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Potential effects of dietary ZnO supported on kaolinite (ZnO-K) to improve biological parameters, reproduction indices, lipid profile and antioxidant enzymes activities for broodstock of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

The current study was carried out to study the effect of zinc oxide (ZnO) supported on kaolinite (ZnO-K) on biological parameters, reproduction indices, semen quality, lipid profile and oxidative biomarkers for broodstock of Nile tilapia. Four tested diets were formulated; Diet 1 was control diet without addition. Diet 2, Diet 3 Diet 4 were supplemented with 30, 60 and 90 mg ZnO-K kg^{-1}, respectively. Sixty females with average weight of 135.19 ± 3.91 g and twenty-four males with average weight of 212.51 ± 13.4 g of Nile tilapia (*Oreochromis niloticus*) were used. All broodstock fish were hand fed experimental diets up to apparent satiation three times daily (9:00, 12.00 and 14.00) for 4 weeks before mating. The highest hepatosomatic index (HSI %) and gonadosomatic index (GSI %) ($P < 0.05$) of male and female were recorded by fish fed Diet 4. The highest reproductive performance of female includes total absolute relative fecundity and fry survival ($P < 0.05$) were recorded for fish fed by Diet 4. Regarding semen quality of male, the highest pH, motility and sperm concentration ($P < 0.05$) were recorded by fish fed diet supplemented with ZnO-K kg^{-1} especially D4. Serum plasma levels of triglyceride cholesterol and high-density lipoprotein (HDL-C) ($P < 0.05$) were at higher levels of broodstock male and female fed by D4. The levels of catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes were significantly increased ($P < 0.05$) in the liver of broodstock male and female fed diet supplemented with higher levels of ZnO-K (D4) while, the highest values of alkaline phosphatase (ALP) ($P < 0.05$) and lower malondialdehyde (MDA) were recorded for fish fed diet without addition (D1). The present result indicated that dietary supplemented with ZnO supported on kaolinite (ZnO-K) at 60 or 90 mg kg^{-1} has a prominent potential in improving biological parameters and reproduction performance, semen quality, serum lipid metabolism and enzymes activates of Nile tilapia broodstock.

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1. Introduction

Tilapia is one of the most widely announced fish globally for fresh water aquaculture and it has emerged as a very promising species in aquaculture in different regions especially, tropical regions, they contribute by 40% of world aquaculture production (FAO, 2018). It is vital to ensure a year-round supply of high-quality breeding material, including brood fish, eggs, and seed production in various stages, in order to sustain viability and the growth of tilapia culture (Cruz et al., 2011; Barua and Das, 2010). Several strategies have been conducted in order to optimize seed production by manipulation of broodstock density, age and size of brood fish, broodstock exchange and conditioning technique (Little et al., 1993; El-Gamal, 2002; Soltan et al., 2007; El-Sayed, 2019). Moreover, nutrition or functional diet for broodstock had positive effects on reproductive performance includes; maturation process of the gametes and energy storage in the form of the yolk (El-Sayed et al., 2005; El-Sayed and Kawanna, 2008; Mañanós et al., 2008; Hajizadeh et al., 2008; Mabroke et al., 2012; Suloma et al., 2017).

Zinc (Zn) is a trace element that has an effect on reproduction and is involved in various food metabolic processes (Jiang et al., 2016; Kocabas and Kutluyer, 2017; Domínguez et al., 2019). In biological system, dietary zinc absorbed by intestine and delivered to the liver in protein bond forms as zinc metallothionein (Thompson et al., 2003; Yamaguchi et al., 2009). Vitellogenin or zinc protein in the liver transported during reproduction process via the blood to the ovary to improve growth of oocytes growth, subsequently developing embryo (Banks et al., 1999). In addition, Zn associated with the secretion of hormone of luteinizing, follicle-stimulating hormone and prolactin (Banks et al., 1999; Salgueiro et al., 2000). Also, it has been contributed in the improvement of sperm quality include semen volume, sperm motility and sperm morphology of fish male (Zhao et al., 2016). According to importance of zinc in fish and broodstock feed mentioned above therefore, urgently supplementation for meeting the nutritional requirements of fish (Tan and Mai, 2001; Kazemi et al., 2020).

Dietary Zn requirements in $ZnSO_4$ form have been noted for different broodstock fish species which were 120 mg kg^{-1} diet for Nile tilapia (Gammanpila et al., 2007), 20–320 mg kg^{-1} diet for adult blunt snout bream diet (*Megalobrama amblycephal*) (Jiang et al., 2016), 40 mg kg^{-1} diet for rainbow trout (*Oncorhynchus mykiss*) (Kazemi et al., 2020) and 50–200 mg kg^{-1} diet for striped catfish (*Plotosus lineatus*) (Aripin et al., 2015). Meanwhile, Zn bioavailability to fish from Zn complexes was higher than that observed for $ZnSO_4$ or ZnO, especially in plant-origin diets (Ashmead, 1992). Therefore, it is mandatory to search for more techniques to enhance its bioavailability in fish.

However, clay mineral is considered as another strategy for enhancing the nutrient bioavailability and growth performance in fish diets (Hu et al., 2008; Yildırım et al., 2009; Hassaan et al., 2020). In particularly, kaolinite is a silicate mineral clay, safe non-conventional feed additives and constructed of three dimensional shape with chemical formula $Al_2Si_2O_5(OH)_4$ (Spotti et al., 2005; EFSA, 2016) which gave a large surface area for nutrient bioavailability. Mostly of kaolinite comprised of kaolin, a plastic raw material that belongs to hydrated silicates of aluminum which have the ability to diminish the absorption of hazardous toxins (Phillips, 1999; Dominy et al., 2004), pathogenic microorganisms and heavy metals (Katsumata et al., 2003; Beck et al., 2015; Harikrishnana et al., 2018) through intestinal mucosa. In this regard, Hu et al. (2014) stated that the growth performance and feed utilization improved by adding ZnO supported on zeolite in Nile tilapia diet. To the best of our knowledge, there are no available reports on the effects of ZnO supported on kaolinite (ZnO-K) on reproductive performance, semen quality, lipid metabolism and oxidative enzymes of broodstock.

Table 1
Ingredients and proximate composition of the basal diets (g/ kg dry diet).

Ingredients	Basal diet (350 g kg^{-1} crude protein)
Fish meal	150
Soybean meal	540
Yellow corn	120
Wheat bran	120
Soybean oil	40
Zn -free Premix[a]	30
Chemical analysis (%)	
Dry matter	89.55
Crude protein	35.0
Crude lipid	7.20
Ash	4.92
Fiber content	5.33
NFE[b]	52.94
GE[c] (MJ kg^{-1})	20.19

[a] Vitamin and mineral mix (mg or g / Kg diet): MnSO4, 40 mg; MgO, 10 mg; K2SO4, 40 mg; KI, 0.4 mg; CuSO4, 12 mg; Ferric citrate, 250 mg; Na2SeO3, 0.24 mg; Co, 0.2 mg; retinol, 40000 IU; cholecalciferol, 4000 IU; α-tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg;;nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; ascorbic acid, 500 mg. ^{2}*B. acidophullus* was prepared to obtain (1.47×10^7 CFU kg^{-1} approximately.

[b] NFE (Nitrogen free extract) = 100- (crude protein + lipid + ash +fibre content).

[c] Gross energy, calculated using gross calorific values of 23.63, 39.52 and 17.15 Kj g^{-1} for protein, fat and carbohydrate.

Therefore, this study aimed to assess the effect of ZnO supported on kaolinite (ZnO-K) on reproductive performance, semen quality, lipid metabolism and oxidative enzymes of Nile tilapia broodstock.

2. Material and methods

2.1. Synthesis of ZnO supported on kaolinite

Using hydrothermal method according Hrenovic et al. (2012) and Mohammady et al. (2021) ZnO supported on kaolinite (ZnO-K) was synthesis. In brief, 0.1 M zinc acetate dehydrate and 10 g of kaolinite was adding under stirring at room temperature. Then, after 12 h stirring, aqueous solution of NaOH (5 M) was slowly added drop-wise to the solution under stirring until the pH 13. The precipitates were dried at 95 °C for 120 min. The precipitate centrifuged and washed twice with double-distilled water and dried at 50 °C.

2.2. Experimental design and diets

The current study was carried out to study the effect of ZnO supported on kaolinite (ZnO-K) on biological parameters, reproduction indices, semen quality, lipid profile and oxidative biomarkers for broodstock of Nile tilapia. Four tested diets were formulated; Diet 1 was control diet without addition. Diet 2, Diet 3 Diet 4 were supplemented with 30, 60 and 90 mg ZnO-K kg^{-1}, respectively. ZnO-K was thoroughly blended with the basal diet containing 350 g kg^{-1} crude protein and 72 g kg^{-1} crude lipid (Table 1), after that, mixing all compounds of each diet, then added 150 ml water kg^{-1} to make a dough of each diet. The dough of each diet was pelleting (2 mm diameter die) via passing it in laboratory pellet machine. After pelleting, the diets dried at room temperature for 24 h, then stored in at 4 °C in refrigerator until use. AOAC (1995) methods were used to estimate the chemical composition of tested diets. After preparing tested diets, the actual concentrations of Zn were 93.75, 116.9, 136.75, and 145 mg kg^{-1} diet for Diet 1 (control), Diet 2, Diet 3 and Diet 4, respectively, and these concentrations were estimated by using atomic absorption spectrophotometer (Uvikon 810; NorthStar Scientific, Bedfordshire, UK).

2.3. Experimental fish and broodstock management

The present experiment was carried out during the spawning season in May 2020 at fish farm of Faculty of Agriculture, Benha University. Nile tilapia broodstock were obtained from commercial farm, Kafer Elshaikh Governorate, Egypt. Fish was sexed and separately stocked in two cement ponds ($2 \times 4 \times 1$ m^3) to acclimate fish on experimental condition for 15-day. During this period, fish were fed commercial broodstock feed (350 g kg^{-1} crude protein). Number of 60 females with average weight of 135.19 ± 3.91 g and 24 males with average weight of 212.51 ± 13.4 g of Nile tilapia (*Oreochromis niloticus*) were used. Females were randomly stocked into twelve breeding hapas ($2 \times 2 \times 1$ m^3 cm each) placed in a cement pond ($2 \times 4 \times 1$ m^3), representing four experimental treatments in triplicates. In addition, males were distributed separately into the same number of hapas of female in the same cement pond. All broodstock fish were hand fed experimental diets up to apparent satiation three times daily (9:00, 12.00 and 14.00) for 4 weeks before mating. Ponds were supplied with fresh water and kept under normal condition. Water quality parameters were day other day measured which values was ranged in acceptable range; temperature was monitored and ranged between 25 and 27 °C in the morning and 28–31°C at afternoon, pH ranged between 7.5 and 8.2, dissolved oxygen > 5.8 mg/l. After 30 days, each hap was stocked with five females (mean weight, 147.4 ± 3.96 g) and two males (mean weight, 235 ± 13.9 g). Broodstock were hand fed their respective experimental diets three time daily at 9:00, 12:00 and 14:00 to apparent satiation throughout the reproductive period. Females were weighted and checked for spawning activity (eggs in buccal cavity) biweekly.

2.4. Fry survival and performance

About of 200 larvae (0.014 average weight) which obtained from each broodstock, were stocked in a 0.5–m^3 fiberglass tank in triplicate groups. All tanks provided with continuous aeration using an air compressor. Fish were hand fed close to apparent satiation seven daily (at 8:00 am, 9:00 am, 10:00 am, 11.00 am, 12:00 pm, 13.00 pm and 14.00 pm) for 30 days. The amounts of feed consumed by fish in each tank during each feeding interval were also recorded. Faeces were siphoned each morning, before the first feeding and about 20% of the water was replaced with fresh water. Water temperature was recorded daily with a mercury thermometer suspended at 15-cm depth. pH was determined by using a pH meter (Orion pH meter, Abilene, Texas, USA). While dissolved oxygen (mg/L) was measured using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). During the feeding trial, the water quality parameters averaged (± standard deviation): Water temperature 26.8 ± 0.3 °C; dissolved oxygen 5.98 ± 0.22; pH values 8.17 ± 0.35; total ammonia 0.16 ± 0.01 mg/L, all tested water quality criteria (temperature, dissolved oxygen, pH value and total ammonia) were within the acceptable limits for rearing Nile tilapia, *O. niloticus* (Boyed, 1990).

2.5. Biological parameters and reproductive performance

Biological parameters and reproductive performance were calculated as following equations:
Condition factor (K)=weight of female or male/ $Length^3$.
Gonado-somatic index (GSI %)= [gonads weight (g)/somatic weight (g)]× 100.
Hepatosomatic index (HSI %)=Liver weight(g) / Whole fish weight (g)× 100.

Absolute fecundity=Mean number of eggs at each spawning per female (Murua et al., 2003).
Relative fecundity=Mean number of eggs at each spawning per female body weight (g) (Qadri et al., 2015).
Survival rate (SR) %= (No. of fish survived at the end of the experiment/ whole number of fish at the beginning)× 100.

2.6. Semen quality

For stripping and collecting milt, the genital area was dried and handily pressure was applied to the fish abdomen, mid- way between the pectoral and pelvic fins, moving posteriorly down to the urogenital papilla (Vanderwael, 1985) as an attempt for milt stripping, this process be repeated 10–15 times for each male. To assessment semen indices such as sperm pH were evaluated immediately using the first squeezed drop of milt and measured by pH tapes according method by Amer et al. (2005). Sperm motility was classified subjected, according to the percentage of motile spermatozoa Viveiros et al. (2001). Spermatozoa concentration was evaluated using a 10 µl subsample of semen with a dilution ratio of 1:20 (one volume of sperm and 20 vol of physiological saline solution) (Billard et al., 1981). The concentration of sperm in the seminal fluid was estimated according to Ruranguwa et al. (2004). The standard methods for determining sperm density (sperm cells/ ml milt) in fish was used to count spermatozoa generally using haemocytometer counting chamber (Büyükhatipoglu and Holtz, 1984).

2.7. Biochemical measurements

Serum cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL–C) and alkaline phosphatase (ALP) were measured using standard Kits (Modern Laboratory Kits). Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and malonaldehyde (MDA) levels were measured using diagnostic kits (Bio-diagnostics, Giza, Egypt) following the manufacturer's instructions according to the methods of Nishikimi et al. (1972), Aebi (1984), Moin (1986), Benzie and Strain (1996), and Uchiyama and Mihara (1978), respectively.

2.8. Statistical analysis

Data were tested for homogeneity and normality tests before analyzed. Afterwards, data were analyzed by using one-way analysis of variance and the differences among means were made by using Duncan's multiple range test using SAS ANOVA procedure (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993). Also, subjected to Nonlinear and linear functions using SAS ANOVA procedure. The differences at $P < 0.05$ were considered significant. The values are presented as means ± standard error of the mean (SEM).

3. Results

3.1. Broodstock biological parameters

Table 2 showed final body weight (FBW), condition factor, gonadosomatic index (GSI) and hepatosomatic index (HSI) in Nile tilapia fed different levels of ZnO-K mg kg^{-1} diet. No significant ($P > 0.05$) differences were found in FBW and K of males and females after feeding different level of 0, 30, 60 and 90 mg kg^{-1} diet. While, HSI of male and female were significantly improved ($P < 0.05$) with increasing the dietary level of ZnO- K (mg kg^{-1} diet), and the highest values (1.38% and 1.46%), respectively, were obtained in fish fed diet containing 90 ZnO-K mg kg^{-1} diet. Also, maximum GSI was obtained in male and female fed diet supplemented with 90 ZnO-K mg kg^{-1} diet.

Table 2
Growth performance of tilapia male and female fed diets supplemented with different levels of ZnO supported on kaolinite (ZnO-K).

	Dietary ZnO-K concentration (mg kg^{-1})				± SEM	P Values
	0	30	60	90		
Male						
Initial body weight (g $fish^{-1}$)	220.9	198.92	224.23	226.9	13.46	0.516
Final body weight (g $fish^{-1}$)	237.69	230.93	239.92	234.30	13.39	0.536
Condition factor (K)	1.63	1.62	1.67	1.68	0.074	0.909
Hepatosomatic index (HSI %)	1.015^{c}	1.15^{b}	1.22^{b}	1.38^{a}	0.022	0.001
Gonadosomatic index (GSI %)	0.465^{b}	0.495^{b}	0.565^{b}	1.45^{a}	0.027	0.003
Female						
Initial body weight (g $fish^{-1}$)	137.97	138.70	134.42	129.69	3.91	0.350
Final body weight (g $fish^{-1}$)	147.42	148.85	140.45	143.76	3.96	0.440
Condition factor (K)	1.70	1.55	1.56	1.58	0.04	0.232
Hepatosomatic index (HSI %)	1.24^{d}	1.35^{b}	1.40^{a}	1.46^{a}	0.01	0.005
Gonadosomatic index (GSI %)	2.36^{b}	5.26^{a}	5.04^{a}	5.90^{a}	0.33	0.014

Means followed by different letters in the same row are significantly different ($P < 0.05$).

3.2. Reproductive performance and fry performance

The reproductive performance of female Nile tilapia broodstock fed diet supplemented with different levels of ZnO-K mg kg^{-1} diet are showed in Table 3. Total, absolute and relative fecundity increased significantly with increasing the ZnO-K supplementation level and the highest records were observed in fish fed diet containing 90 mg ZnO-K kg^{-1} diet. Fry survival increased significantly with increasing supplementation level of ZnO-K, where, the best level was showed in fish fed diet supplemented with 90 mg ZnO-K kg^{-1} diet (Fig. 1) where, the relationship was linearly according to this equation: = 0.1078x + 73.51; $R^2 = 0.9667$. The relationship between dietary ZnO-K and fry weight was polynomial and was best represented by the following equation: $y = -1E\text{-}05x^2 + 0.0017x + 0.1888$; $R^2 = 0.9793$ (Fig. 2).

3.3. Quality of semen

Characteristics of semen quality; Ph, motility % and concentration for Nile tilapia fed different levels of ZnO- K mg kg^{-1} diet are present in Table 4. Data showed that semen pH ranged from 7 to7.5 and had a significant difference ($P < 0.05$) among treatments. The highest pH value (7.5) was obtained in fish fed diet containing 90 ZnO-K mg kg^{-1} diet. The motility of sperms significantly affected by dietary ZnO-K, which was higher in all diets containing ZnO-K than control group. The highest motility % was obtained in fish fed diet supplemented with 90 mg ZnO-K kg^{-1} diet. Also, sperm concentration significantly improved by dietary ZnO-K kg^{-1} diet. Diet supplemented with 60 mg or 90 mg ZnO-K kg^{-1} diet achieved the highest concentration of sperm (5.20×109 and 5.65×109), respectively with insignificant differences.

3.4. Lipid metabolism for broodstock

Serum lipid profile in male and female brood stock of Nile tilapia are presented in Table 5. Male of Nile tilapia fed diet supplemented with ZnO- K mg kg^{-1} diet exhibited significantly higher content of triglyceride, cholesterol and HDL-C. Diet supplemented with 90 ZnO- K mg kg^{-1} diet released the highest value of cholesterol and HDL-C content for male and female than other dietary diet. While, the highest content of triglyceride was observed in female fed control diet and diet supplemented with ZnO- K mg kg^{-1}.

3.5. Oxidative responses

Oxidative enzymes activity in serum of Nile tilapia brood stock fish fed different levels of ZnO- K mg kg^{-1} diet are presented in Table 6. Activates of oxidative enzyme of male and female tilapia fish fed dietary supplemented with ZnO-K were improved. The higher records of CAT, GPX, SOD were detected in fish diet supplemented with 90 ZnO- K mg kg^{-1}. While control has higher values of ALP and lower MDA compared to other treatments supplemented with different levels of ZnO- K.

4. Discussion

Zinc (Zn) is an essential micronutrient in the animal body and play a vital role in biological activities and also have been used in the fish diet in numerous studies for improving growth, metabolism, immune response, and enzyme function in fish (Antony et al., 2016; NRC, 2011). Moreover, dietary Zn supplementation had an effective role in reproductive performance and fertility of fish (Jiang et al., 2016; Thompson et al., 2002). Addition of Zn could be improved the growth of fish at sexual maturity phase and it have been maintained the gonadal development of broodstock (Jiang et al., 2016). In the present study, biological indices; HSI and GSI of males and females were significantly increased with increasing ZnO-K supplementation levels in the broodstock diet. Meanwhile, final body weight and condition factor (K) did not affect by dietary ZnO-K supplementation. This improvement in HSI and GSI may be associated with kaolinite is a good carrier for increase the releasing rate of ZnO subsequently increase the bioavailability of ZnO in the intestinal tract of fish (Hu et al., 2014; Swain et al., 2016; Onuegbu et al., 2018; Mohammady et al., 2021). Previous studies reported that the K has a positive effect on spawning, and also positively correlated with HSI and GSI was found (Uka and Edun, 2011; Uka and Sikoki, 2016). HSI and GSI indices are a good indicator of fish gonad maturation, which liver organ have an important role for vitellogenin synthesis at the reproduction period of fish where it can be expressed the estradiol receptors determination (Orlando et al., 2017). (Arellano-Martínez and Ceballos-Vázquez, 2001). Aripin et al. (2015) found that GSI of Walking catfish (*Clarias macrocephalus*) was significantly higher in fish fed diet supplemented with zinc amino acid. Also, Thompson et al. (2002) showed that zinc with amino acid

Table 3
Fecundity parameters and fry performance of female tilapia fed diets supplemented with different levels of ZnO supported on kaolinite (ZnO-K).

	Dietary ZnO-K concentration (mg kg^{-1})				± SEM	P Values
	0	30	60	90		
Absolute fecundity	2281.3^{b}	7965.9^{a}	7678.8^{a}	2987^{b}	568.8	0.0107
Relative fecundity	3.078^{b}	10.666^{a}	11.040^{a}	4.113^{b}	0.632	0.0055
Fry per (m^2)	331.13^{c}	327.13^{c}	386.00^{b}	444.00^{a}	21.01	0.0431
Fry weight (g)	0.18720^{b}	0.2270^{a}	0.2256^{a}	0.2392^{a}	0.001	0.0001

Means followed by different letters in the same row are significantly different ($P < 0.05$).

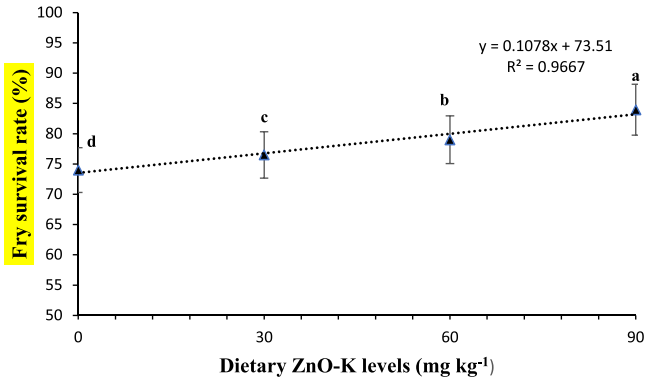

Fig. 1. The relationship between the fry survival rate (%) and dietary ZnO-K for Nile tilapia broodstock based on linear or quadratic regression analysis.

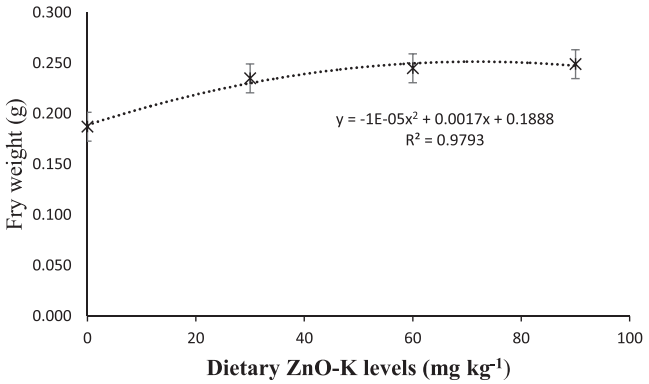

Fig. 2. The relationship between the fry weight (g) and dietary ZnO-K for Nile tilapia broodstock based on linear or quadratic regression analysis.

Table 4
Semen quality of male tilapia fed diets supplemented with different levels of ZnO supported on kaolinite (ZnO-K).

	Dietary ZnO-K concentration (mg kg^{-1})				±SEM	P Values
	0	30	60	90		
pH of Semen	7.00^{b}	7.00^{b}	7.00^{b}	7.50^{a}	0.001	0.0001
Motility (%)	24.00^{c}	61.00^{b}	65.50^{ab}	80.50^{a}	3.851	0.0067
Sperm concentrate (Sperm /ml milt)	2.6 × 10^{9b}	2.03 × 10^{9b}	5.20 × 10^{9a}	5.65 × 10^{9a}	0.299	0.0071

Means followed by different letters in the same row are significantly different ($P < 0.05$).

Table 5
Lipid metabolism profile of male and female tilapia fed diets supplemented with different levels of ZnO supported on kaolinite (ZnO-K).

	Dietary ZnO-K concentration (mg kg^{-1})				±SEM	P Values
	0	30	60	90		
Male						
Triglyceride (mmol L^{-1})	190.5^{c}	188^{d}	240.5^{b}	288^{a}	0.18	0.0001
Cholesterol (mmol L^{-1})	170.5^{a}	112.5^{d}	131.5^{c}	149.5^{b}	0.18	0.0001
HDL-C$^{\#}$ (mmol L^{-1})	63.5^{d}	70.5^{c}	79.5^{b}	94^{a}	0.18	0.001
Female						
Triglyceride (mmol L^{-1})	367^{a}	257^{d}	289^{c}	348^{a}	0.20	0.0001
Cholesterol (mmol L^{-1})	108^{d}	121^{c}	146^{b}	159^{a}	0.35	0.0001
HDL-C$^{\#}$ (mmol L^{-1})	44^{d}	53^{c}	63.5^{b}	74^{a}	0.18	0.0001

Means followed by different letters in the same row are significantly different ($P < 0.05$).
$^{\#}$HDL-C = high-density lipoprotein-cholesterol.

Table 6
Antioxidant enzymes and alkaline phosphatase of male and female tilapia fed diets supplemented with different levels of ZnO supported on kaolinite (ZnO-K).

	Dietary ZnO-K concentration (mg kg^{-1})				±SEM	P Values
	0	30	60	90		
Male						
Catalase (CAT U/g protein)	0.6500	0.9300	0.5300	0.8250	0.0900	0.1456
Malonaldehyde MDA (nmol/g tissue)	13.90^{d}	110.93^{b}	45.585^{c}	279.40^{a}	0.7907	0.0001
Glutathione peroxidase (GPX U/g protein)	25448^{a}	17257.5^{d}	19183^{c}	32391^{a}	0.2887	0.0001
Superoxide dismutase (SOD U/g protein)	118.62^{d}	274.035^{a}	259.485^{b}	240.350^{c}	1.1244	0.0001
Alkaline phosphatase (U/L)	69.65^{a}	55.260^{b}	25.20^{c}	15.070^{d}	1.0633	0.0001
Female						
Catalase (CAT U/g protein)	0.8650^{b}	0.7550^{b}	0.8350^{b}	1.6700^{a}	0.0286	0.0005
Malonaldehyde MDA (nmol/g tissue)	13.085^{d}	65.90^{a}	31.835^{c}	45.135^{b}	1.0513	0.0002
Glutathione peroxidase (GPX U/g protein)	25662^{d}	27392.5^{b}	26049^{c}	33967.5^{a}	0.8660	0.0001
Superoxide dismutase (SOD U/g protein)	117.590^{c}	160.965^{b}	251.200^{a}	247.315^{a}	1.1905	0.0001
Alkaline phosphatase (U/L)	71.425^{a}	48.175^{c}	48.815^{c}	58.415^{b}	0.8898	0.0009

Means followed by different letters in the same row are significantly different ($P < 0.05$).

can accumulated in the liver then transported via bloodstream to any organs. Contrary, condition factor and (HSI) of blunt snout bream, *Megalobrama amblycephala* showed no significant differences among diets supplemented with different levels of $ZnSO_4$ (Jiang et al., 2016).

Total absolute and relative fecundity in the present study were significantly improved in broodstock fed diet supplemented with ZnO-K compared with control diet. The improvement in reproductive parameters could be attributed to one of the following scenario: i) The shape, size, optical, and electrical characteristics of ZnO-K were improved Zn bioavailability and their absorption, which increased their application and incorporation to enhance growth and feed efficiency (Mohammady et al., 2021), additionally, ii) ZnO can stimulate the somatic growth through stimulation cell division in fish body (Şıklar et al., 2003). Similarly, Salgueiro et al. (2000) showed that addition of Zn significantly ($P < 0.05$) could be improved the fertility of female broodstock. Increased the bioavailability of Zn in intestinal track increased the efficiency of vitellogenesis process, subsequently improved egg growth, development of embryo and larvae after fertilization (Banks et al., 1999; Thompson et al., 2002). Therefore, the nutrition of broodstock reflects the quality of larva production (Orlando et al., 2017). In the same context, our finding showed higher number of fry per cubic meter, fry weight, and fry survival in tilapia broodstock fed dietary supplemented with 90 mg kg^{-1} ZnO-K. Correspondingly with our finding, Aripin et al. (2015) found that Walking Catfish (*Clarias macrocephalus*) female broodstock fed diet dietary supplemented with zinc amino acid had significantly ($P < 0.05$) higher fertilization rate, egg production fecundity, hatching rate, and larva survival rate compared with the control group.

The present study found that an improvement in semen quality includes pH, motility and sperm concentration in tilapia broodstock fed dietary supplemented with ZnO-K. This improvement in semen quality may related to Zn play an important role in ATP synthesized in sperm mitochondria for energy supply to flagella motility of Japanese ell (*Anguilla japonica*) (Yamaguchi et al., 2009; Turner, 2006). Moreover, zinc has an effective role in testosterone production where it can be induced Leydig cell to produce testosterone which it was a limiting factor for quality and quantity of sperm (Syarifuddin et al., 2017). Zn supplementation positively affected the sperm characterization includes the spermatogenesis process, sperm motility in Japanese eel (*A. japonica*) (Yamaguchi et al., 2009). Moreover, the highest spermatocrit percentage and motility duration were detected in rainbow trout broodstock male fed diet supplemented with Zn compared with control (Kazemi et al., 2020). Previous studies showed that Zn improved the sperm motility of Nile tilapia (Gammanpila et al., 2007) and adult blunt snout bream (*Megalobrama amblycephal*) (Jiang et al., 2016). In consistent with the present data Kaliky et al. (2019) reported that dietary supplementation of Zn at a dose of 200 mg kg^{-1} enhanced the quality and quantity of sperm includes semen volume, sperm motility, sperm viability, and sperm concentration of catfish.

Lipid mobilization from liver contributes to oocyte growth; moreover, it exhausted as a source of energy during the vitellogenesis process (Bon et al., 1997; Johnson et al., 1991). Thus, lipid profile; triglyceride, cholesterol and HDL-C play an important role in reproduction and gonadal maturation during the spawning process of fish (Hiramatsu et al., 2015; Shankar and Kulkarni, 2007). In this context, Kocaman et al. (2005) noted that major source of energy during reproduction in liver and yolk sac originated from triglycerides. The present study showed higher levels of triglyceride and HDL-C in broodstock male and female fed diet supplemented with high inclusion level 90 mg kg^{-1} ZnO-K while, cholesterol was decreased in male and female fed dietary supplemented with different levels of ZnO-K compared with control. The reduction of cholesterol in broodstock may be due to the consumption of cholesterol for synthesis natural lipid in the oocyte of fish via fatty acids transporter protein such as srb1, fatp1 and fabp1 (Hiramatsu et al., 2015). Therefore, our finding appeared an improvement in total, absolute and relative fecundity where ZnO-K supplementation with higher levels induced all parameters related to reproductive performance. Contrary with our finding dietary $ZnSO_4.7H_2O$ supplementation have been reduced triglyceride while cholesterol and HDL-C in the serum of blunt snout bream, (*Megalobrama amblycephala*) broodstock did not affect (Jiang et al., 2016). The contrary between present results and previous study s may be due to the bioavailability of ZnO-K was higher than $ZnSO_4.7H_2O$.

Zn plays crucial role in improving the antioxidant defines to protect the membrane cells form oxidation process as well as prevents cells damage (Powell, 2000; Zago and Oteiza, 2001; Mohammady et al., 2021; Ibrahim et al., 2021a & b). In the present study activates

of antioxidant enzymes of tilapia broodstock includes CAT, SOD, GPX were significantly increased in fish fed dietary supplemented with ZnO-K, whereas, values of liver MDA were decreased in tilapia broodstock fed dietary supplemented with ZnO-K. This may be due to dietary supplemented with ZnO-K decreased the hepatic oxidative stress of tilapia broodstock. In this context, fish fed diet supplemented with high zinc level had higher activates of antioxidant enzymes includes SOD, CAT, T-AOC and GSH-Px of Carp fish serum (Feng et al., 2011), serum of tilapia (Huang et al., 2015) and liver of blunt snout bream, *Megalobrama amblycephala* broodstock (Jiang et al., 2016). On the other side, MDA at low levels reflects the antioxidant status of fish where, it can be used an indicator of oxidative stress (Devasena et al., 2001). In addition, dietary supplemented with zinc have a positive effect on serum Alkaline phosphatase (ALP) and also ALP is an indicator of zinc status of animal and fish (Yousef et al., 2002, Shinde et al., 2013) where, ALP is a Zn-dependent metalloenzyme. In the present study, levels of serum ALP of tilapia male and female were decreased in fish fed dietary supplemented with ZnO-K compared to the control. Contrary with our finding, Jiang et al. (2016) showed that fish fed diet supplemented with zinc had significant levels of serum ALP of blunt snout bream broodstock, tilapia (Wu et al., 2015; Sá et al., 2004), Atlantic salmon (*Salmo salar*) (Maage and Julshamn, 1993), and grass carp (*Ctenopharyngodon idella*) (Liang et al., 2012).

In conclusion, the current results recommend that additional ZnO-K is compulsory in broodstock Nile tilapia feeds, and about 60 or 90 mg kg^{-1} dietary ZnO-K is essential for optimal reproductive performance. Nevertheless, further studies are needed to confirm whether the improvement in performance of reproductive will recompense for the rise in feed cost resulted in ZnO-K supplementation.

CRediT authorship contribution statement

Conceptualization, Data curation, Formal analysis: **Mohamed R. Soaudy, Eman Y. Mohammady, Marwa M. Ali, Mohamed A. El-ashry, Mohamed S. Hassaan**. Funding acquisition, Investigation, Methodology, Project administration, Resources, Software: **Souady**. Project administration, Resources, Software: **Mohamed R. Soaudy, Eman Y. Mohammady, Marwa M. Ali, Mohamed A. El-ashry, Mohamed S. Hassaan**. Validation, Visualization, Writing – original draft, Writing – review & editing: **Mohamed Hassaan**.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

Data of the present article are not available.

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