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Effect of including dried microalgae *Cyclotella menegheniana* on the reproductive performance, lipid metabolism profile and immune response of Nile tilapia broodstock and offspring

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A R T I C L E I N F O

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A B S T R A C T

Cyclotella menegheniana is a diatom microalga often found in both marine and freshwater environments, characterized by a high-quality nutritional profile, including fatty acids, protein, and carotenoids, it is also a promising bioresource for improving food and animal feed nutrition. In this study, two separate experiments were conducted. In the first experiment, the utility of including *C. menegheniana* in the diet was evaluated for its impact on the reproductive efficiency, hematobiochemical biomarkers, and oxidative enzymes of broodstock Nile tilapia, *Oreochromis niloticus*, fish fed diet T2 (with 10 g kg^{-1} *C. menegheniana* supplementation) recorded the highest ($P \leq 0.05$) gonadosomatic index (GSI) for female tilapia, whereas fish fed diet T3 (with 15 g kg^{-1} *C. menegheniana* supplementation) recorded significantly higher ($P \leq 0.05$) values of egg diameter, egg volume, sperm motility, and sperm concentration compared to the remaining dietary treatments. In addition of 15 g kg^{-1} *C. menegheniana* significantly improved hemoglobin (Hb) and hematocrit (Htc) values of male and female broodstock. The content of triglyceride decreased in the blood of the broodstock fed diets supplemented with *C. menegheniana*. The lowest level of ALT and AST for male was observed in T3 group, while the highest one ($P < 0.05$) was in the control group. Complements component (C3), (C4) and IgM were significantly ($P < 0.05$) higher in fish fed diet T2 and T3 compared with other treatments. The final body weight, weight gain and specific growth rate of fries (F1 generation–II experiment) produced by fish received 15 g *C. menegheniana* significantly increased ($P < 0.05$), while fish received either 10 or 15 g *C. menegheniana* significantly improved ($P < 0.05$) feed conversion ratio and protein efficiency ratio. In addition, fish received diets enriched with *C. menegheniana* significantly improved fry survival rate. In conclusion, the use of *C. menegheniana* as a feed additive stimulates the immune system and improves the blood and reproductive parameters of Nile tilapia broodstock, and it has a positive influence on the growth and feed conversion of Nile tilapia offspring

1. Introduction

The importance of aquaculture as a viable method for sustainable aquatic feed production and availability is expanding worldwide (Mohammady et al., 2023; Ali et al., 2023). One of the factors that contributes to in the sustainability of the aquaculture sector is the reproduction of fish from broodstock (Soaudy et al., 2021). However, the use of non-specific diets for brooders results in low production yields from broodstock (Khanzadeh et al., 2016). Because breeder nutrition affects reproductive performance, using more appropriate feed that has been supplemented by mixing bioactive natural sources can be crucial to improving the reproductive abilities of broodstock (Chong et al., 2004; Kumaraguruvasagam et al., 2007). Manufactured diets containing naturally functioning components that can enhance fish immunity then improve reproductive efficiency (Hassaan et al., 2016, Wan et al., 2018; Hoseinifar et al., 2018; Saeed et al., 2021, Hassaan et al., 2021a). Microalgae can be exploited as a natural source of bioactive chemicals because of their rich polyunsaturated fatty acid content (PUFA), notably

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the n-3 series and crude protein (Christaki et al., 2011; Abd El-Hack et al., 2019; Hassaan et al., 2021a). Microalgae have the potential to become a substantial renewable energy source since they are a large source of oils and other compounds that may be utilized as feedstock to manufacture biofuels and high-value products (Moreno-Garcia et al., 2017). Microalgae may thrive in a variety of wastewater, including those produced by aquaculture systems such as farming systems and hatcheries (Malibari et al., 2018; Dourou et al., 2020). Also, they enhance the pharmacological, immunostimulatory, antioxidant, and growth performance in aquatic animals (Pistelli et al., 2021; Taalab et al., 2022). The diatom *C. menegheniana* has been proposed as a species deserving of further research due to its harmless nature, right size, and potential for heterotrophic development (Wood et al., 1999; Pahl et al., 2010a). It has been consumed by aquatic animals, the focus of these investigations was not on its reproductive performance (Webb and Chu, 1983; De Pauw and Persoone, 1988). Due to the diatom *C. menegheniana cryptica's* high concentration of Phytosterols and carotenoids, the total amount of vitamins is significantly enhanced immune response and antimicrobial activity (Del Mondo et al., 2020; Pistelli et al., 2021). This study is considered as the first attempt to demonstrate the effects of using *C. menegheniana* as a feed additive on the reproductive efficiency, immune response, haematological, and blood serum biochemical parameters of Nile tilapia (*Oreochromis niloticus*) broodstock (experiment I), as well as the impact on the survival of their fries (F1 generation experiment II).

2. Materials and methods

2.1. Analysis of bioactive compounds and vitamins

The analysis of *C. menegheniana's* bioactive components was conducted following the protocol outlined in Smerilli et al. (2019), while the estimation of vitamin content was based on the method described in Pistelli et al. (2021) and described in Table 1.

2.2. The amino acid and fatty acid profile

The analysis of lipid extraction, fatty acids and amino acids was conducted following the protocol outlined in Radwan (1978) and Harold et al. technique (1981) and described in Table 2&Table 3.

2.3. Experimental design and diets

The formulation of four isonitrogenous (34.47%, crude protein) and isoenergetic (19.96 MJ kg^{-1}, gross energy compounds was done (Table 4). Dried *C. menegheniana* powder was added to each diet in the following amounts: 0 g kg^{-1} (Control), 5 g kg^{-1} (T1), 10 g kg^{-1} (T2), and 15 g kg^{-1} (T3). *C. menegheniana* species were taken from the National Institute of Oceanography and Fisheries (NIOF) farm in El-Qanater El-Khyria, Egypt. It was examined morphologically by a phytoplankton specialist in the lab. Under a microscope and confirmed. Ingredients were mixed for five minutes in a feed mixer equipped with a homogenous mixture grinder. The components were combined, the dried *C. menegheniana* species added, and the mixture was then homogenized. Afterwards, homogenization was followed by mixing in the fish oil, vitamins, and minerals. Using a pellet mill (2 mm die), dry pellets of prepared diet were created. The pellets were kept at −20 °C until usage after being dried for 4 hours at 60 °C. According to the AOAC (1995), the chemical composition of formulated diets was estimated.

Table 1
Chemical composition and analysis of bioactive compounds and Vitamin A, Vitamin (B1) in *cycolotella menegheniana*.

Items	Result
Dry matter	89.17
Crude protein	11.70
Crude fat	8.20
Ash	13.41
[2]Total carbohydrate	66.96
Total flavonoids (μg mg^{-1})	0.2
Total carotinods (μg mg^{-1})	1.3
Total sterols (μg mg^{-1})	40
Vitamin (A mg100 g^{-1})	457.19
Vitamin B1 (g kg^{-1})	0.00048

Table 2
Amino acid analysis of *Cycolotella menegheniana*.

Items	Hydrolyzed amino acid composition (%)
Essential amino acid	
Arginine	0.26
Histidine	0.11
Lysine	0.28
Methionine	0.07
Leucine	0.39
Isoleucine	0.24
Threonine	0.22
Phenylalanine	0.19
Valine	0.31
Proline	0.23
Tyrosine	0.11
Cystine	0.10
Non-essential amino acid	
Glutamate (GLU)	0.67
Aspartatic (ASP)	0.50
Serine (SER)	0.23
Glycine (GLY)	0.26
Alanine (ALA)	0.29

Table 3
Fatty acid analysis of *Cycolotella menegheniana*.

Items	Hydrolyzed fatty acid composition (%)	
	Name	Relative distribution
C10:0	Capric acid	0.88%
C12:0	lauric acid	0.58%
C13:0	Tetradecenoic acid	0.44%
C14:0	Myristic acid	6.45%
C14:1 ω 7	Myristioleic acid	0.32%
C15:0	Pentadecanoic acid	5.13%
C15:1 ω 6		0.83%
C16:0	Palmitic acid	25.36%
C16:1 ω 9		2.63%
C16:1 ω7	Palmitoleic acid	22.59%
C16:1 ω 5		0.47%
C 17:0	Heptadecanoic acid	2.60%
C 16:3 ω 4	Hexa decatrienoic acid	1.03%
C 18:0	Stearic acid	4.54%
C18:1 ω 13		1.75%
C 18:1 ω 9	Oleic acid	6.92%
C 18:1 ω 7	Vaccinic acid	7.71%
C 18:1 ω 5	Octadecosaenoic acid	3.32%
C 18:2 ω 7		0.34%
C 18:2 ω 6	Linoleic acid	2.43%
C 18:2 ω 4		0.42%
C 20:1 ω 11	Eicosaenoic acid	1.42%
C 20:1 ω 9	Gadolic acid	0.68%
Non identified fatty acid		%

2.4. Fish and rearing conditions

The propagation of Nile tilapia was done in the Lakes and fish resources protection and development Agency, fish hatchery at Sahary, Aswan, Egypt. About, 192 broodstock (144 females and 48 males) were obtained from the fish hatchery farm. Once the broodstock was sexed and transported to twelve concrete ponds (3×1×1 m) and maintained separately, males were stocked in four concrete ponds with a stocking of 48 males each pond. For females, which were set in each pond in three

Table 4
Chemical and Fatty acid composition (% total fatty acids) of the experiment diets (g 100 g^{-1} diet).

Ingredients	Experimental diets			
	Control	T1	T2	T3
Fish Meal	10	10	10	10
Soybean Meal	45	45	45	45
Yellow Corn	20	20	20	20
Bran	13	12.5	12	11.5
Fish Oil	4	4	4	4
Corn Gluten	8	8	8	8
Cyclotella menegheniana	0	0.5	1	1.5
Vitamin &Minerals	2	2	2	2
Proximate analysis				
Dry matter	89.16	89.02	88.68	88.87
Crude protein	34.26	34.38	34.69	34.56
Ether extract	7.62	7.69	7.68	7.81
Ash	6.56	6.65	6.49	6.89
Total carbohydrate[2]	51.56	51.28	51.14	50.74
Gross energy (MJ kg^{-1})[3]	19.94	19.95	20.01	19.95
Fatty acid composition (% total fatty acids)				
C11:0	ND	ND	ND	0.093
C12:0	ND	ND	0.071	0.084
C13:0	0.117	0.127	0.203	0.211
C14:0	2.262	2.014	1.819	1.914
C14:1	0.100	0.085	0.204	0.069
C15:0	0.566	0.560	0.368	0.845
C15:1	0.262	0.439	0.235	0.118
C16:0	20.804	21.785	23.422	21.534
C16:1	2.688	2.481	0.139	2.444
C17:0	0.509	0.512	0.569	0.512
C17:1	0.272	0.234	0.287	0.221
C18:0	4.353	4.427	5.471	4.683
C18:1 C	26.651	28.121	29.782	28.611
C18:2 C	36.410	35.025	33.790	35.558
C18:3 ω 3	0.644	0.702	0.987	0.742
C20:0	0.832	0.960	0.202	0.257
C20:1	0.133	ND	ND	ND
C20:2	0.488	ND	ND	ND
C20:3 ω 3	0.375	ND	0.249	ND
C20:4	ND	0.410	ND	ND
20:05	2.526	2.110	2.193	2.098

Vitamin and mineral mixture kg-1 of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate (FeSO4.7H2O, 20% Fe), 65 mg; manganese sulfate (MnSO4, 36% Mn), 89 mg; zinc sulfate (ZnSO4.7H2O, 40% Zn), 150 mg; copper sulfate (CuSO4.5H2O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), Total carbohydrate =100-(CP + EE+ Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively according to Brett (1973).

replicates (12 females), the same procedure was followed. Fish took 5 weeks to adjust to their new surroundings before mating and fed basal diet throughout this period. Males and females were weighed after the adaption period; a male's average body weight was 440 g, while a female's average body weight was 286 g. They were subsequently stocked at a rate of four fish per cubic metre (4 broodstock ponds), with a female: masculine sex ratio of 3:1, and given diets that are being tested for a month. 2% of their body weight was provided to broodstock twice each day at 90:00 am and 3:00 pm. According to Matter (2011), the experiment's conditions were maintained for the collecting of female eggs for the following biweekly. For 60 days, seeds were collected and counted (two batches). Each pond's water volume (20%) was refilled every day with new water after the accumulated excreta was removed. Parameters relating to water quality were checked every week. A mercury thermometer suspended at a depth of 15 cm was used to measure the water's temperature every day. The pH was measured using a pH metre (Orion pH meter, Abilene, Texas, USA). YSI model 56 oxygen metre was used to test dissolved oxygen (mg L^{-1}) (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA).

2.5. Morphometric index

Both at the beginning and end of the experiment, each fish in each pond had their initial body length (cm), initial body weight (g), and final body weight (g) measured. The ovary was weighed to determine the Gonadosomatic index (GSI) values at the end of the study, and females were randomly chosen from each repeat. The GSI index and the condition factor for male and female fish values are determined as stated in the footnote of Table 5.

2.6. Semen quality and egg diameter/volume of Nile tilapia broodstock

In order to strip the fish's genitalia and collect the milt, the area was dried, and then the fish's abdomen was gently pressed starting at the posterior region and working downward to the urogenital papilla (Vanderwael, 1985). Each male from each replication group underwent this technique 10–15 times. Using the methodology described by Amer et al. (2005), the pH of semen was promptly assessed using the first squeezed drop of milt. According to the percentage of sperm motility, and motile spermatozoa was characterized (Viveiros, et al., 2001). Twenty volumes of physiological saline solution and one volume of sperm and were used to dilute a 10 μl subsample of semen in order to determine the concentration of spermatozoa (Billard et al., 1981). According to Ruranguwa et al., the amount of sperm in the seminal fluid was estimated (2004). Using a hemocytometer counting chamber, spermatozoa were counted to determine sperm density (sperm cells per milliliter) (Buyukhatipoglu and Holtz, 1984). According to Rodríguez Barreto et al. (2014) egg volume (mm^3) and egg diameter (mm) were measured.

2.7. Nile tilapia broodstock reproductive

Following estimates were made for the reproductive performance: Absolute fecundity = Mean number of seeds produced by each female during each spawning for the first (I) and second hatching (II). Relative fecundity = Mean number of seeds per female body weight at each spawning for the initial (I) and second (II) hatchings (g). System productivity for initial (I) and second hatching (II) = Mean number of seeds per day/pond size. Ovulation synchronized by administration of the HCG protocol as described by Fernandes et al. (2013).

2.8. Blood sampling and hematological indices

Blood samples were taken from each replication after 30 days of experimental diet. A fish's caudal vein was used to collect blood after the fish were put to sleep with 100 mg L^{-1} MS 222. Hematocrit (Htc), red blood cells (RBCs), Hemoglobin (Hb), and white blood cells were

Table 5
Condition factor and gonadosomatic index of broodstock Nile tilapia fed experimental diets.

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Condition factor (male)	0.11^{b}	0.24^{a}	0.18^{ab}	0.21^{a}	0.0198	0.0440
Condition factor (Female)	0.16^{a}	0.12^{b}	0.15^{ab}	0.15^{ab}	0.006	0.077
Gonadosomatic index (GSI)	3.02^{d}	3.21^{c}	3.69^{b}	3.98^{a}	0.056	0.002

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05). Gonadosomatic index = weight of gonad/weight of fish 100; Condition factor = Weight (g)/L3, where L is the fish's length (cm).

measured in blood samples that had been combined with the anticoagulant 10% Ethyl enediaminetetraacetate (EDTA). By using Hemoglobin kits which is a standardized approach of the cyanmethemoglobin method, Hb was measured and the total count of WBCs was performed using the indirect method (Martins et al., 2004). Htc was estimated as stated by Reitman and Frankel (1957). The following equations from Lewis et al. (2001) were used to calculate the mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

2.9. Serum biochemical indices

Fish from each replication were given a caudal vein puncture to obtain blood samples. Formerly, 3-aminobenzoic acid ethyl ester was used to anaesthetize the fish (100 mg L^{-1} MS 222; Sigma Aldrich, Egypt). The blood samples were centrifuged at 3000 rpm for 10 minutes after clotting at 4°C. Before usage, the non-hemolyzed serum was obtained and kept at 20°C. Reitman and Frankel's (1957) method was used to assess the levels of serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT). Henry's calorimetric and enzymatic determine cation methods were used to measure serum creatinine (1974). According to Wotton and Freeman (1974) and Henry (1964), respectively, total serum protein and albumin were determined. However, according to Coles, protein was subtracted from total albumin to determine globulin (1974). According to Friedewald et al. (1972), serum triglycerides, total cholesterol, HDL-C, and LDL-C were calculated.

2.10. Immunological assays and Ionogram of Nile tilapia broodstock

Using the C3 and C4 kit, serum complements component (C3) and (C4) activity were measured (Zhejiang Elikan Biological Technology Co., Ltd, Wenzhou, Zhejiang, China). According to Thomas, methods for C4 and C3 activity analysis included measuring the rise in turbidity following the immune responses of C3 and C4 and its elevated antibody (1998). Using commercial kits made by Pasteur labs, serum phosphorus, calcium, sodium, and potassium were analyzed spectrophotometrically (Egyptian American Co. for Laboratory Services, Egypt).

2.11. Offspring and rearing conditions

In the first period, 100 fries totalling 11 g were collected from each replicate and put in a hapa (1×1×1 m) fixed in a concrete pond. To estimate the feed efficiency for growth during a 30-day period, fries were supplied at a ratio of 10% of the stock biomass, with the feeding ratio being modified every 10 days. After collected the hapa's accumulated excrement, fresh water was added to the pond's water volume every day, making up 30% of its total volume. Fry was fed a commercial meal containing 40% protein from Aller Aqua Egypt in Cairo, Egypt, for 30 days.

2.12. Growth performance and feed utilization of fries

At the start and end of the feeding trail, each hapa's initial body weight for live mass (IBW) and final body weight for live mass (FBW) were recorded. Weight gain (WG), specific growth rate (SGR %), Feed conversion ratio (FCR), and survival rate (SR), where survival rate (SR) = All fries - death fries. Table 11 lists the additional formulas and provides an explanation for each.

2.13. Data analysis

All data were presented as means with standard errors (SE). Using SAS statistical software, growth, hematological, blood chemistry, and hormone data were evaluated. Duncan's multiple range tests were then used to compare variations in individual means (Statistical Analysis System, 1993. To account for the statistical difference in means, a probability of 0.05 was used. Prior to analysis, arcsine-transformed percentage values were normalized.

3. Results

3.1. Condition factor and gonadosomatic index

Fish fed diets supplemented with different doses of *C. menegheniana* enhanced ($P \leq 0.05$) the gonadosomatic index (GSI) and condition factor (K) of female and male versus basal diet respectively (Table 5). The highest value of K was noticed in T3 diet, while the superior value of GSI noted in female tilapia fed T2 diet.

3.2. Egg diameter, egg volume and

In terms of egg diameter (mm^3) and egg volume inclusion of dried *C. menegheniana* improved the egg diameter ($P \leq 0.05$) for female. Fish fed diets T3 recorded the uppermost worth ($P \leq 0.05$) of egg diameter (2.35 mm^3) versus basal diet. In addition, fish fed wither T2 or T3 recorded the superior ($P \leq 0.05$) egg volume (Table 6).

3.3. Semen quality

The characteristics of semen quality are described in Table 7. In details fish fed wither T2 or T3 recorded the highest ($P \leq 0.05$) pH values, while the lowermost value was noticed in control group. The sperm motility of male varied between 50% and 75%. However, the highest ($P \leq 0.05$) value of sperm motility observed in fish fed T3 diet (75.0%) versus others ($P \leq 0.05$). In addition, inclusion of Dried *C. menegheniana* enhanced the sperm concentration. Though the sperm concentration values vary between 2.88×10^9 and 4.43×10^9. However, the superior value of sperm concentration reported in male fed T3 group, while the least value was recorded in the basal diet ($P \leq 0.05$; Table 7).

3.4. Reproductive parameters

Table 8 shows the reproductive performance parameters after the first and second hatch, inclusion of dried *C. menegheniana* improved the reproductive performance parameters of broodstock versus basal diet ($P \leq 0.05$). Though, the highest value of system productivity (I, 69.52), relative fecundity (I, 10.42), and absolute fecundity (I, 1042.83), observed in T3 diet. Follow the same patterns, the superior value ($P < 0.05$) of absolute fecundity (II, 491.50), relative fecundity (II, 4.91) and system productivity (II, 32.77) were obtained in T3 group.

3.5. Hematological parameters

Table 9 shows that Inclusion of *C. menegheniana* improved the hematological parameters of male and female broodstock. The lowest Hb and Htc values were obtained in fish fed basal diet, while there were no differences ($P \geq 0.05$) of the RBCs, MCV, MCH, and MCHC among Fish given varying doses of *C. menegheniana*; Table 9). The uppermost number of neutrophils of male and female were detected in T2 group (9.00%) and T3 group (10.00%) respectively. The superior level ($P \geq 0.05$) of lymphocytes was obtained by male and female from T1, T2 and T3, but

Table 6
Egg diameter and egg volume female Nile tilapia fed experimental diets.

Items	Experimental diets				±SE	P-Value
	Control	T1	T2	T3		
Egg diameter	2.19^b	2.29^b	2.32^a	2.35^a	0.0515	0.0183
Egg volume	5.03^b	5.25^b	5.37^a	5.53^a	0.0282	0.06379

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different ($P > 0.05$).

Table 7
Semen quality of Nile tilapia male fed experimental diets.

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
pH of semen	7.0[b]	7.01[b]	8.1[a]	8.0[a]	0.060	0.046
Sperm motility (%)	50.0	60.0	70.0	75.0	2.330	0.016
Sperm concentration	2.88×10^{9d}	2.98×10^{9c}	3.21×10^{9b}	4.43×10^{9a}	0.135	0.000

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

Table 8
Reproductive parameters of Nile tilapia male and female fed experimental diets.

Items	Fecundity after the first (I) and second hatch (II)				±SE	P-Value
	Control	T1	T2	T3		
Absolute fecundity I	609.67[cb]	475.16[c]	709.00[b]	1042.83[a]	1.725	0.015
Relative fecundity I	5.86[b]	4.68[b]	6.76[b]	10.42[a]	0.509	0.013
System productivity I	40.64[cb]	31.68[c]	47.26[b]	69.52[a]	3.450	0.015
Absolute fecundity II	364.16[b]	07.83[ab]	16.50[ab]	491.50[a]	19.123	0.064
Relative fecundity II	3.46[c]	4.92[a]	4.42[b]	4.66[a]	0.108	0.008
System productivity II	24.27[b]	27.19[ab]	27.77[ab]	32.77[a]	1.276	0.065

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).

Table 9
Hemoglobin and hematocrit values of male and female Nile tilapia fed experimental diets.

Items	Experimental Diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Hemoglobin (g/dl)	7.90[c]	9.13[b]	10.5[b]	12.12[a]	0.26	0.002
Hematocrit %	17.12[c]	19.12[b]	20.13[b]	22.30[a]	1.11	0.001
RBCs[a](× 10^6 μL^{-1})	2.80	2.30	2.26	2.50	0.02	0.091
MCV[b](fl)	101.70	98.80	99.11	102.00	6.21	0.061
MCH[c] (pg)	30.35	30.00	30.08	30.00	1.12	0.069
MCHC[d] (%)	29.80	32.30	30.35	31.25	1.11	0.085
WBCs	32.20[c]	33.00[c]	47.80[b]	54.00[a]	1.19	0.001
Neutrophils	5.00[a]	9.00[b]	10.00[a]	9.00[b]	0.98	0.012
Lymphocytes	82.00[b]	88.00[a]	91.00[a]	92.00[a]	6.36	0.001
Monocytes	2.00[b]	3.00[b]	3.00[b]	5.00[a]	0.25	0.024
Eosinophils	1.00[c]	2.00[b]	2.00[b]	3.00[a]	0.03	0.003
Female						
Hemoglobin (g/dl)	8.99[c]	10.12[b]	11.12[ab]	13.60[a]	0.35	0.001
Hematocrit %	18.19[c]	19.90[b]	21.91[ab]	22.9[a]	1.02	0.001
RBCs[a] (× 10^6 μL^{-1})	2.16	2.73	2.83	2.06	0.03	0.063
MCV[b] (fl)	97.22	95.60	98.36	105.70	7.13	0.075
MCH[c](pg)	30.09	30.03	30.05	30.09	1.18	0.062
MCHC[d] (%)	30.95	31.41	30.55	30.78	1.12	0.091
WBCs[e]	30.00[c]	42.60[b]	42.00[b]	52.30[a]	1.12	0.002
Neutrophils	7.00[c]	9.00[b]	9.00[b]	10.00[a]	0.98	0.031
Lymphocytes	82.00[b]	89.00[a]	90.00[a]	89.00[a]	3.69	0.041
Monocytes	3.00[b]	2.00[c]	3.00[b]	6.00[a]	0.52	0.003
Eosinophils	1.00[b]	2.00[b]	2.00[b]	3.00[a]	0.00	0.005

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05)
[a] RBCs = Red blood cells
[b] MCV = Mean corpuscular volume;
[c] MCH = Mean corpuscular hemoglobin;
[d] MCHC = Mean corpuscular hemoglobin;
[e] WBCs = White blood cells.

the lowermost value recorded in male fed the basal diet. The Monocytes male and female fed T3 diet recorded the highest value (P ≤ 0.05; 5.0% and 6.0%), respectively. However, male and female fed T3 diet detected the greatest eosinophil value (3.00%) versus others (P < 0.05; Table 9).

3.6. Lipid metabolism

Diets enriched with dried *C. menegheniana* given to fish improved the broodstock serum cholesterol, triglyceride, HDL-C and LDL-C content versus basal diet (Table 10). The lower content of triglyceride for male was observed in T3 and T2 diets versus others. Triglyceride content of female was lower (P≤0.05) in T3 group, while the uppermost level of HDL-C was noted in male from T2 and T3 groups (110.00 and 90.00 mg dl^{-1}, respectively). The maximum HDL-C level for female was observed in T3 group (69.00 mg dl^{-1}). The highest value of LDL-C was recorded for male and female fed the control diet (78.00 mg dl^{-1} and 83.00 mg dl^{-1}). While, the lowest value of LDL-C of male and female (22.00 and 53.00 mg dl^{-1}) were recorded for fish fed T3 diet.

3.7. Serum biochemical parameters

Inclusion *C. menegheniana* improved liver enzymes activity as ALT,

Table 10
Lipid profile of Nile tilapia male and female fed experimental diets.

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Cholesterol (mg dl^{-1})	220.00[a]	230.00[b]	180.90[c]	170.00[d]	1.12	0.001
Triglycerides (mg dl^{-1})	360.00[a]	220.60[b]	110.00[c]	90.00[c]	10.30	0.001
HDL-C[a] (mg dl^{-1})	50.00[d]	42.90[c]	54.00[b]	78.00[a]	2.12	0.002
LDL-C[b] (mg dl^{-1})	78.00[a]	67.18[b]	36.90[c]	22.00[d]	2.33	0.001
Female						
Cholesterol (mg dl^{-1})	235.00[a]	199.80[b]	185.60[b]	132.00[c]	10.13	0.003
Triglycerides (mg dl^{-1})	169.00[a]	167.20[a]	154.00[b]	115.00[c]	3.69	0.012
HDL-C[a] (mg dl^{-1})	46.50[d]	51.00[c]	60.00[b]	79.80[a]	1.89	0.001
LDL-C[b] (mg dl^{-1})	88.50[a]	81.36[b]	76.20[b]	53.00[c]	5.96	0.025

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different (P > 0.05).
[a] HDL-C = High-density lipoprotein -C;
[b] LDL-C = Low-density lipoprotein-C

AST and ALP (Table 11). The lowest level of ALT and AST for male and female was noticed in T3 group, while the highest value was found in the control group. ALP at its greatest level ($P \geq 0.05$) in male and female fed either T2 or T3, while the lowest was seen with the basal diet. The highest level of globulin and total protein content was obtained of female and male fed T3 diet. The greater level of albumin was detected in T2 for male while in T2 and T3 for female (Table 11).

3.8. Immune responses

In inclusion of dried *C. menegheniana* enhanced (Table 12; $P \leq 0.05$) the serum of C3, C4, IgM and IgG of broodstock versus basal diet. The highest level of C3 content was obtained in male from T3 group, and female fed T2 and T3. The higher level of C4 (Table 12; $P \geq 0.05$) for male and female was detected in T2 and T3 respectively. The higher level of IgM content was obtained by male and female fed T3 diet. Also, the highest level of IgG for male was perceived in T3 group, also, the same trend was shown in IgG for Nile tilapia female.

3.9. Ione serum

Ca and K contents of Nile tilapia broodstock did not show any significant changes (Table 13; $P \geq 0.05$). Inclusion of dried *C. menegheniana* shows the uppermost value of Na for female ($P < 0.05$). While, the highest value of Na for male was noticed in T2 and T1 groups ($P < 0.05$; Table 13).

3.10. Fry performance

The fry produced by broodstock from T3 group show the greatest weight gain (WG; 14.85 g), final body weight (BW; 0.1175 g), and specific growth rate (SGR) (Table 14). The fish survival (FS) of fries produced by broodstock ranged from 90.50 to 96.50. The highest value of FR was recorded for fries produced by broodstock from T3 group. The best FCR value and highest PER were obtained for fries produced by broodstock from T3 group ($P < 0.05$) Table 14.

4. Discussion

Microalgae consider as a promising ingredients that act as an ideal candiate of food function group, *C. menegheniana* consider a member of microalgae group that contain plenty of bioactive compounds as polyphenol and phytochemicals (Table 1) which act as growth boosters, anti-

Table 11
Serum biochemical parameters of Nile tilapia affected by experimental diets.

Items	Experimental diets				±SE	P-value
	Control	T1	T2	T3		
Male						
ALT[a] (UL^{-1})	100.00^{a}	92.30^{b}	70.00^{c}	48.00^{d}	1.12	0.001
AST[b] (UL^{-1})	68.60^{a}	50.50^{b}	40.30^{c}	30.30^{d}	1.58	0.012
ALP[c] (UL^{-1})	20.00^{b}	31.60^{b}	35.00^{a}	34.00^{a}	3.60	0.001
Total protein (UL^{-1})	2.56^{d}	3.50^{c}	4.00^{b}	4.49^{a}	0.02	0.001
Albumin (UL^{-1})	1.49^{c}	1.74^{b}	2.10^{a}	1.65^{b}	0.03	0.001
Globulin (UL^{-1})	1.07^{c}	1.76^{c}	1.90^{b}	2.84^{a}	0.05	0.021
Female						
ALT[a] (UL^{-1})	138.40^{a}	110.00^{b}	98.50^{c}	78.50^{d}	1.32	0.001
AST[b] (UL^{-1})	60.70^{a}	42.50^{b}	33.00^{c}	24.20^{d}	1.54	0.001
ALP[c] (UL^{-1})	22.40^{c}	25.50^{c}	28.60^{a}	32.50^{a}	2.36	0.023
Total protein (UL^{-1})	2.62^{b}	4.30^{a}	3.65^{b}	4.60^{a}	0.03	0.013
Albumin (UL^{-1})	0.96^{c}	1.65^{b}	2.51^{a}	2.63^{a}	0.03	0.001
Globulin (UL^{-1})	1.66^{c}	2.65^{a}	1.14^{c}	1.97^{b}	0.01	0.001

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different ($P > 0.05$).
[a] ALT = alanine aminotransferase;
[b] AST = aspartate aminotransferase;
[c] ALP = alkaline phosphatase

Table 12
Immune responses of Nile tilapia male and female fed experimental diets.

Items	Experimental Diets				±SE	P-value
	Control	T1	T2	T3		
Male						
C3	3.00^{c}	4.80^{b}	5.00^{b}	6.00^{a}	0.56	0.002
C4	1.00^{c}	1.30^{b}	2.10^{a}	2.60^{a}	0.02	0.001
IgM	12.00^{b}	15.00^{b}	19.00^{a}	20.00^{a}	0.98	0.002
IgG	7.00^{d}	8.50^{c}	11.70^{b}	12.50^{a}	0.79	0.004
Female						
C3	2.51^{b}	3.00^{b}	4.50^{a}	4.10^{a}	0.23	0.002
C4	1.24^{c}	1.60^{b}	2.00^{a}	2.94^{a}	0.12	0.014
IgM	12.60^{a}	12.97^{a}	18.00^{b}	22.50^{a}	0.97	0.001
IgG	6.20^{c}	9.80^{b}	10.40^{b}	13.00^{a}	0.96	0.027

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different ($P > 0.05$).

Table 13
Serum calcium, sodium and potassium of male and female Nile tilapia fed experimental diets.

Items	Experimental Diets				±SE	P-value
	Control	T1	T2	T3		
Male						
Calcium (Ca, mg dl^{-1})	8.50	9.00	8.80	8.60	0.65	0.056
Sodium (Na, mg dl^{-1})	149.00^{b}	150.00^{a}	154.00^{a}	148.00^{b}	6.36	0.0425
Potassium (K, mg dl^{-1})	6.00	8.00	7.00	5.30	0.35	0.223
Female						
Calcium (Ca, mg dl^{-1})	8.20	8.64	7.10	8.80	0.38	0.225
Sodium (Na, mg dl^{-1})	146.00^{b}	150.00^{a}	152.00^{a}	152.00^{a}	10.12	0.0452
Potassium (K, mg dl^{-1})	4.80	5.21	4.60	4.50	0.46	0.259

Values (± SE, N= 3). Means in within same row sharing the same superscript are not significantly different ($P > 0.05$).

Table 14
Growth performance and feed utilization of offspring hatched from broodstoock fed experimental diets.

Items	Experimental groups				±SE	P-Value
	Control	T1	T2	T3		
Initial body weight IBW (g)	0.110^{a}	0.113^{a}	0.118^{a}	0.116^{a}	0.004	0.624
Final body weight FBW (g)	0.959^{b}	1.026^{b}	1.042^{b}	1.155^{a}	0.008	0.005
Weight gain WG (g)	0.845^{b}	0.914^{b}	0.924b	1.04^{a}	0.001	0.002
Specific growth rate SGR (% d^{-1})	7.21^{b}	7.36^{b}	7.29^{b}	7.69^{a}	0.050	0.001
Fry survival (%)	90.50^{b}	94.00^{ab}	95.50^{a}	96.50^{a}	0.854	0.049
Feed intake FI (g $fish^{-1}$)	1.150	1.250^{a}	1.115	1.255	0.032	0.112
Feed conversion ratio FCR	1.216^{b}	1.368^{a}	1.2061^{b}	1.207^{b}	0.030	0.027
Protein efficiency ratio PER	2.746^{a}	2.437^{b}	2.764^{a}	2.762^{a}	0.068	0.096

Values (± SE). Means in within same row sharing the same superscript are not significantly different ($P > 0.05$).

inflammatory, anti-bacterial and immunostimulating that boost growth performance and immunological response (Reyes-Becerril et al., 2013; Sarker et al., 2018). The current research revealed the greatest K value was in T3 group. In addition, GSI was high in female given diets enriched with varying amounts of *C. menegheniana* The current outcomes are in parallel with Martínez-Pita et al. (2016) who detected that *Mytilus galloprovincialis* fed diet enriched with microalgae enhanced condition factor and GSI versus basal diet. Alike, Wahbi and Sangak (2017) demonstrated that diet supplemented with *Spirulina spp.* improved GSI of Nile tilapia. However, the results are inconsistent with (Khanzadeh et al., 2016) who found no variation in condition factor of rainbow trout fed diet supplemented with *Spirulina spp.* Furthermore, condition factor was not affected by inclusion *Spirulina spp* in gourami *spp* (Khanzadeh et al., 2016). The current research revealed that the sperm motility and sperm concentration of male fed experimental diets containing *C. menegheniana* were improved. The present results are in parrallel with Putri and Budi (2020) who stated that inclusion of 3% *Spirulina platensis* enhanced the sperm characteristics and performance of Silver rasbora. The present results could be attributed to the properties of *C. menegheniana which* contains i) high amount of bioactive compounds as polyphenol and phytochemical (Budi, 2020)., ii) Microalgae contains high-value biochemical compounds as AAs and PUSFAs particularly *w*-3 series (Christaki et al., 2011)., iii) The manner of action of the bioactive compounds in *C. menegheniana* that acts as antioxident, anti-inflammatory and antibacterial agents (Xu et al., 2010).

In this study, it was shown that *C. menegheniana* significantly influenced the reproductive performance parameters of Nile Tilapia after first and second hatch. Egg size was also significantly higher in females fed with diet supplemented with *C. menegheniana.* The current findings are consistent with Güroy et al. (2012) who revealed that addition of 2.5% *Spirulina sp.* in the yellowtail diet enhanced reproductive performance. Furthermore, using of *Spirulina sp.* in fish diets enhancing zebrafish reproduction (Martins et al., 2020). Similar results yellow tail cichlid exhibited a considerable increase in hatching rate and egg production when given up to 10% *Spirulina sp.* versus diet free of microalgae (Guroy et al., 2012). James et al. (2006) and Carneiro et al. (2020) reported that 8% inclusion of *Spirulina sp.* increased swordtail (*Xiphophorus helleri*) reproductive performance. The enhancement of reproductive performance of Nile tilapia given *C. menegheniana*-enriched diets could be attributed to different theory as follows: i) the presence of bioactive compounds as carotenoids, ascorbic acid, polyphenol and PUFA that have positive impacts on egg quality, quality and production (Scabini et al., 2011)., ii) *Spirulina sp*. has a high concentration of LA and ALA, the precursor various fatty acids involved on prostaglandin synthesis and ovulation (Chong et al., 2004).

The present study has shown that improving hematological parameters: Hb, Htc, RBCs count, MCV, MCH, MCHC, and WBCs and its constituents and a decline of neutrophils percent of fish fed dried *C. menegheniana.* The present findings are consistent with Saleh et al. (2020) who noted a considerable rise in the RBCs and WBCs of fish enriched with *Amphora sp.* compared with the control group. However, the current outcomes are inconsistent with Aly et al. (2014) who observed that the haematological analyses of Nile tilapia fed diets supplemented with *Chlorella ellipsoidea* and *Scenedesmus bijuga* did not reveal significant differences. Thus, the mode of *C. menegheniana* on hematology parameters still needs more investigations to discover the mechanism of this action.

The present study revealed that diets supplemented with dried *C. menegheniana* significantly improved the serum cholesterol content compared with the control diet for broodstock. Cholesterol, triglyceride, LDL-C, and HDL-C were significantly lower in male and female of T3 group compared with other treatment diets. The present study are in parallel with Rahimnejad et al. (2017) who found the lowest values of lipid profile components as compared to other animals fed the greatest quantities of microalgae. Follow the same patterns, inclusion of 150 g *Chlorella vulgaris* kg^{-1} *in P. Olivaceus* resulting in reduction of serum cholesterol (Rahimnejad et al., 2017). Alike, (Carneiro et al., 2020) found that Zebrafish fed 50 g kg^{-1} diet of *Chlorella sp.* represent the lowest levels of Cholestrol. A similar decline in cholesterol level was found in diets enriched with 160–200 g kg^{-1} *Chlorella* powder provided to crucian carp (Xu et al., 2014) and to juvenile ayu (*Plecoglossus altivelis*) (Nakagawa et al., 1983; Nematipour et al., 1990). The presnt results could be attributed to: i) microalgae stimulates lipid metabolism functions in Nile tilapia broodstock (Kay and Barton, 1991)., ii) the existence of bioactive compounds in *Chlorella* sp. that enhancing intestinal tract fat assimilation (Cherng and Shih, 2005) and iii) *Chorella* sp. Phytosterols have hypocholesterolemic properties (Luo et al., 2015).

In the present study, the inclusion of dried *C. menegheniana* enhanced ALT, AST and ALP of broodstock. The lowest level of ALT and AST for male was obtained by male and female from T3 group. The present findings are in accordance with Mekkawy et al. (2020) who found that inclusion of *Amphora sp.* affected catfish blood biochemical constituents. On the contrary, the current findings are incompatible with Ayoub et al. (2019) who detected no significant variations in liver enzymes activity when fish fed diet enriched by. *Coffeaeformis-supplemented* diets. The discrepancy between prior and current outcomes is related to increased supplementation amounts employed in the current study.

Broodstock fed a diet high in *C. menegheniana* sp. produced the greatest levels of total protein content, albumin, and globulin. The current findings are in line with Kumala et al. (2018) and Saleh et al. (2020), who observed that *O. niloticus* given diets supplemented with algae, *Gracilaria verrucosa* resulted in a significant rise in total serum protein content (Saleh et al., 2020). Follow the same patterns, serum protein levels in Nile tilapia fed bergamot oil-supplemented diets were likewise higher (Kesbiç et al., 2020).

It is an urgent matter to strengthen the immune system to control disease outbreaks and pathogens infection (Chen et al., 2013; Hassaan et al., 2018, 2019; 2021b; Mohammady et al., 2022; Saleh et al., 2022). The present study showed that broodstock diet supplemented with dried *C. menegheniana sp.* increased activity and levels of immunoglobulin (IgG and IgM) and complement (C3 and C4). The present results are consitent with Cerezuela et al. (2012) who reported enhancement of immunological parameters as IgD, MHC-I, and IgM in gibel carp serum (*Carassius gibelio*) fed diets supplemented with chlorealla. In addition, Zhang et al. (2014) showed that ingesting *Chlorella* sp. dramatically increased serum IgD and IgM levels in gibel carp (*Carassius gibelio*). The present could be attributed to different scenario as follows: i) Inclusion of diatoms as *C. vulgaris* improve liver functions and antioxidant levels., ii) Addition of microalgae dramatically boost the expression of immune-related genes and inflammatory response (Onofrejova et al., 2010., iii) Microalgae contains plenty of bioactive compounds stimulate immune system response (Bernal et al., 2011).

The current research clearly shows that inclsuion of *C. menegheniana* significantly improve growth and feed efficiency for 30 days. The present data are in parallel with Kiron et al. (2012) and Carboni et al. (2012) who found that inclusion of algae boosts the survival rate and growth performance of aquatic animals. Furthermore, inclusion of algae and its effective components promote growth performance as well as the survival of raising organisms (Sang et al., 2010; Kiron et al., 2012). Also, Sørensen et al. (2016) found that diatom *Phaeodactylum* can replace up to 6% of the fishmeal without any negative consequences on growth of Atlantic salmon. Follow the same patterns, (Namaei Kohal et al., 2018) found that inclusion of *Arthrospira platensis* close to 10% of the diet improved growth and reproductive performance in shrimp. On the contrary, Dallaire et al. (2007) observed that rainbow trout fed diets enriched with algae resulting decline of the growth. The present results could be attributed to the following theories: i) *C. menegheniana* used as prebiotics that modulate intestinal microbiota community toward boost the growth of helpful bacteria while inhibiting the development of harmful bacteria (Reyes-Becerril et al., 2014; Rasdi et al., 2015)., ii) the bioactive compounds present of Cyclotella recognized as a growth promoters (Kiron et al., 2012)., iii) Inclusion of macroalgae and diatoms

enhance the colour, availability, size and quality of the diet that support larval growth., iv) The composition of microalgae and its relatively high digestibility improve feed intake and availability (Sarker et al., 2018).

5. Conclusion

The present work attempts highlight the importance of using *C. menegheniana* as feed additives for broodstock and larva's diet and the best recommended level of *C. menegheniana* varied between 10 g kg^{-1} to 15 g kg^{-1}. In conclusion, the use of *C. menegheniana* as a feed additive stimulates the immune system and improves the blood and reproductive parameters of Nile tilapia broodstock, and it has a positive influence on the growth and feed conversion of Nile tilapia offspring

CRediT authorship contribution statement

Hosam E Elsaied: Data curation, Conceptualization. **Sylwia Jarmołowicz:** Formal analysis. **Ahmed M. Abdel-Moez:** Data curation, Conceptualization. **Ehab El-Haroun:** Formal analysis, Data curation, Conceptualization. **Eman Y. Mohammady:** Conceptualization. **Marwa M. Ali:** Data curation, Conceptualization. **Mohamed S. Hassaan:** Data curation, Conceptualization.

Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

Data will be made available on request.

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