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Effects of dietary supplementation with benthic diatom *Amphora coffeaeformis* on blood biochemistry, steroid hormone levels and seed production efficiency of Nile tilapia *Oreochromis niloticus* broodstock

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Abstract

Aquafeed additive quality and quantity remain pivotal factors that constrain the sustainability and progress of aquaculture feed development. This study investigates the impact of incorporating the benthic diatom *Amphora coffeaeformis* into the diet of Nile tilapia (*Oreochromis niloticus*) broodstock, on the blood biochemistry, steroid hormone (SH) levels and seed production efficiency. Broodstock females displaying mature ovary indications were initially combined with males at a ratio of three females to one male. A total of 384 adult Nile tilapia (288 females and 96 males) were used, with 32 fish (24 females and eight males) assigned to each of 12 concrete tanks (8 m^3; 2 m × 4 m × 1 m), with three replicate tanks for each dietary treatment, throughout a 14-day spawning cycle until egg harvest. Fish were fed one of four different dietary treatments: $AM_{0\%}$ (control diet), and $AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ enriched with the diatom *A. coffeaeformis* at levels of 20, 40 and 60 g/kg of diet respectively. At the trial's conclusion, total protein, albumin, triglyceride and creatinine), SHs (follicle-stimulating hormone, luteinizing hormone, free testosterone, total testosterone, progesterone and prolactin) and seeds production efficiency of Nile tilapia improved significantly ($p < 0.05$) in alignment with the increment of *A. coffeaeformis* supplementation. The findings propose that including *A. coffeaeformis* at levels ranging from 4% to 6% could be effectively employed as a feed additive during the Nile tilapia broodstock's spawning season.

KEYWORDS

aquaculture; feed additive; follicle-stimulating hormone; hormone-promoters; luteinizing hormone; progesterone, prolactin; testosterone, progesterone

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1 | INTRODUCTION

According to the statistical report of FAO (Action, 2020), Nile tilapia ranked as the third most produced species worldwide, contributing about 9% to the total global aquaculture production, with the largest aquaculture yield (4,407,000 tons of live weight). Egypt is one of the largest aquaculture producers worldwide. In 2022, Egypt ranked as the sixth largest global aquaculture producer, contributing around 1,592,000 tons to the total world aquaculture production. Out of this, 352,000 tons originated from marine aquaculture, while 1,240,000 tons came from inland aquaculture, primarily driven by Nile tilapia (Munguti et al., 2022). Currently, several factors significantly limiting aquaculture production and development such as climate change, pandemics, wars, global economic and political issues (Ahmed & Azra, 2022), aqua-diet high production cost with low quality, unstable water quality, low fish immunity, widespread fish diseases, low productivity of broodstock and high mortality rate in fish larvae (Abo-Taleb, Ashour, et al., 2020; Abo-Taleb, Zeina, et al., 2020; Abo-Taleb et al., 2021; Mabrouk et al., 2022; Magouz, Essa, Matter, Mansour, Alkafafy, & Ashour, 2021). Therefore, several strategies have been recently applied to sustain and develop aquaculture production (Alprol et al., 2021; Magouz, Essa, Matter, Mansour, Ahmed, & Ashour, 2021; Yilmaz, 2019), especially in Nile tilapia aquaculture (Ashour et al., 2021; Olajuyigbe et al., 2020).

The high cost of high-quality aquaculture feeds, crucial for sustaining the growth and health of Nile tilapia broodstock, poses a significant constraint on the production and maintenance of this species. These specialised diets, designed to offer a comprehensive and balanced nutritional profile, constitute more than 50% of the production costs for tilapia fry (Mansour, Ashry, et al., 2022). Aquaculture diet can impact water quality, growth performance, fish health and immunity negatively, potentially increasing the risk of infections (Abu-Elala et al., 2020; Lieke et al., 2020). In order to foster and support aquaculture endeavours, the utilisation of feed additives has emerged as a primary strategy in aquaculture, attributed to the enhanced value they bring to aqua diets (Karim et al., 2022; Mansour, Ashour, Alprol, & Alsaqufi, 2022; Marimuthu et al., 2022; Mohammadian et al., 2022).

Many feed additives have been used in aqua diets such as algae derivatives, seaweed extracts, binders, antimicrobials, antioxidants, enzymes, immunostimulants probiotics (Mohammadi et al., 2022), photogenic and prebiotics which improve growth performances and nutrient utilization (Abu-Elala et al., 2021; Alemayehu et al., 2018; Ali et al., 2020; Mandey & Sompie, 2021; Suphoronski et al., 2019; Yao et al., 2020; Yilmaz, 2019). Due to their high content of bioactive molecules (Sarker et al., 2020), microalgal cells are used in several bioindustries including, food supplements (Fais et al., 2022; Vieira et al., 2020), pharmaceuticals (Shao et al., 2019), cosmetics (Arad & Yaron, 1992; Mourelle et al., 2017; Zhuang et al., 2022), phytoremediation (Abdelsalam et al., 2019; Abou-Shanab et al., 2014; Alprol et al., 2023; Essa et al., 2018; Mansour et al., 2022a; 2022b), antimicrobial activities (Osman et al., 2010; Osman et al., 2020), biodiesel (Abomohra & Elshobary, 2019; Elshobary, El-Shenody, & Abomohra, 2021; Elshobary, Zabed, et al., 2021; M. Abdelsalam et al., 2019) and aquaculture feed-additives (Mabrouk et al., 2022; Mansour, Ashour, Abbas, et al., 2022; Sharawy et al., 2022). Recently, several studies have focused on utilising microalgae and/or their extracts in aqua diets due to their antioxidant and effective biological substrates, which are used to support the growth and overall activity of fish in a biological system (Abbas et al., 2023; Glodde et al., 2018; Kumosani et al., 2017). Diatoms, which belong to the Bacillariophyceae class, are the largest single-celled microalgae found in various aquatic environments. These microorganisms play a critical role in the phytoplankton communities of marine, brackish and fresh waters (Anantharaj et al., 2011; Bhosle et al., 1993). *Amphora coffeaeformis* is a benthic diatom rich in nutrients including proteins, lipids, minerals and various bioactive compounds. These compounds include phenolics, polyphenols, flavonoids, carotenoids, vitamins, phytol, neophytadiene, 2,6-dimethyl-4[3H]-quinazolinone, metals, sulfated polysaccharides and pigments (Chtourou et al., 2015; Karawita et al., 2007; Mekkawy et al., 2020). As reported by several studies, due to their bioactive materials, *A. coffeaeformis* has potent antibacterial, higher 1,1-diphenyl-2-picrylhydrazyl activities, anticancer, anti-inflammatory, antiviral and antioxidants activities which promote this species to be utilised in the manufacturing of food supplements, pharmaceuticals, cosmetics, animal and aqua feed additives (Abu Affan et al., 2007; Alwaleed et al., 2021; El-Sayed et al., 2018; Kuczynska et al., 2015; Lee et al., 2009; Mohamed Shawky, 2020; Yousof et al., 2021). *A. coffeaeformis* has been successfully used as a live feed in both natural habitats and aquaculture applications for a variety of species such as gastropods, mollusks, bivalves, sea urchins, crustaceans, zooplankton and various fish larvae (Courtois de Viçose et al., 2012; Kaparapu, 2018). There have been relatively few studies on the use of *A. coffeaeformis* as an aqua feed additive for aquatic animals, specifically fingerlings and juvenile Nile tilapia (Ayoub et al., 2022; Ismail et al., 2021; Saleh et al., 2020), juvenile African catfish (*Clarias gariepinus*) (Mekkawy et al., 2020) and larvae and postlarvae of the whiteleg shrimp (*Litopenaeus vannamei*) (Khwancharoen et al., 2020; 2021). To our best knowledge, no previous study was conducted to investigate the impact of dietary supplementation of the diatom *A. coffeaeformis* on the broodstock of Nile tilapia (Bhujel et al., 2001; El-Sayed et al., 2003; El-Sayed, 2006). Several studies have found that a high-quality diet for Nile tilapia broodstock leads to enhanced spawning performance and a higher yield of seeds (El-Sayed & Kawanna, 2008; Gunasekera et al., 1997). Therefore, the current study is the first study aimed to investigate how supplementing the diet of Nile tilapia The *A. coffeaeformis* dehydrated powder, produced according to the method described by (El-Sayed et al., 2018) was obtained from the National Research Center in Egypt broodstock with *A. coffeaeformis* can influence blood biochemistry, steroid hormone (SH) levels in both males and females and seed production efficiency during the spawning season.

2 | MATERIALS AND METHODS

2.1 | Diatom species, *A. coffeaeformis*

The *A. coffeaeformis* dehydrated powder, produced according to the method described by (El-Sayed et al., 2018) was purchased

from the National Research Center in Egypt. The powder's biochemical composition, including protein, carbohydrate, lipid, ash and fibre content, was determined using the guidelines outlined by AOAC (2003). The *A. coffeaeformis* nutritional profile used in this study was found to be 33.5% protein, 10.7% carbohydrate, 7.4% lipid, 37.9% ash and 3.2% fibre (% of dry weight basis). After analysis, the *A. coffeaeformis* powder was stored in plastic bags at 20°C until further use.

2.2 | Water quality indices parameters

During the study period (14-day spawning cycle and 21-day equipping period), water quality parameters of dissolved oxygen (DO, mg L^{-1}), ammonia (NH_3, mg L^{-1}), nitrite (NO_2, mg L^{-1}) and nitrate (NO_3, mg L^{-1}), were recorded three times a week. Moreover, temperature (°C), salinity (ppt) and pH were recorded daily (at 1.00 PM), following the protocol guidelines by APHA (2005). All recorded water quality indices of temperature (25.32–27.32°C), pH (7.21–7.75), salinity (0.88–0.98), DO (6.58–6.98 mg L^{-1}), NH_3 (0.098–0.111 mg L^{-1}), NO_2 (0.145–0.192 mg L^{-1}) and (NO_3 0.185–0.227 mg L^{-1}) were within the recommended ranges of the cultural requirements for Nile tilapia broodstock during the spawning season (Wilson, 1991). In the current experiment, the irrigation water of the El-slam Canal was the main source of water. The rate of daily freshwater change was 30%. Unconsumed feed and wastes were removed by siphoning every day.

2.3 | Nile tilapia (*Oreochromis niloticus*) broodstock

2.3.1 | Experimental fish and design

The present study was carried out at a private Tilapia hatchery in the Port Said Governorate of Egypt. The breeding stock of Nile Tilapia was obtained from a commercial farm within the same region. Prior to the start of the feeding trial, the fish underwent a 21-day acclimation period to adapt to the experimental conditions and were fed a control diet during this phase. This interval also facilitated gonad development, allowing the fish to enter a spawning cycle. Following this period, during which the female ovaries were confirmed to be in the pre-spawning maturation stage, males and females were grouped together at a ratio of three females to one male for a 14-day spawning cycle. At the start of the trial, a total of 384 fish (96 males and 288 females) were randomly distributed across 12 indoor concrete tanks, each measuring 8 m (2 m × 4 m × 1 m) in size. The fish were stocked at a density of 32 individuals per tank (eight females and 24 males). The tanks were equipped with aeration, and 30% of the water volume was replaced daily. Once the resulting larvae were produced, they were gently collected and transferred to another tank.

2.3.2 | Experimental Diet

A reference diet was formulated, to which four different inclusion levels of the diatom *A. coffeaeformis* were added (as shown in Table 1). The first diet, denoted as $AM_{0\%}$, served as the control, while the other three diets ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$) were supplemented with *A. coffeaeformis* at levels of 20, 40 and 60 g per kilogram of the diet respectively. The addition of *A. coffeaeformis* to diets was performed as previously described by Mabrouk et al. (2022). The fish

TABLE 1 Composition analysis (%) of the experimental diets supplemented with *Amphora coffeaeformis* as feed additives for Nile tilapia during spawning season.

	Experimental diets			
Diets Composition and analysis	**$AM_{0\%}$**	**$AM_{2\%}$**	**$AM_{4\%}$**	**$AM_{6\%}$**
Composition (% of dry weight)				
Fish meal	14	14	14	14
Yellow corn	25	25	25	25
Soybean meal	20	20	20	20
Wheat bran	15	15	15	15
Rice bran	15	15	15	15
Corn gluten	7	7	7	7
Soya oil	3	3	3	3
DCP ($CaHPO_4$)	0.7	0.7	0.7	0.7
Premix mixer[a]	0.3	0.3	0.3	0.3
Total	100	100	100	100
Diatom *A. coffeaeformis* supplementation levels (g kg^{-1})	0	20	40	60
Biochemical composition (% of dry weight)[a]				
DM	93.55			
CP	29.98			
EE	9.25			
CF	4.73			
Ash	7.58			
NFE	48.74			
GE (kj kg^{-1} diet)	4963			
DE (kj kg^{-1} diet)	3520			

Note: All previous elements were calculated according to the reported guideline of AOAC (2003). Each 1-kg premix contains (mg kg^{-1}): P-amino benzoic acid (9.48), D-biotin (0.38), inositol (379.20), niacin (37.92); Ca-pantothenate (56.88), pyridoxine-HCl (11.38), riboflavin (7.58), thiamine-HCl (3.79), L-ascorbyl-2-phosphate Mg (296.00), folic acid (0.76), cyanocobalamin (0.08), menadione (3.80), vitamin A-palmitate (17.85), a-tocopherol (18.96), calciferol (1.14), K_2PO_4 (2.011), Ca_3 $(PO_4)_2$ (2.736), Mg $SO_4.7H_2O$ (3.058) and $NaH2PO_4.2H_2O$ (0.795). Abbreviations: CF, crude fibre; CP, crude protein; DE, digestible energy; DM, dry matter; EE, ether extract; GE, gross energy; NFE, nitrogen-free extract.

[a]AM0%, AM2%, AM4% and AM6% are diets supplemented with diatom (*A. coffeaeformis*) at levels of 0, 20, 40 and 60 g kg^{-1} diet respectively.

were fed by hand three times a day (at 9 AM, 12 PM and 4 PM) at a rate of 3% of their wet body weight.

2.4 | Tested parameters

2.4.1 | Blood serum analysis

At the end of the spawning cycle, six fish (three males and three females) from each replicate were anaesthetised (using TMS buffered Tricaine Methanesulfonate at the dose of 30 mg L^{-1}) and blood serum samples were collected for biochemical analysis. The blood samples were collected into sterilised tubes, using a hypodermic syringe (3 mL with a 22 gauge needle and a heparinized tube), kept at room temperature for 30 min and centrifuged at 3000 rpm for 15 min. The collected serum was preserved at −20°C for further analysis. The total protein level (g dL^{-1}) (Lowry et al., 1951), and albumin level (g dL^{-1}) (Wotton & Freeman, 1982) were determined and the difference between the values of total protein and albumin was used to calculate the globulin level (g dL^{-1}). The triglyceride level (mg dL^{-1}) (McGowan et al., 1983), glucose level (mg dL^{-1}) (Henry, 1964) and cholesterol level (mg dL^{-1}) (Naito & Kaplan, 1984) were determined using calorimetric kits supplied by El-Nasr Pharmaceutical Chemicals, following the manufacturer's instructions. Additionally, the activities of serum glutamic pyruvate transaminase (GPT, U mL^{-1}) (Kim & Seo, 1998) and creatinine (U mL^{-1}) (Park et al., 2001) were determined using specific commercial kits (Biodiagnostic), according to the manufacturer's instructions.

2.4.2 | SHs aspects

At the end of the experiment, six fish (three males and three females) were randomly selected from each replicate group to determine SHs. Both males and females were tested for follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Only males were tested for total testosterone (TT) and free testosterone (FT) hormones. Conversely, only females were tested for prolactin (PRO) and progesterone (PRG) hormones. The SHs were determined using ELISA assay (Abraham, 1977), an Enzyme-linked immune sorbent, known as the Immulite/Immulite 1000 system (Beitins et al., 1976). Using specific commercial kits, SHs of FSH (RAB0660-1KT), LH (SE120071), TT (SE120120), FT (SE120120), PRO (RAB0408-1KT) and PRG (SE120087) were determined, according to the manufacturer's instructions.

2.5 | Female's seed productivity

After the 14-day spawning experiment, the broodstock (males and females) were meticulously collected and transferred to alternate ponds, while reducing the water volume. The eggs were collected following the method outlined by El-Sayed et al. (2012). The number of larvae produced per female was determined using the following equation:

$$\text{Number of fries per female} = \frac{\text{Total number of seeds/tank}}{\text{Total number of females/tank}}. \quad (1)$$

The improvement average ratio (%) of the number of seeds from mothers fed the control diet to the supplemented diets was conducted as the following equation:

$$\text{The improvement average ratio}(\%) = \frac{Sn - Sc}{Sc} \times 100, \quad (2)$$

where Sn and Sc are the numbers of seeds that come from mothers fed the supplemented diets ($AM_{0\%}$, $AM_{0.4\%}$ and $AM_{0.6\%}$) and the control diet (N_0) respectively.

2.6 | Statistical analysis

The hypotheses of homoscedasticity and normality were checked before the statistical analysis of data. All data were in mean ± standard deviation (SD). All data were analysed by the SPSS computer software package programme. To compare the significant differences among means, at the level of $p < 0.05$, a one-way analysis of variance test, followed by Duncan's multiple range tests was carried out. Finally, the GraphPad Prism program (version 9) was used to perform the graphical figures of the SHs and the broodstock seeds production figures.

3 | RESULTS

3.1 | Blood biochemistry

Table 2 presents the results of the blood serum biochemical analysis of *O. niloticus* broodstock fed with different inclusion levels of *A. coffeaeformis*. In comparison to the control diet ($AM_{0\%}$), compared to the control diet ($AM_{0\%}$), fish that were fed diets supplemented with *A. coffeaeformis* showed significantly higher ($p < 0.05$) levels of protein, albumin, triglycerides and creatinine. However, there were no significant differences ($p < 0.05$) observed in globulin, cholesterol, GPT and glucose between fish fed with the control diet ($AM_{0\%}$) and diets supplemented with *A. coffeaeformis* ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$).

3.2 | Steroid hormones

Figure 1 demonstrates the influence of diets supplemented with varying levels of *A. coffeaeformis* on the concentrations of FSH and LH hormones in *O. niloticus* broodstock (males and females). Figure 1 illustrates that fish who were fed with supplemented diets ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$) demonstrated a significant ($p < 0.05$) improvement in both FSH and LH values in both males and females compared to fish

TABLE 2 Blood biochemical parameters of Nile tilapia broodstock fed with diets supplemented with *Amphora coffeaeformis* during the spawning season.

Blood Biochemistry Indices	Experimental diets			
	$AM_{0\%}$	$AM_{2\%}$	$AM_{4\%}$	$AM_{6\%}$
Total protein (g dL^{-1})	4.51 ± 0.11^{c}	4.74 ± 0.05^{bc}	4.91 ± 0.12^{b}	5.23 ± 0.09^{a}
Albumin (g dL^{-1})	1.86 ± 0.03^{c}	2.02 ± 0.04^{b}	2.15 ± 0.03^{b}	2.78 ± 0.07^{a}
Globulin (g dL^{-1})	2.64 ± 0.14	2.72 ± 0.09	2.75 ± 0.11	2.45 ± 0.16
Triglyceride (mg dL^{-1})	199.7 ± 2.9^{b}	283.3 ± 8.8^{a}	276.3 ± 29.9^{a}	321.7 ± 5.9^{a}
Cholestrol (mg dL^{-1})	189.7 ± 2.03	206.7 ± 12.1	192.3 ± 3.7	194.1 ± 6.7
Creatinine	0.34 ± 0.01^{c}	0.39 ± 0.012^{b}	0.39 ± 0.012^{b}	0.44 ± 0.015^{a}
GPT (U mL^{-1})	43.33 ± 2.3	46.67 ± 2.4	42.33 ± 2.4	45.01 ± 1.5
Glucose (mgdL^{-1})	166.01 ± 6.6	167.33 ± 10.7	183.33 ± 8.6	171.33 ± 6.2

Note: $AM_{0\%}$, $AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ are diets supplemented with diatom *A. coffeaeformis* at levels of 0, 20, 40 and 60 g kg^{-1} diet respectively. The presented data are Means ± SD ($n = 3$). Different letters ($a > b > c$) in each row indicate significant differences ($p < 0.05$), while the absence of letters means no significant differences (AOAC, 2003).

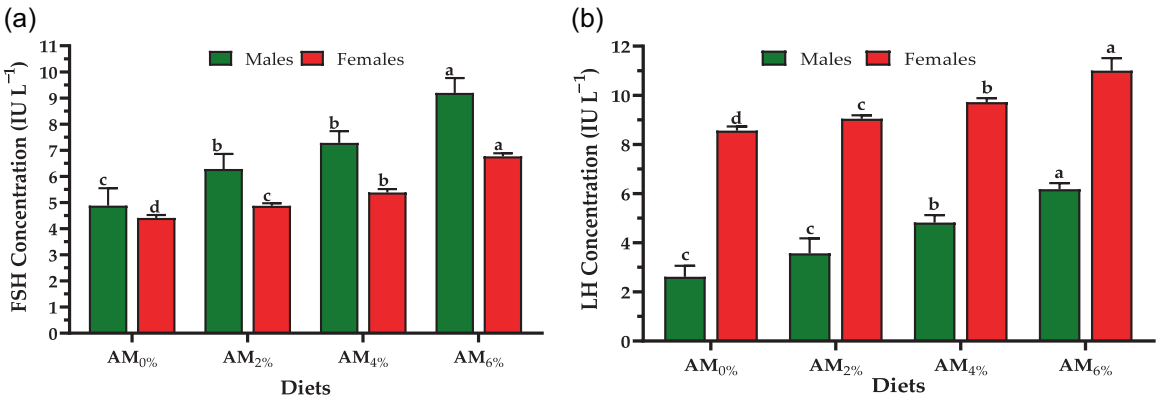

FIGURE 1 Effect of diets supplemented with several doses of diatom *Amphora coffeaeformis* on the concentrations of (a) follicle-stimulating hormone (FSH) and (b) luteinizing hormone (LH), of *O. niloticus* broodstock (males and females). $AM_{0\%}$, $AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ are diets supplemented with diatom (*A. coffeaeformis*) at levels of 0, 20, 40 and 60 g kg^{-1} diet respectively. The presented data are means ± standard deviation ($n = 3$). Different letters ($a > b > c > d$) in each column indicate significant differences ($p < 0.05$).

fed with the control diet ($AM_{0\%}$). The impact of diets supplemented with different levels of *A. coffeaeformis* on the concentrations of FSH and LH hormones in *O. niloticus* broodstock (both males and females) is shown in Figure 1. Fish that were fed supplemented diets ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$) exhibited a significant ($p < 0.05$) enhancement in both FSH and LH values for both males and females, in comparison to those fed the control diet ($AM_{0\%}$) (Figure 1). On the other hand, for the FSH hormone, males had a stronger response to increasing supplementation levels than females (Figure 1). Figure 2, which pertains to males only, illustrates that as the levels of dietary supplementation increased, there was a corresponding increase in the concentrations of both TT and FT hormones in fish. The data in Figure 2 indicate that fish fed with diets supplemented with diatom *A. coffeaeformis* ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$) demonstrated significant ($p < 0.05$) improvements in the concentrations of total and FT hormones when compared to fish fed with the control diet.

In the case of females, the increase in diet supplementation levels, lead to a proportional and significant ($p < 0.05$) increase in the concentrations of PRG and PRO hormones among females fed diets supplemented with diatom *A. coffeaeformis* ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ respectively), in comparison to fish fed the control diet (Figure 3a,b). Figure 3 illustrates significant ($p < 0.05$) improvements in the concentrations of PRG and PRO hormones in fish (females only) fed with diets supplemented with diatom *A. coffeaeformis* ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ respectively), compared to fish fed the control diet (Figure 3a,b).

3.3 | Broodstock seeds production

Regarding the seed production, differences were observed among females fed each of the four dietary treatments (Figure 4). As

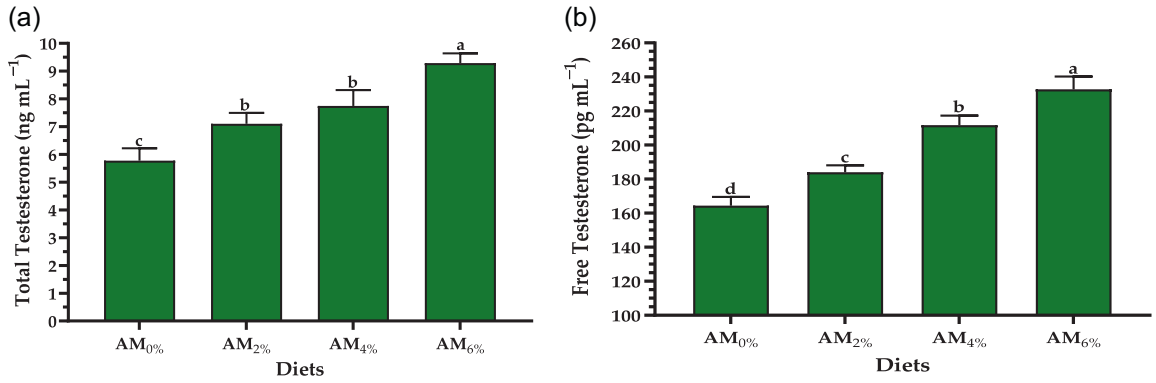

FIGURE 2 Effect of diets supplemented with several doses of diatom *Amphora coffeaeformis* on the concentrations of (a) total testosterone hormone and (b) free testosterone hormone of *Oreochromis niloticus* broodstock (males and females). $AM_{0\%}$, $AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ are diets supplemented with diatom *A. coffeaeformis* at levels of 0, 20, 40 and 60 g kg^{-1} diet respectively. The presented data are means ± standard deviation ($n = 3$). Different letters ($a > b > c >$ d) in each column indicate significant differences ($p < 0.05$).

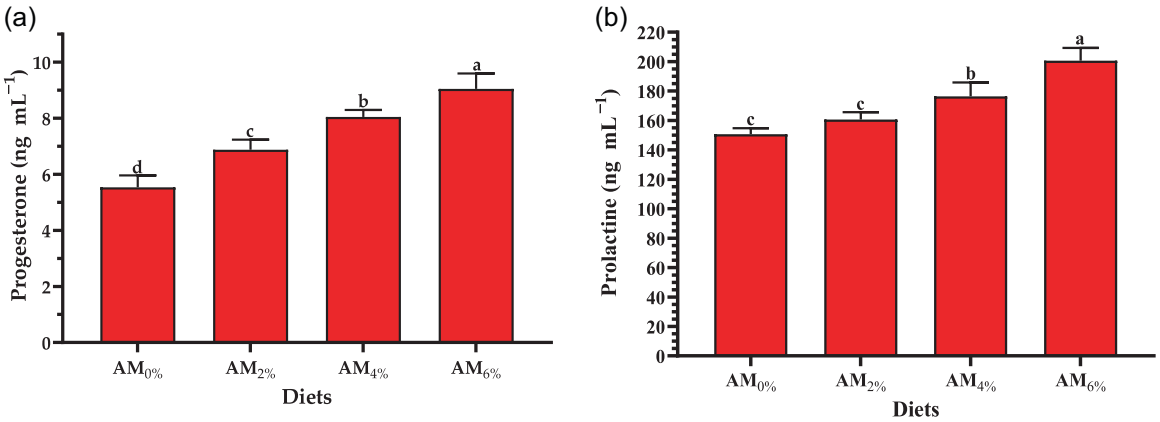

FIGURE 3 Effect of diets supplemented with several doses of diatom *Amphora coffeaeformis* on the concentrations of (a) progesterone and (b) prolactin hormones of *Oreochromis niloticus* broodstock (females only) of *O. niloticus*. $AM_{0\%}$, $AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$ are diets supplemented with diatom *A. coffeaeformis* at levels of 0, 20, 40 and 60 g kg^{-1} diet respectively. The presented data are means ± standard deviation ($n = 3$). Different letters ($a > b > c > d$) in each column indicate significant differences ($p < 0.05$).

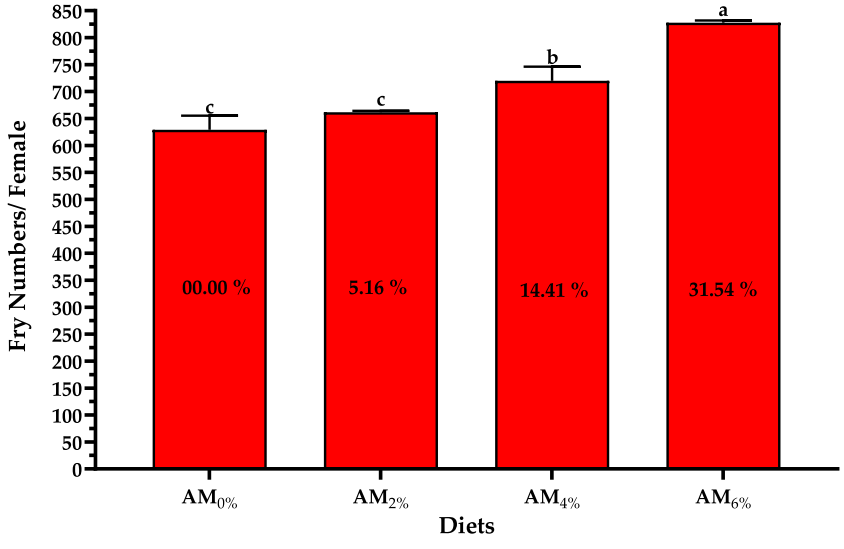

FIGURE 4 Effect of diets supplemented with several doses of diatom *Amphora coffeaeformis* on the seed production efficiency of *Oreochromis niloticus*. $AM_{0\%}$, $AM_{0.2\%}$, $AM_{0.4\%}$ and $AM_{0.6\%}$ are diets supplemented with diatom *A. coffeaeformis* at levels of 0, 2, 4 and 6 g kg^{-1} diet respectively. The presented data are means ± standard deviation (n = 3). Different letters (a > b > c) in each column indicate significant differences ($p < 0.05$). The percentages that exist in the bars are the improved average ratios (%) in seed production for females fed with supplemented diets ($AM_{0.2\%}$, $AM_{0.4\%}$ and $AM_{0.6\%}$) compared to the control diet.

previously observed for other parameters, female Nile tilapia produced significantly ($p < 0.05$) more offspring's when fed diets supplementated with increasing levels of A. coffeaeformis ($AM_{2\%}$, $AM_{4\%}$ and $AM_{6\%}$) than to those fed the control diet ($AM_{0\%}$). There are significant ($p < 0.05$) improvements in seed productivity for females on the supplemented diets compared to those on the control diet.

4 | DISCUSSION

The current study aimed to evaluate the impact of dietary supplementation with the benthic diatom *A. coffeaeformis* on the blood biochemistry, steroid hormonal aspects and seed production efficiency of Nile tilapia during the spawning season. However, the current study did not aim to investigate either growth performance, nutrient utilization and biochemical composition of the Nile tilapia, due to the study period (14 days) of the spawning season.

As shown in Table 2, the current results showed significant improvements in total protein, albumin, triglyceride and creatinine in fish-fed diets supplemented with *A. coffeaeformis*, compared to the control diet. However, no significant improvements were observed in globulin, cholesterol, GPT and glucose. The use of blood serum indices, such as total protein, albumin, triglyceride, cholesterol, GPT, glucose and creatinine, are important tools to evaluate the effectiveness of feed additives (Akbary & Aminikhoei, 2018; Akbary & Molazaei, & Aminikhoei, 2018; Madibana et al., 2017). The study by (Saleh et al., 2020) found that the inclusion of *A. coffeaeformis* (25–100 g kg^{-1}) in the diets of Nile tilapia fingerlings led to significant improvements in red blood cells, white blood cells, superoxide dismutase, catalase, aspartate transaminase, alanine transaminase, total serum protein, lymphocytes, monocytes, eosinophils and lysozyme. However, no significant improvements were observed in albumin and globulin. F. Ayoub et al. (2019) found that the inclusion of *A. coffeaeformis* (10–30 g kg^{-1}) in the diets of Nile tilapia fingerlings (initial weight of 7.8 g) led to significant improvements in lysozyme activities, total serum protein, albumin and globulin, but no significant improvements in aspartate transaminase and alanine transaminase. Another study by Ayoub et al. (2022), found that the inclusion of *A. coffeaeformis* (10–30 g kg^{-1}) in the diets of Nile tilapia fingerlings (initial weight of 25.8 g) led to significant improvements in antioxidant and immunological indices. These findings may be attributed to the bioactive compounds present in *A. coffeaeformis*, which promote its successful use as a feed additive in the diets of Nile tilapia (Abu Affan et al., 2007; Alwaleed et al., 2021; F. Ayoub et al., 2019; Ayoub et al., 2022; El-Sayed et al., 2018; Kuczynska et al., 2015; Lee et al., 2009; Shawky, 2020; Saleh et al., 2020; Yousof et al., 2021).

The understanding of the relationship between SHs and the diet of Nile tilapia broodstock, and how it affects seed production efficiency, is essential for achieving efficient and successful reproduction (Ajiboye, 2015; Qiang et al., 2021). In this study, the inclusion of *A. coffeaeformis* at levels of 2%, 4% and 6% in the diet significantly ($p < 0.05$) improved the SH values and seed productivity (number of seeds produced per female and the average improvement ratio compared to the control diet) in female Nile tilapia. The current results indicate that the gradual inclusion of *A. coffeaeformis* in the diet led to a gradual improvement in SHs values (LH, FSH, PRO, PRG, TT and FT) and seed productivity in female Nile tilapia. The study by Hassaan (2022) found that the inclusion of *Cyclotella* spp. (10–15 g kg^{-1}) in the diet of Nile tilapia, broodstock improved seed production and SHs values, as well as significantly increasing the gonadosomatic index and fecundity. These findings align with the study of Promya and Chitmanat (2011), who used *Arthrospira* as a replacement for artificial hormones in the diet of Nile tilapia broodstock. Joshua and Zulperi (2020) reviewed the significant impact of *Chlorella vulgaris* and *Arthrospira platensis* in improving reproduction and hormonal spawning aspects in several aquatic animals' broodstock during the spawning season.

5 | CONCLUSIONS

This study concluded that the gradual increase of *A. coffeaeformis* in the diet of Nile tilapia broodstock resulted in significant improvements in blood biochemistry, SHs and seed production efficiency. The current work suggested that *A. coffeaeformis* at a level of 4%–6% can be effectively used as a feed additive during the spawning season for Nile tilapia broodstock.

AUTHOR CONTRIBUTIONS

Mohamed Mabrouk: Conceptualisation; methodology, software; validation; formal analysis, investigation; resources; data curation; writing—original draft preparation; visualisation; supervision; funding acquisition. **Mohamed Ashour**: Conceptualisation; methodology, software; validation; formal analysis, investigation; resources; data curation; writing—original draft preparation; writing—review and editing; visualisation; project administration; funding acquisition. **Mohamed F. Abdelghany**: Conceptualisation; methodology; validation; investigation; resources. **Mohamed A. Elokaby**: Conceptualisation; methodology; software; validation; investigation; resources. **Abdel-Wahab A. Abdel-Warith**: Writing—review and editing; funding acquisition. **Elsayed M. Younis**: Writing—review and editing; funding acquisition; **Ehab El-Haroun**: Resources; writing—review and editing; supervision. **Ahmed G. A. Gewida**: Conceptualisation; methodology; validation; investigation; resources. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Availability of data is available by the authors upon request.

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SUPPORTING INFORMATION

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