spawning period) for each replicate
(3 days × 3 tanks × 4 males = 36 samples for each group).

To assess spermatozoa density, semen was mixed with buffered formalin 4%, pH: 7.0 at the ratio of 1 : 20000. Then, 10-μL sample was loaded into a hemocytometer chamber, covered with a coverslip, and left for spermatozoa sedimentation for 10 min. Finally, spermatozoa were counted by use of a dark-field microscope (Motic BA210, Speed Fair Company, Hong Kong, China) at 100x magnification. The spermatocrit was also measured using microhaematocrit capillary tubes (75 mm in length and 1.1–1.2 mm in diameter) that were filled with semen and were centrifuged (3000 rpm for 10 min) and defined as the ratio of spermatozoa volume to total volume of sperm × 100.

2.8. Antioxidant Capacity Analyses. Samples of the liver, testes, and semen were defrosted, weighed, and then homogenized in ratio of 1 to 9 (w/v) of cold potassium phosphate buffer (0.1 M, pH = 7.4, 4°C) at 10,000 rpm for 60 s close to ice. The homogenate was centrifuged (9,000 rpm, 30 min, 4°C); the supernatant was removed and aliquoted (5 × 500 μL) and kept at -80°C.

The catalase activity (CAT) was measured according to Koroluk et al. [51]. First, 5 μL of tissue homogenate or serum samples were mixed with 150 μL of reaction solution (10 mM H_2O_2 diluted in 0.05 mM Tris-HCl buffer, pH 7.8, room temperature) and incubated for 10 min. The reaction was halted with 50 μL ammonium molybdate (4%), and the optical density absorbance was measured at 410 nm.

The activity of superoxide dismutase (SOD) was assayed as described by Kono [52]. For SOD activity evaluation, 500 μL of homogenate or serum samples were mixed with reaction solution (1300 μL carbonate buffer (pH: 10.2), 500 μL of nitro blue tetrazolium (60 μM), 100 μL of Triton X-100 (0.6%), and 100 μL of hydroxylamine hydrochloride (20 mM, pH: 6.0)). The optical density absorbance was measured at 540 nm for 5 min at room temperature.

Glutathione peroxidase activity (GPx) was assayed according to Maraschiello et al. [53]. To evaluate GPx activity, 20 μL of samples (serum or tissue homogenate) was blended with 150 μL of a reaction mixture (1 mM NADPH, 1 mM glutathione, 1.2 U glutathione reductase, 50 mM Tris-HCl, and 5 mM EDTA). 20 μL of cumene hydroperoxide was added into the mixture for initiating the reaction. The optical density absorbance was measured at 340 nm for 5 min at room temperature against a blank consisting of all the reagents except the sample and a positive control containing all reagents+bovine erythrocyte GPx.

Table 2: Body characteristics of the male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentrations of selenium nanoparticles (Se-N). Data are mean ± SD ($n = 24$).

	FM-based diet	Se-N supplemented PP-rich diets (mg/kg)				
		0	0.5	1.0	2.0	4.0
IBW (g)	197.0 ± 17.6	193.2 ± 24.8	213.0 ± 3.82	203.4 ± 21.2	209.2 ± 6.6	207.6 ± 6.4
FBW (g)	207.8 ± 12.6	201.2 ± 11.3	221.1 ± 9.4	210.9 ± 38.1	214.1 ± 7.6	215.6 ± 20.8
SGR (%IBW/day)	0.06 ± 0.0	0.05 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.03 ± 0.0	0.04 ± 0.0
K (%)[1]	2.1 ± 0.7	2.0 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.2	2.0 ± 0.3
HSI (%)[2]	2.8 ± 1.2	2.3 ± 0.9	2.2 ± 1.0	2.3 ± 0.5	2.6 ± 1.2	2.0 ± 0.5
GSI (%)[3]	4.9 ± 1.3	4.8 ± 2.3	3.2 ± 1.8	3.7 ± 2.0	3.1 ± 1.8	3.4 ± 0.7
Survival (%)[4]	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

Abbreviations: IBW: initial body weight; FBW: final body weight; K: Fulton's condition factor; HSI: hepatosomatic index; GSI: gonadosomatic index. [1]$K = (\text{FBW/standard length}^3) \times 100$. [2]HSI = (liver weight (g)/FBW) × 100. [3]GSI = (gonad weight (g)/FBW) × 100. [4]Survival = (number of fish in each group remaining on day 90/initial number of fish) × 100.

Total antioxidant capacity (TAC) was measured according to Benzie and Strain [54]. In this regard, 100 μL of serum or homogenate samples were mixed with 3 mL of the reactive solution (-
10 mM TPTZ (2, 4, 6 − tripyridyl − s − triazine) + 40 mM HCl + FeCl3 (20 mM) + acetate buffer 0.3 M, pH 3.6). The optical density absorbance was measured at 593 nm for 5 min at room temperature. Calculations were performed using a calibration curve of $FeSO_4{\cdot}7H_2O$ (100 to 1000 $\mu M\,L^{-1}$).

The production of malondialdehyde (MDA) as lipid peroxidation index was measured in the samples according to Buege and Aust [55]. In this regard, 200 μL of tissue homogenates or serums were reacted with 2 mL of thiobarbituric acid reagent (-
0.375%thiobarbituric acid + 15%trichloroacetic acid + 0.25 N HCl). The mixture was boiled for 15 min, cooled, and centrifuged. The optical density absorbance of the supernatant was measured at 532 nm. Lipid peroxidation was expressed as thiobarbituric acid concentration, by using 1,3,3,3 tetraethoxypropane as a standard. The total protein content of the serum or tissue homogenates was determined by the Bradford method [56].

2.9. Statistics. Data were analyzed by applying the SPSS software version 23.0. Kolmogorov-Smirnov and Leven tests were applied for evaluation of normality and homogeneity of variance, respectively. A one-way ANOVA followed by Duncan's post hoc analyses were performed to evaluating the effects of dietary Se-N on physiological responses at $P < 0.05$ for all statistical tests. The Pearson product moment correlation test was used to determine any correlation among reproductive performance and sperm's antioxidative parameters. In all cases, $P < 0.05$ was considered as significant.

3. Results

3.1. Diet Se Concentrations and Fish Growth. The actual Se concentrations were measured to be 1.34 ± 0.10 in FM-based diet and 0.67 ± 0.08, 1.09 ± 0.05, 1.48 ± 0.18, 2.60 ± 0.14, and 4.82 ± 0.10 mg/kg in PP-rich diet supplemented by Se-N at 0, 0.5, 1, 2, and 4 mg/kg, respectively. There was no mortality during the experiment. In addition, biomass of fish, Fulton's condition factor, hepatosomatic index, and gonadosomatic index did not show significant differences at the end of the experiment ($P > 0.05$; Table 2).

3.2. Tissue Se Concentrations. Increased dietary Se-N levels in the PP-rich diet resulted in increases in Se concentrations in the liver, testis, and semen ($P < 0.05$; Figure 1). The highest Se concentrations in the liver, testes, and semen were observed in fish fed 4 mg/kg Se-N, and the lowest concentrations were observed in fish fed 0-0.5 mg/kg Se-N. Compared to FM-based diet, Se concentrations in the liver, testes, and semen were higher in fish fed PP-rich diet containing 4, 4, and ≥2 mg/kg Se-N, respectively ($P < 0.05$; Figure 1).

3.3. Semen Quality and Fertility Assessment. Semen quality of A. arabicus differed among experimental groups (Table 3). Higher spermatozoa density, percentage of spermatozoa motility evaluated at 60 or 300 s postactivation in seawater, and spermatocrit were observed in fish fed with Se-N supplemented PP-rich diets than those of fish fed FM-based diet ($P < 0.05$). Among the fish fed various Se-N levels in the PP-rich diet, spermatozoa density, percentage of spermatozoa motility at 60 and 300 s postactivation, and spermatocrit were significantly higher at $4 \geq 2 \geq 1$ and 0.5 mg/kg Se-N, respectively ($P < 0.05$). However, semen volume and mass did not differ among fish fed various Se-N levels in the PP-rich diet ($P > 0.05$). In contrast to these parameters, the highest semen volume and mass were observed in fish fed FM-based diet, which were higher than those of fish fed PP-rich diet containing Se-N ($P < 0.05$). Further analyses revealed positive correlations between spermatozoa density and dietary Se levels ($r = 0.62$, $P < 0.05$) and Se levels in semen ($r = 0.82$, $P < 0.01$) (Table 4). There were no correlations between semen volume or spermatocrit with dietary Se levels or Se levels in the semen ($P > 0.05$; Table 4).

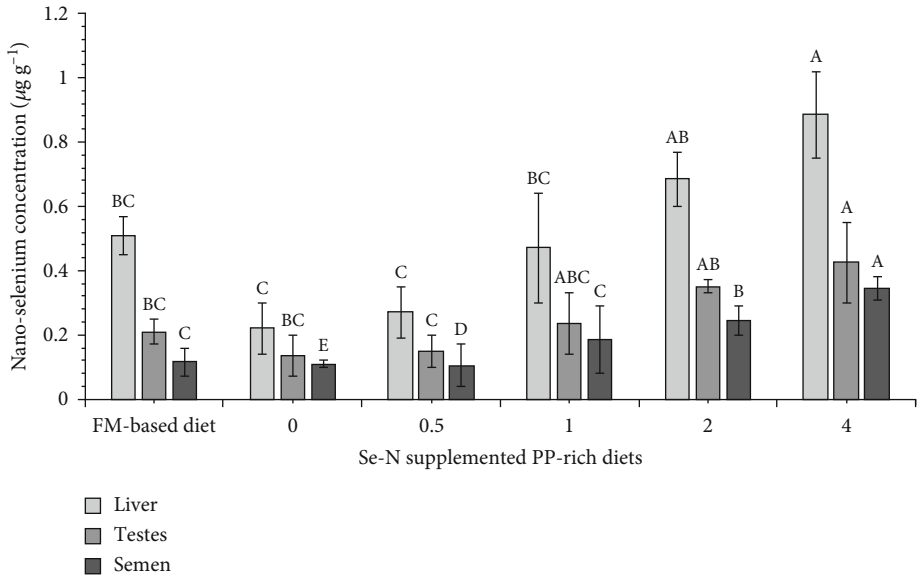


Figure 1: Actual selenium (Se) concentrations in the liver, testes, and semen of male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentration of selenium nanoparticle (Se-N). Data are mean ± SD ($n = 3$). For each tissue, values with different letters show significant differences ($P < 0.05$).

Table 3: Semen quality in male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentrations of selenium nanoparticles (Se-N). Data are mean ± SD ($n = 36$). For each parameter, values with different superscripts are significantly different ($P < 0.05$).

	FM-based diet	Se-N supplemented PP-rich diets (mg/kg Se-N)				
		0	0.5	1.0	2.0	4.0
Spermatozoa density ($\times 10^9$/mL)	32.9 ± 1.3^b	31.9 ± 2.9^b	26.7 ± 3.0^b	34.6 ± 2.3^b	42.1 ± 1.9^a	38.2 ± 1.6^a
Sperm motility 60 s (%)	36.66 ± 20.8^b	55.0 ± 10.3^b	55.0 ± 17.3^b	80.0 ± 10.5^a	70.0 ± 15.8^a	85.0 ± 16.7^a
Sperm motility 300 s (%)	8.3 ± 7.4^c	23.3 ± 5.8^b	25.0 ± 13.2^b	48.7 ± 6.5^b	40.0 ± 15.8^b	70.0 ± 15.0^a
Semen volume (mL)	1.6 ± 0.7^a	0.7 ± 0.4^b	0.7 ± 0.5^b	0.4 ± 0.3^b	0.8 ± 0.2^b	0.5 ± 0.3^b
Semen mass (g)	1.5 ± 0.7^a	0.6 ± 0.3^b	0.5 ± 0.5^b	0.3 ± 0.2^b	0.6 ± 0.2^b	0.4 ± 0.3^b
Spermatocrit (%)	2.5 ± 0.5^b	2.8 ± 1.3^b	4.0 ± 1.0^a	2.7 ± 0.6^b	1.3 ± 0.3^c	3.0 ± 1.0^b

Further analyses of the spermatozoa motility kinematics showed that the percentage of spermatozoa motility and percentage of spermatozoa with straight movement were higher in Se-N supplemented PP-rich diet compared to that of FM-based diet ($P < 0.05$; Figure 2). Also, the percentage of spermatozoa motility and percentage of spermatozoa with straight movement were higher in fish fed PP-rich diets containing Se-N, which were significant at ≥1 or ≥2 mg/kg Se ($P < 0.05$; Figure 2(a)). Moreover, the percentage of spermatozoa with pendulum-like motility was decreased in the males fed Se-N supplemented PP-rich diet at ≥2 mg/kg Se ($P < 0.05$; Figure 2(a)).

The longevity of spermatozoa motility was higher in Se-N supplemented PP-rich diet compared to that of FM-based diet ($P < 0.05$; Figure 2(b)). Also, Se-dependent longevity of spermatozoa motility was observed, which was higher in fish fed PP-rich diet at 4 mg/kg Se compared to those fed 0 and 0.5 mg/kg Se. Positive correlations were observed between the longevity of spermatozoa motility and dietary Se levels ($r = 0.57$, $P < 0.05$) and Se levels in the semen ($r = 0.69$, $P < 0.01$) (Table 4).

A Se-dependent fertilization rate was observed in fish fed PP-rich diet (Figure 3). It was higher in fish fed 4 mg/kg Se-N compared to those fed 0-1 mg/kg Se-N ($P < 0.05$). The fertilization rate was lower in fish fed PP-rich diet containing 0 and 0.5 mg/kg Se-N compared to those fed FM-based diet ($P < 0.05$). Further analyses revealed positive correlations (Table 4) between fertilization rate and dietary Se levels ($r = 0.70$, $P < 0.01$), Se levels in semen ($r = 0.58$, $P < 0.05$), spermatozoa density ($r = 0.68$, $P < 0.01$), and percentage of spermatozoa motility with straight movement ($r = 0.57$, $P < 0.05$) (Table 4).

The hatching rate showed highest value in fish fed 2 mg/kg Se-N in the PP-rich diet compared to those of fish fed 0-1 mg/kg Se-N ($P < 0.05$; Figure 3). The hatching rate was lower in fish fed PP-rich diet containing 0.5 and 1 mg/kg Se-N compared to those fed FM-based diet ($P < 0.05$). The hatching rate was positively correlated with spermatozoa

Table 4: Correlation among dietary nano-Se concentration, sperm quality, and fertility in the male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a plant protein-rich diet (PP-rich diet) supplemented by various concentrations of selenium nanoparticles (Se-N).

	Dietary Se level	Semen Se level	CAT activity	SOD activity	GPX activity	TAC	MDA	Spz density	Semen volume	Sct	Motility	Longevity	Straight movement	Pendulum movement	FR	HR
Dietary Se level	1	0.82**	-0.12	0.36	0.65**	0.65*	0.36	0.62**	-0.23	-0.12	0.43	0.57*	0.81**	-0.81**	0.70**	0.38
Semen Se level	0.82**	1	-0.16	0.16	0.25	0.46	0.43	0.64**	-0.40	-0.28	0.47	0.69**	0.71**	-0.71**	0.58*	0.36
CAT activity	-0.12	-0.16	1	0.48*	0.32	0.22	0.28	-0.10	0.42	-0.06	0.09	-0.12	-0.10	0.10	0.01	-0.13
SOD activity	0.36	0.16	0.48*	1	0.40	0.60**	0.57*	-0.27	-0.09	0.28	-0.04	0.17	-0.01	0.01	0.11	-0.28
GPX activity	0.65**	0.25	0.32	0.40	1	0.21	0.60*	0.41	-0.02	0.04	0.19	0.63**	0.47	-0.47	0.47	0.23
TAC	0.65*	0.46	0.22	0.60**	0.21	1	-0.38	0.04	-0.46	0.02	0.47	0.63**	0.22	-0.22	0.61*	0.07
MDA	0.36	0.43	0.28	0.57*	0.60*	-0.38	1	0.22	-0.23	-0.11	0.38	0.45	-0.52*	0.47	0.42	0.05
Spz density	0.62**	0.64**	-0.10	-0.27	0.41	0.04	0.22	1	-0.12	0.50*	0.44	0.43	0.67**	-0.67**	0.68**	0.72**
Semen volume	-0.23	-0.40	0.42	-0.09	-0.02	-0.46	-0.23	-0.12	1	-0.12	-0.28	-0.75**	-0.19	0.19	0.17	0.26
Sct	-0.12	-0.28	-0.06	0.28	0.04	0.02	-0.11	0.50*	-0.12	1	-0.31	-0.19	-0.22	0.22	-0.41	0.63*
Motility	0.43	0.47	0.09	-0.04	0.19	0.47	0.38	0.44	-0.28	-0.31	1	0.76**	0.68**	-0.68**	0.15	0.06
Longevity	0.57*	0.69**	-0.12	0.17	0.63**	0.63**	0.45	0.43	-0.75**	-0.19	0.76**	1	0.61*	-0.61*	0.05	0.07
Straight movement	0.81**	0.71**	-0.10	-0.01	0.47	0.22	-0.52*	0.67**	-0.19	-0.22	0.68**	0.61*	1	-1**	0.57*	0.47
Pendulum movement	-0.81**	-0.71**	0.10	0.01	-0.47	-0.22	0.47	-0.67**	0.19	0.22	-0.68**	-0.61*	-1**	1	-0.57*	-0.47
FR	0.70**	0.58*	0.01	0.11	0.47	0.61*	0.42	0.68**	0.17	-0.41	0.15	0.05	0.57*	-0.57*	1	0.79**
HR	0.38	0.36	-0.13	-0.28	0.23	0.07	0.05	0.72**	0.26	0.63*	0.06	0.07	0.47	-0.47	0.79**	1

Abbreviations: Se: selenium; CAT: catalase; SOD: superoxide dismutase; GPX: glutathione peroxidase; TAC: total antioxidant capacity; MDA: malondialdehyde; Sct: spermatocrit; SPZ: spermatozoa; FR: fertilization rate; HR: hatching rate. CAT, SOD, GPx, TAC, and MDA activities were measured in the semen. *Correlation is significant at $P < 0.05$ (two tailed). **Correlation is significant $P < 0.01$ (two tailed).

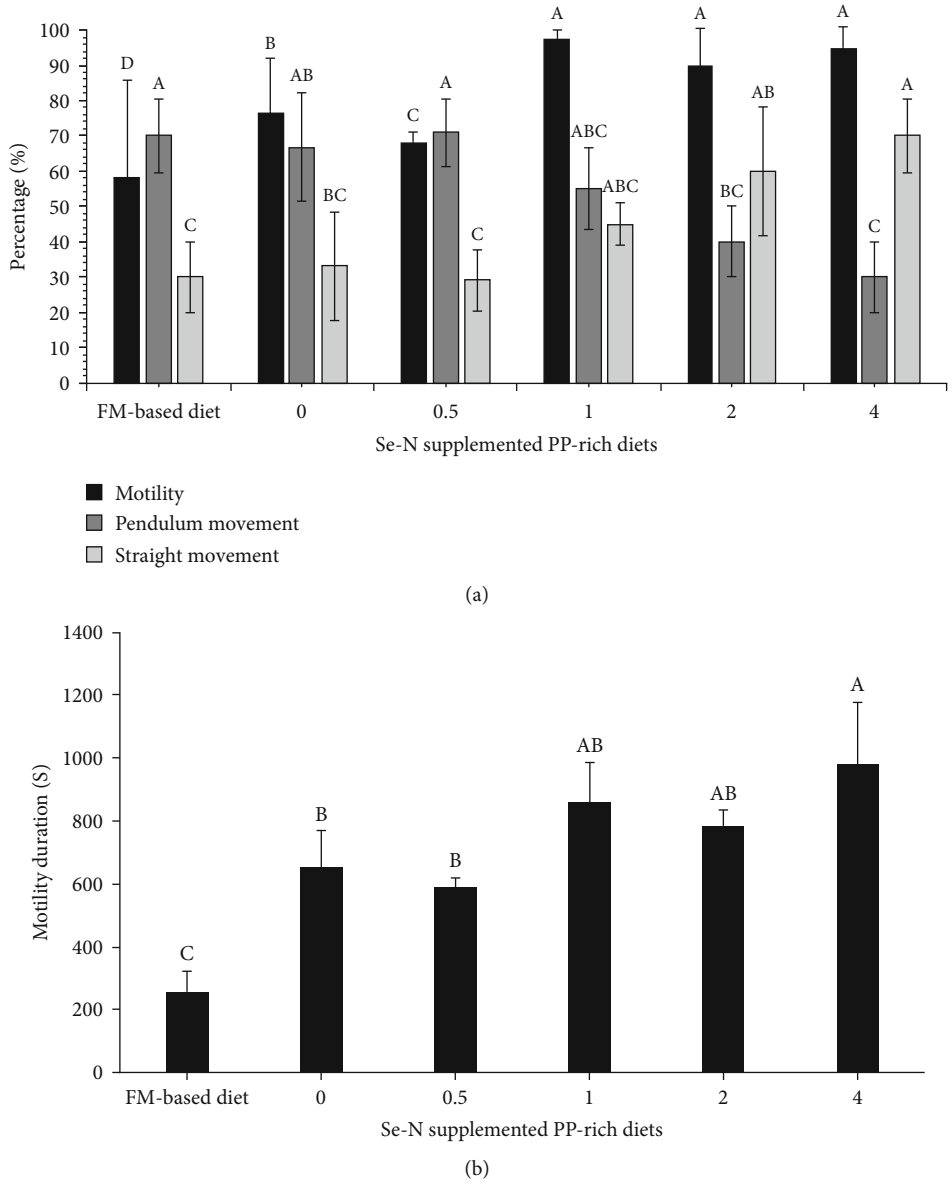


Figure 2: (a) Longevity of sperm motility in male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentration of selenium nanoparticle (Se-N). Data are mean ± SD ($n = 36$). For each parameter, values with different letters show significant differences ($P < 0.05$). (b) Sperm motility kinetics in male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentration of selenium nanoparticle (Se-N). Data are mean ± SD ($n = 36$). For each parameter, values with different letters show significant differences ($P < 0.05$).

density ($r = 0.72$, $P < 0.01$), spermatocrit ($r = 0.63$, $P < 0.05$), and fertilization rate ($r = 0.79$, $P < 0.01$) (Table 4).

3.4. Antioxidant Capacity. Various antioxidant activities (CAT, SOD, GPx, and TAC) were assessed in different tissues to better understand protective roles of Se against oxidative stress. The CAT activity remained unchanged in testes and semen of fish fed Se-N supplemented PP-rich diet and showed no differences compared to that of FM-based diet ($P > 0.05$; Figure 4(a)). However, CAT activity was affected in the liver and serum ($P < 0.05$, Figure 4(a)). In both liver and serum, the highest CAT activity was observed in fish fed FM-based diet. In the liver, CAT activity showed decrease in fish fed PP-rich diet supplemented with 0.5 and 2 mg/kg Se-N compared to that of FM-based diet ($P < 0.05$). In serum, CAT activity showed decrease in fish fed PP-rich diet supplemented with 0 mg/kg Se-N compared to that of FM-based diet. However, CAT activities did not show significant differences in the liver or serum of fish fed different concentrations of Se-N in the PP-rich diet ($P > 0.05$).

Activity of SOD was affected in the semen and serum ($P < 0.05$) but did not change in the testes and liver ($P > 0.05$, Figure 4(b)). In semen, the highest activity of SOD was observed in fish fed PP-rich diets supplemented with 4 mg/kg Se-N, which was higher than those of fish fed 0-2 mg/kg Se-N ($P < 0.05$). In serum, SOD activity was

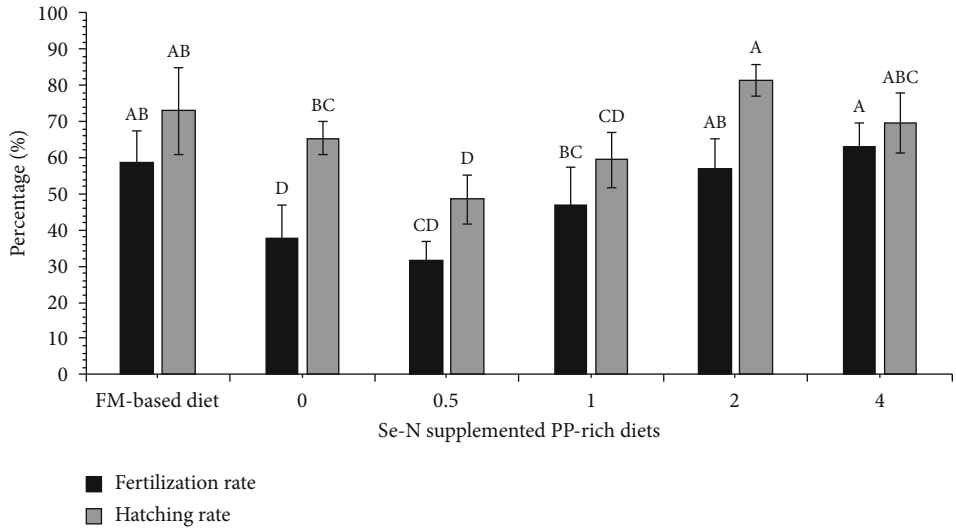


Figure 3: Fertility of male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentration of selenium nanoparticle (Se-N). Data are mean ± SD ($n = 27$). For each parameter, values with different letters show significant differences ($P < 0.05$).

lowest in fish fed PP-rich diets supplemented with 4 mg/kg Se-N, which was lower than those of fish fed 0 and 1 mg/kg Se-N as well as FM-based diet ($P < 0.05$).

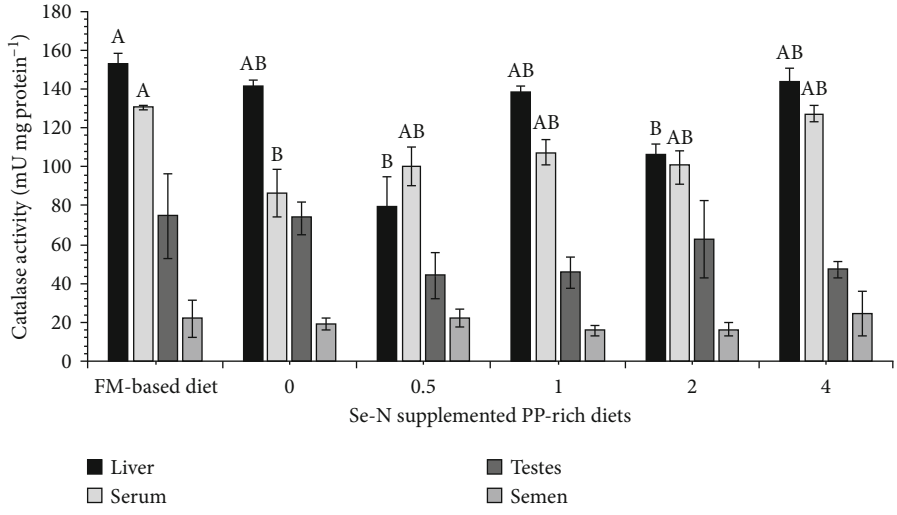
The activity of GPx was without changes in the testes ($P > 0.05$), but it was affected in the liver, serum, and semen ($P < 0.05$, Figure 4(c)). In semen, the highest GPx activity was observed in fish fed PP-rich diet supplemented with 4 mg/kg Se-N, which was higher than those of fish fed 0 and 0.5 mg/kg Se-N ($P < 0.05$). Similarly, the highest GPx activity was observed in the liver of fish fed PP-rich diet supplemented with 4 mg/kg Se-N; it was higher than those of fish fed the FM-based or 0-1 mg/kg Se-N in PP-rich diets ($P < 0.05$). In serum, the highest GPx activity was observed in fish fed PP-rich diet supplemented with 2 and 4 mg/kg Se-N, which was higher than those fed FM-based and 0.5 mg/kg Se-N ($P < 0.05$).

The TAC was affected in the liver, testes, and semen ($P < 0.05$), but it was without changes in the serum ($P > 0.05$; Figure 4(d)). In testes, the highest TAC activity was observed in fish fed PP-rich diet supplemented with 4 mg/kg Se-N, which was higher than fish fed FM-based diet or PP-rich diet with 0-1 mg/kg Se-N ($P < 0.05$). In semen, TAC activity was the highest in fish fed PP-rich diet with 4 and 1 mg/kg Se-N, which was higher than fish fed FM-based diet or PP-rich diet with 0, 0.5, and 2 mg/kg Se-N ($P < 0.05$). In the liver, the highest TAC was in fish fed PP-rich diet with 1 mg/kg Se-N, which was higher than those of fish fed FM-based or PP-rich diet with 0, 0.5, 2, and 4 mg/kg Se-N ($P < 0.05$). Also, the TAC activity was higher in fish fed PP-rich diets supplemented with 2 and 4 mg/kg Se-N than those of fish fed FM-based or PP-rich diet with 0 and 0.5 mg/kg Se-N ($P < 0.05$).

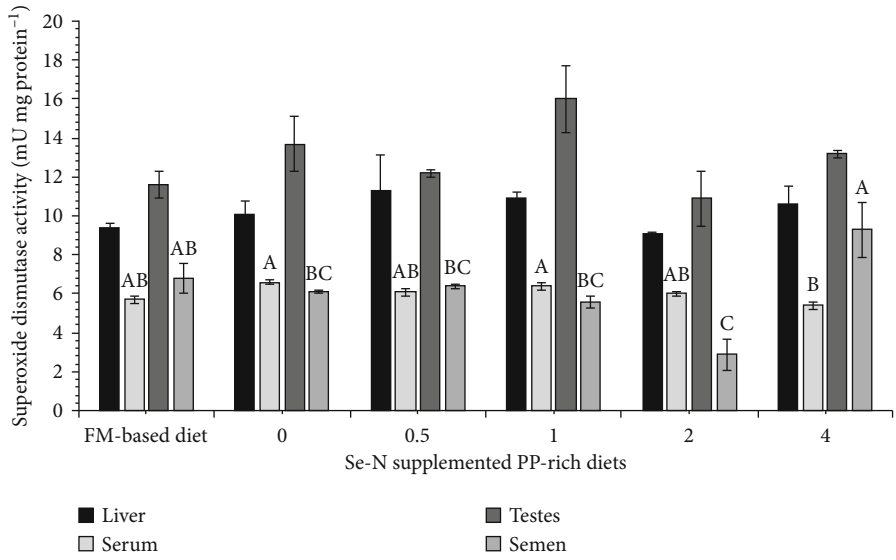
Significant changes in MDA levels were observed in the liver, serum, testes, and semen (Figure 4(e)). In the testes, the highest of MDA levels was observed in fish fed FM-based diet, which was higher than those of fish fed PP-rich diets ($P < 0.05$). Among Se-N supplemented groups, the MDA levels were also higher at 0 and 4 mg/kg Se-N compared to 0.5 and 2 mg/kg Se-N ($P < 0.5$). The MDA level was the highest in semen of fish fed PP-rich diet without Se-N, higher than those of fish fed FM-based diet or PP-rich diets containing different concentrations of Se-N ($P < 0.05$). The liver MDA levels in fish fed PP-rich diets supplemented with 1 and 2 mg/kg Se-N/kg were higher than those fed 0 and 0.5 mg/kg Se-N ($P < 0.05$). In serum, the levels of MDA were higher in fish fed FM-based diet or fed PP-rich diets containing 0, 0.5, and 1 mg/kg Se-N compared to those of fish fed PP-rich diet supplemented with 2 and 4 mg/kg Se-N ($P < 0.05$).

4. Discussion

4.1. Selenium Retention and Growth. Results of the present study show that inclusion of Se-N higher than 1 mg/kg in a PP-rich diet positively affected sperm kinetics resulting in higher fertilization or hatching rates. This might be due to the improvement of antioxidant defense system in the semen of A. arabicus. There were positive correlations between dietary Se-N concentration and semen Se content, spermatozoa density, longevity of sperm motility, straight movement, and fertilization rate (Table 4). Moreover, a negative correlation was noticed between dietary Se-N and pendulum movement of sperms suggesting dietary Se had truly a vital role in male fertility in A. arabicus. We observed that Se retention increased in the liver, testes, and semen of males when the concentrations of Se-N increased in the PP-rich diet. Regardless of the form of Se supplemented in a diet, the dose-dependent Se retention has been reported in other marine fish species such as cobia (Rachycentron canadum) [57], Malabar grouper (Epinephelus malabaricus) [58], Atlantic salmon (Salmo salar) [59], and gilthead sea bream (Sparus aurata) [60]. Although less studies have been conducted to investigate Se effects on semen quality and fertility, feeding European seabass (Dicentrarchus labrax) with diets



(a)



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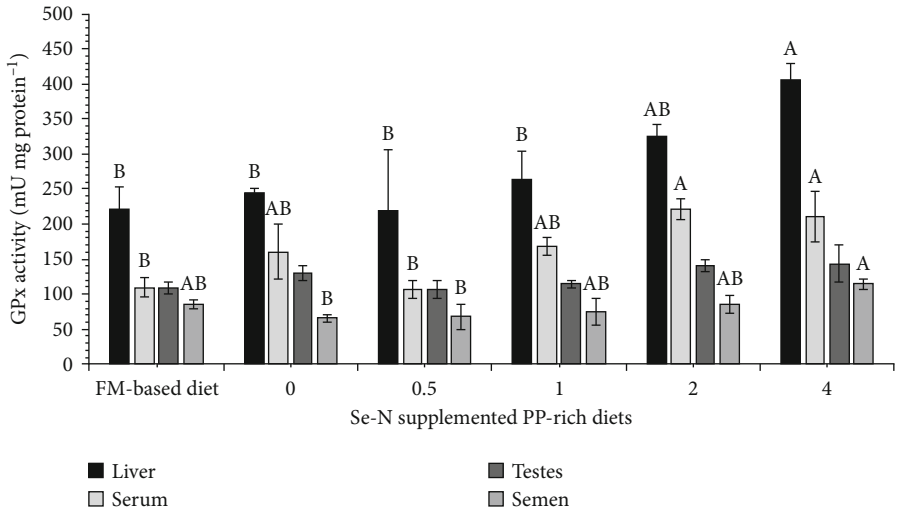

(c)

Figure 4: Continued.

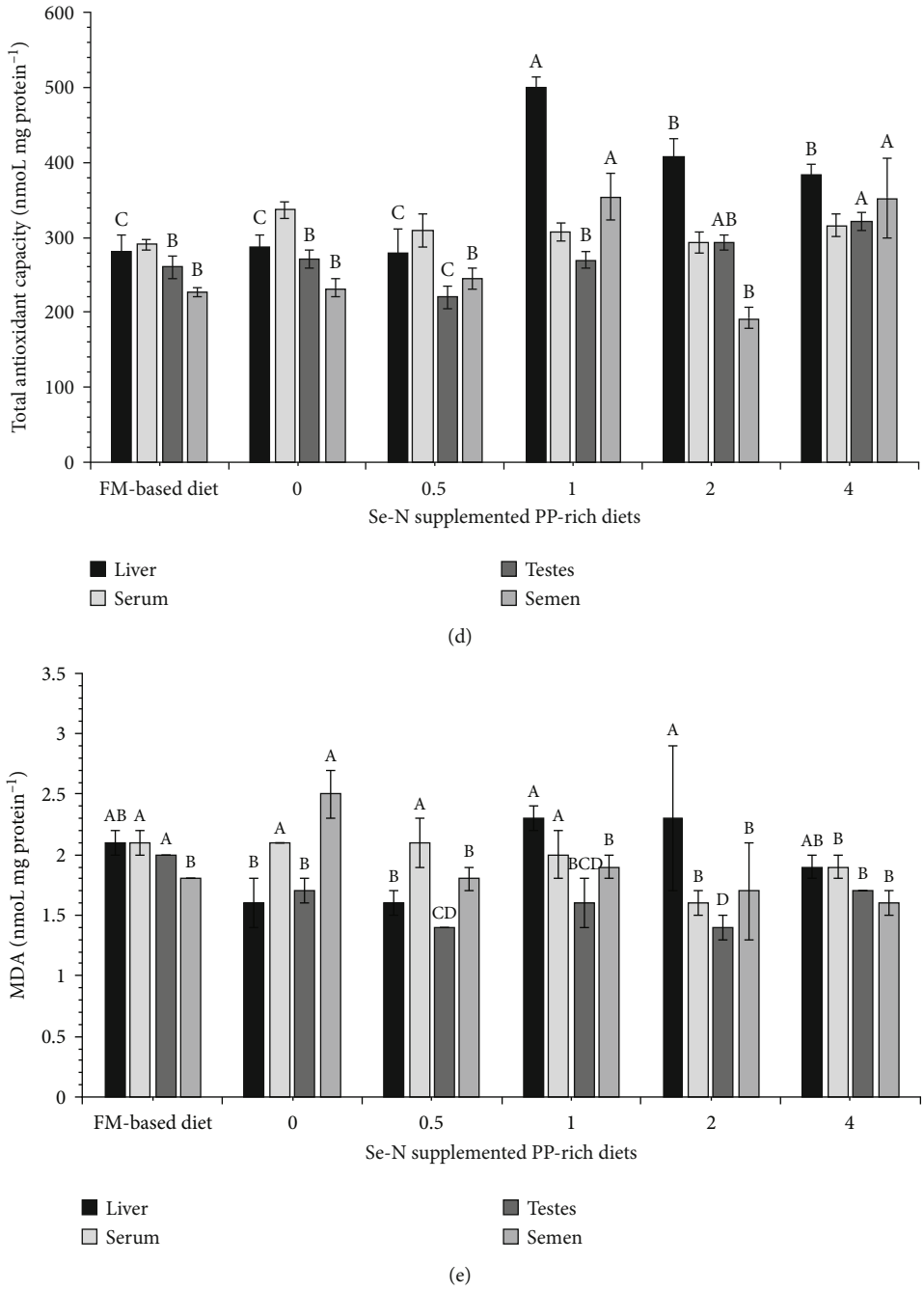

Figure 4: (a) Catalase, (b) superoxide dismutase, (c) glutathione peroxidase, (d) total antioxidant capacity, and (e) malondialdehyde concentration in the liver, serum, testes, and semen of male Arabian yellowfin sea bream (Acanthopagrus arabicus) fed a fishmeal- (FM-) based diet and a plant protein-rich diet (PP-rich diet) supplemented by various concentration of selenium nanoparticle (Se-N). Data are mean ± SD ($n = 9$). For each parameter, values with different letters show significant differences ($P < 0.05$). Parameters with no superscripts show no significant differences among groups ($P > 0.05$).

supplemented with Se, Zn, or vitamins (C and E) showed significant increases in sperm viability and reduced lipid peroxidation in semen with increase in dietary Se concentration [61]. In African catfish, Se concentration increased in testis when the fish were exposed to sodium selenite (0.1 mg/L water) for 30 d [23]. In contrast to aforementioned studies Wischhusen et al. [36] reported that supplementing diets with inorganic (i.e., sodium selenite) or organic (i.e., hydroxy-selenomethionine) sources (0.7 mg/kg) did not affect total Se content in O. mykiss males. The differences in Se retention among species in response to Se-supplemented diet may suggest a species-specific response.

The results showed that growth and somatic indices of A. arabicus were not affected by supplementation PP-rich diet with Se-N, indicating low level of Se (0.67 mg/kg) might be enough for meeting basic physiological needs of this species during reproduction. These show that feeding the male A. arabicus with PP-rich diet supplemented by Se-N was

without effects on somatic and testicular growth factors. It should be mentioned that there is a priority for gametogenesis during spawning season, and subsequently main portion of dietary nutrients and energy are channeled to gonadogenesis rather than somatic growth [62, 63]. Similarly, supplementing a PP-based diet with Se did not affect growth parameters in O. mykiss brooders [36]. In addition, it has been reported that supplementing a diet with 2-10 mg/kg of selenomethionine during entire life cycle of cutthroat trout (Oncorhynchus clarki bouvieri) (2.5 years, from fry stage to maturity) did not affect growth performances [64].

4.2. Selenium Effects on Fertilization Rate. In the present study, sperm quality parameters including sperm count, straight movement, and longevity of sperm motility were positively correlated with the semen Se concentration in the males fed Se-N supplemented diets what eventually resulted in higher fertilization rate, particularly in those fed with 2 and 4 mg Se-N/kg PP-rich diets. Selenium has vital role in male fertility, and it is an indispensable element for the spermatogenesis, sperm maturation, and its normal motility. Also, Se has structural role in selenoproteins, which comprise a large amount of sperm's keratinous mitochondrial capsule [65]. Dietary Se deficiency can reduce sperm motility by inducing morphological alterations and abnormal chromatin condensation that consequently leads to fertilization failure [66]. It has been confirmed that, dietary Se deficiency drastically increases abnormal spermatozoa with head morphological defects in mice that coincides with increasing the prevalence of chromosomal abnormalities in spermatocytes [67].

Sperm motility is the best biomarker for linking up diet quality with successful reproduction in fish [68]. In the present study, inclusion of Se-N in PP-rich diet over 1 mg/kg increased the percentage of sperm motility, longevity, and motile spermatozoa with straight movement and decreased pendulum-like motile spermatozoa, what suggest that inclusion of Se with diet resulted in improvement of sperm quality. To assume, we observed a positive correlation between fertilization rate and percentage of motile sperm with straight movement. Moreover, positive relationships were also found between percentage of sperm motility and longevity of sperm motility and percentage of sperm with straight movement. It is worth to note that sperm motility kinematics as key determinants of male fertility have been reported in masu salmon (Oncorhynchus masou) [69], turbot (Scophthalmus maximus) [70], and common carp [71].

In the present study, spermatozoa density increased with increasing dietary Se-N level in PP-rich diets, and there was also a positive correlation between spermatozoa density and fertilization rate. These may suggest that supplementing PP-diet with Se-N improved spermatogenesis. Likewise, Jahanabadi et al. [72] reported that supplementing diet with 2 mg/kg Se-N significantly enhanced semen volume, spermatozoa density, sperm movement longevity, fertilization, and hatching rates in O. mykiss males; however, they reported that Se-N was without effects on spermatocrit and sperm motility percentages. In African catfish males treated with Se (sodium selenite, 0.1 mg/L) for 30 d, spermatocrit, semen volume, sperm density, sperm movement longevity, and sperm with straight and rapid movement increased and percentage of dead sperm decreased, what was associated with marked increase of selenium content, total antioxidant capacity, CAT, SOD, and GPx activities in testes [23]. In Senegalese sole males, diets enriched with Se and vitamin E improved sperm motility and velocity what coincided with increasing GPx Se-dependent activity in blood plasma, enhancing docosahexaenoic acid levels in sperm and reducing lipid peroxidation in milt [73]. These findings suggest that Se protects sperm against oxidative stress and results in improvement of sperm quality. In this context, selenoproteins not only modulate the steroidogenesis pathway but also counteract intracellular oxidative stress and apoptosis induction in testes, which consequently improves spermatogenesis in males [22].

4.3. Selenium Role in Antioxidative Defense. Reactive oxygen species are capable of damaging DNA, mitochondria, and axoneme of spermatozoa by peroxidation of LC-PUFA in the membrane what results in apoptosis, reduction of motility, and diminished fertility [28]. As an effective antioxidative protection, fish spermatozoa possess various endogenous antioxidant enzymes (including CAT, SOD, and GPx) [74, 75]. Superoxide dismutase converts superoxide radical to hydrogen peroxide, which is consequently transformed to water by CAT and GPx [76]. As a fundamental component in numerous glutathione-dependent enzymes, Se has a vital role in the antioxidative defense of animals [77]. For instance, dietary Se supplementation in Turkeys exposed to low dose of Hg, testicular SOD, CAT, and GPx activities was restored to normal ranges suggesting Se can ameliorate oxidative stress [78]. In fish, Se-promoted antioxidant enzyme activities have been shown in pacu (Piaractus mesopotamicus) [79], juvenile meagre (Argyrosomous regius) [80], red sea bream (Pagrus major) [81], common carp [82–84], Nile tilapia (Oreochromis niloticus) [85], and rainbow trout [86].

Catalase is a metalloprotein oxidoreductase in both the sperm membrane and seminal plasma and plays a crucial role in protecting sperm membrane integrity and its viability against ROS in fish [74]. In the present study, the liver and serum CAT activity was decreased in fish fed low dietary Se level (0 or 0.5 mg/kg Se-N in PP-rich diet) (Figure 4(a)). The Se-enhanced CAT activity was consistent with other studies on Asian seabass (Lates calcarifer) at 4 mg/kg [87] and of European seabass at 0.5-1 mg/kg diet [88, 89]. No changes in the testicular and semen CAT activity may suggest that CAT may not have a pivotal role in antioxidative protection of semen in A. arabicus, similar to that of brown trout (Salmo trutta) [90]. Furthermore, we observed no correlations between the semen CAT activity and sperm motility kinematic and fertility. However, there was a positive correlation between semen CAT and SOD activities that suggests CAT fulfills the SOD activity in neutralizing ROS in semen.

The SOD (including intracellular or extracellular copper/zinc-SOD and manganese-SOD) is metalloenzyme well-known as the first line of antioxidant defense system in animals, including fish [91]. Former studies demonstrated positive effects of dietary Se-N on SOD activity in marine fish species [87–89]. In the present study, the semen SOD

activity was significantly enhanced at 4 mg Se-N/kg diet but there were no correlations between SOD and dietary Se-N level, sperm production, and motility kinematics. However, there was a positive correlation between semen SOD and TAC activities that may suggest physiological contribution of SOD as an antioxidant to protect sperm against oxidative stress in A. arabicus, similar to that of brown trout [90].

Antioxidant defense of Se is also exerted by synthesis of Se-dependent GPx [92]. It has been shown that deficiencies in dietary Se generally suppress GPx activity in aquatic species, and the liver GPx activity is considered as a reliable marker of dietary Se deficiency [19]. Wischhusen et al. [36] reported that parental Se nutrition with hydroxyl-selenomethionine upregulated the expression of antioxidative protein genes (i.e., SelP, GPx, and methionine Sulfoxide Reductase B2) that resulted in increased GPx activity in the progeny. In the present study, there was a positive correlation between GPx activity in the semen and dietary Se-N, MDA level, and longevity of sperm motility indicating GPx can strengthen sperm tolerance to ROS. In this context, Li et al. [93] reported that GPx activity was the main antioxidant enzyme responding to lipid peroxidation in sperm of common carp.

Total antioxidant capacity, which is the sum of enzymatic and nonenzymatic antioxidants, is considered as a reliable index for determining male sperm quality and fertility in teleosts, and its value in seminal plasma varies among species suggesting a species-specific physiological characteristic [94]. It is assumed that TAC in seminal plasma of fish is strongly related with nonenzymatic factors (mainly ascorbic acid, glutathione, and uric acid) rather than enzymatic ones [94]. In the present study, there was a positive correlation between TAC activity in the semen and dietary Se-N suggesting dietary Se-N can enhance tolerance of sperm against ROS, mainly due to increasing the concentration of selenoproteins in semen.

It has been demonstrated that augmentation of MDA value due to lipid peroxidation can drastically cause mortality of spermatozoa as a consequence of inducing DNA fragmentation, as previously demonstrated in Senegalese sole [73] and common carp [95]. Former studies have shown that dietary Se supplementation reduced lipid peroxidation in juvenile rainbow trout juveniles [96, 97], European sea bass larvae [98], or in common carp [99]. In the present study, the semen MDA in fish fed PP-based diet without Se-N markedly increased compared to the other groups, indicating that dietary Se deficiency enhances lipid peroxidation, what may result in oxidative damage to the membrane fluidity and integrity of spermatozoa. In addition, there were negative correlations among semen MDA and percentage of sperm that showed straight movement indicating lipid peroxidation can damage effective motility of the spermatozoa in A. arabicus males.

5. Conclusion

In conclusion, our results show that supplementing PP-rich diets with Se-N could promote sperm quality and fertility potential in A. arabicus males. Spermatozoa density, the percentage of sperm motility, the percentage of spermatozoa with straight movement, the longevity of sperm motility, and fertilizing ability of sperm increased in fish fed a PP-rich diet containing Se-N. These improvements were associated with higher activities of antioxidant enzymes that protect spermatozoa against oxidative stress. Among different antioxidant enzymes, a positive correlation was found between GPx activity in the semen and longevity of sperm motility. These results suggest Se-N, by increasing the synthesis of selenoproteins, exerts its antioxidative protection on spermatozoa and consequently enhances semen quality in these fish. Further studies are required to evaluate the effects of dietary Se-N at molecular level (e.g., transcriptomics, metabolomics, and proteomics) to elucidate the mode of action of this trace element on reproductive performance of A. arabicus.

Data Availability

The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

Special thanks are due to Mr. Sadegh Saffari who helped us during husbandry and sampling.

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