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Nutritional and immunological evaluation of *Nannochloropsis oculata* as a potential Nile tilapia-aquafeed supplement

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Abstract

Nannochloropsis oculata (*N. oculata*) is a marine microalga containing bioactive compounds and a high omega-3 polyunsaturated fatty acid (ω-3 PUFAs). Therefore, it is very promising for nutraceutical and the functional food industry applications. Three groups of Nile tilapia (forty-five fish/group) were fed on basal diets or diets containing 5% (N5) or 10% (N10) of the microalga *N. oculata* for seven weeks. Fish growth performance, proximate composition, and lipid (fatty acids/ FAs and lipoproteins) profile were estimated. In addition, the expression pattern of some lipid metabolism and immune-relevant genes were assessed. An enhancement in whole body crude protein and growth indices of Nile tilapia was observed on both the supplemented groups N5 and N10. Higher levels of high-density lipoproteins (HDL); and lower levels of the low-density lipoproteins (LDL) were evident in both supplemented groups, while the cholesterol and triglycerides (TG) levels were similar among groups. Ω-3 PUFAs were the significant FAs profile of tilapia fed on *N. oculata*-supplemented diets in terms of eicosapentaenoic acid, docosahexaenoic acid, and n3/n6 ratio. Concerning the gene expression pattern, heat-shock protein70, glutathione-S-transferase, glutathione peroxidase, and interleukin-1β *(IL-1β)* were elevated significantly in both supplemented groups. *IL-10* is only upregulated in the N10 group. The lipid metabolism-related gene expression showed downregulation of only fatty acid synthase (*FAS*) in both supplemented groups, with no statistical changes in Peroxisome proliferator-activated receptor alpha (*PPARα)*. Tumor necrosis factor-*α* (*TNF-α)*, Transforming growth factor-β1 (*TGF-β1),* and the apoptotic related genes [*caspase3* and Proliferating cell nuclear antigen (*PCNA*)] showed insignificant changes among groups. The histopathological examination of the intestine, liver, and spleen supports our findings and confirms the benefits and safeness of *N. oculata* dietary inclusion. Collectively, *N. oculata* is a very promising nutraceutical for improving fish health and sustainability of aquaculture production.

Keywords Fish, Fatty acids, Growth, Histomorphology, Immunity, Microalgae

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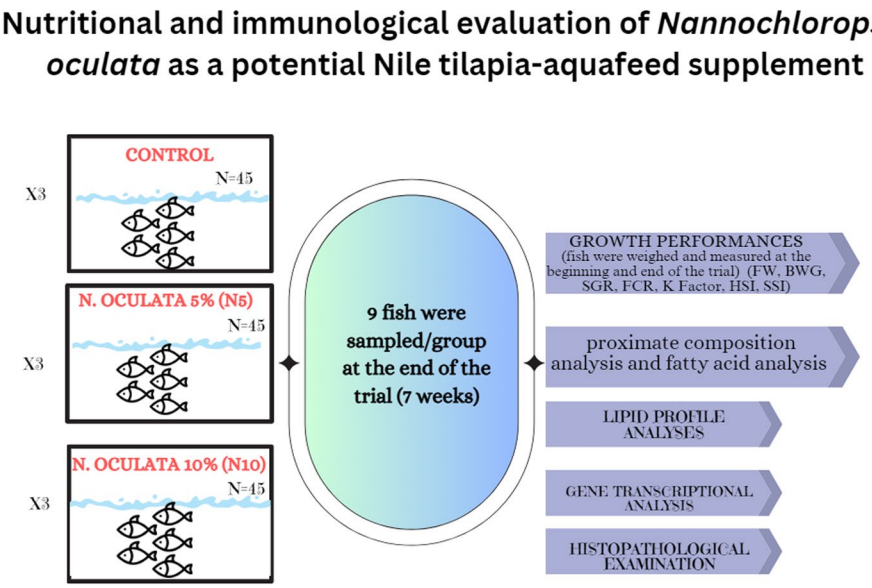
Graphical Abstract



Introduction

The inclusion of dietary supplements rich in fatty acids (FAs) is an approach of promising benefits for animal health and body composition. However, the imbalance of lipid metabolism by promotion or suppression is the leading cause of lipotoxicity, where the balance between lipolysis and lipogenesis is critical and delicate [1, 2]. Excessive lipolysis and/or lipogenesis via insulin resistance of the adipose tissues were reported to cause lipotoxicity [3]. Subcutaneous fats release plasma free-FAs, mainly during fasting, and the excess fat, triglycerides (TG), accumulate in non-adipose tissues such as the heart, liver, pancreas, and muscle, promoting cell dysfunction since lipids are the primary targets for the reactive oxygen species (ROS), which implies oxidative stress [4, 5]. Therefore, feed supplementation with fat sources should be accurately balanced.

Microalgae are unicellular eukaryotic algae known as resources for FAs, pigments, and other several bioactive metabolites of high nutritive as well as biomedical values [6–8]. They are photosynthetic microorganisms that require low light and nutrients to produce a high amount of energetic biomass and lipids during short periods [9]. They are rich in all the essential amino acids and used as bio-supplies for the polyunsaturated fatty acids (PUFAs), mainly that cannot be synthesized by the vertebrates' bodies such as C18 PUFA, linoleic (2n-6), and α-linolenic acids (3n-3), which could be then converted into physiologically active long-chain (C20-24) PUFA essential for the normal growth and development [10]. Of them, the marine microalgae *Nannochloropsis* spp., which considered the most promising microalgal feedstock for fish owing to their high rate of biomass accumulation and their high oil, PUFAs, proteins, and carbohydrates content; therefore, they are frequently used in algal nutraceutical, biodiesel, and biofuel production, and the functional food industry applications [11–17]. It contains a high level of FAs, which are affected by nitrogen and phosphorus levels in their growth media, which makes it very promising for nutrition and the applications demanding high lipid concentrations such as biodiesel production and fish dietetics [18–21].

Nannochloropsis oculata (*N. oculata*) is characterized by its high productivity, FA, and TG–FA content. In addition, *N. oculata* is a strong bio-sourced for the nutraceutical valuable eicosapentaenoic acid and the biodiesel feedstock [22, 23]. The eicosapentaenoic acid (EPA, 20:5n-3) (215 g/kg total fat) and some of the docosahexaenoic acid (DHA, 22:6n-3) (32 g/kg total fat) are the main fatty-acid components of *N. oculata* [24]. Supplementation of fish feed with both FAs improved lipid metabolism, significantly enriched FA profile, and improved fish antioxidant capacity and hematological characteristics [25]. On the other hand, *N. oculata* in fish diets stimulate the production of functional growth hormone [26]. Furthermore, *N. oculata* possessed considerable immunomodulatory efficacy as its sterol rich fraction was found to elicit anti-inflammatory and anti-cancer

activities, and its water-soluble polysaccharides showed in vitro immunostimulatory efficacy, which makes it promising for several biomedical applications [27–29]. Further, n3-long chain (LCPUFAs); mainly EPA and DHA could be linked to the membrane fluidity of fish cells [30] that play a role in fish immune defense during inflammatory or other immune responses [31–33]. Thus, the immune response reported in fish can be modified depending on the dietary lipid source [34–36].

To the best of our knowledge, information about the hypolipidemic effect of *N. oculata* is lacking, additionally, our unique approach to fully evaluate the nutritional, biochemical and immunological aspects provide a better understanding for *N. oculata* as an aquafeed supplement.

Materials and methods

Ethics statement

All fish in the experimental protocols were reared and handled in accordance with the guidelines of the local Administrative Panel on Laboratory Animal Care and Committee of Mansoura University with the code number (R/108), which specifically approved this study.

Experimental fish

One hundred and thirty-five apparently healthy Nile tilapia (*Oreochromis niloticus*) with initial body weight (34.42 ± 1.47 g; mean ± SD) were purchased from a private fish farm in Kafr El-Shiekh governorate, Egypt, and delivered alive to the fish diseases and management laboratory at Mansoura University. Sample size was calculated according to Krejcie and Morgan [37] and a G*Power analysis. In 500 L fiberglass aquariums, fish were acclimated for two weeks. Fish were fed a commercial diet ad libitum at the time (Uccma feed, Egypt; crude protein, 32%; crude lipid, 6.2%; crude fiber, 5.7%). According to Noga [38], evaluations of fish health were conducted during the acclimatization phase, and only fish with a healthy general look and activity level were used. During the period of acclimatization, no clinical symptoms or mortalities were seen.

Diet preparation

For the supplemented feed, *N. oculata* dried powder was purchased from the National Research Centre, Cairo, Egypt; the proximate composition and fatty acid profiles of *N. oculata* are presented in Table 1. Three different isonitrogenous and isolipidic diets [non-supplemented, 5% *N. oculata* (N5), or 10% *N. oculata* (N10)] were formulated (Table 2) for the basal and *N. oculata*-supplemented diets. The different diet components were mixed with oil, and water was added to make a stiff dough, extruded through a mincer, and allowed to dry, broken up into pellets, and stored in clean dried plastic bags at 4 °C until use. The fatty acid profiles of experimental diets are presented in Table 3.

Table 1 Nutrient composition (crude material %) and fatty acid composition (percentage of total fatty acid methyl esters (FAMEs) of *Nannochloropsis oculata (N. oculata)*

Nutrient composition of *N. oculata* (%)		
Crude Protein	46.3	
Crude Lipid	7.5	
Crude ash	11.5	
Crude Fiber	5.5	
Ca	3.25	
P	1.17	
[% of FAMEs] composition of *Nannochloropsis oculata (N. oculata)*		
Saturated fatty acids (SFA)		
Mystiric acid	C14:0	2.83
Palmitic acid	C16:0	28.81
Stearic acid	C18:0	11.54
Monounsaturated fatty acids (MUFA)		
Palmitoleic acid	C16:1n7	3.93
Oleic acid	C18:1n9	10.32
Polyunsaturated fatty acids (PUFA)		
Omega-6		
Linoleic acid (LA) (ω-6)	C18:2n6	16.93
Omega-3		
α-Linolenic acid (ALA) (ω-3)	C18:3n3	10.12
EPA; Eicosapentaenoic acid (ω-3)	C20:5n3	9.16
∑n-SFA		43.18
∑n-MUFA		14.25
∑n-6 (ω-6)		16.93
∑n-3 (ω-3)		19.28
n3/n6		1.14

Σ SFA is the sum of saturated fatty acids, Σ MUFA is the sum of monounsaturated fatty acids, Σn-3 is the sum of n-3 polyunsaturated fatty acids, and Σ n-6 is the sum of n-6 polyunsaturated fatty acids

Experimental design

Post 2-weeks acclimation period. Nile tilapia fish were randomly assigned to 3 groups, namely: group 1 (control) fed basal diet, group 2 (N5) fed a diet supplemented with *N. oculata* (5%), and group 3 (N10) fed a diet supplemented with *N. oculata* (10%). Fifteen fish were randomly assigned to each aquarium tank (80 × 40 × 30 cm) (n = 45 fish/group). Electric aerators and an underwater internal power filter were used to maintain the oxygen level in all aquarium tanks. Fish were fed 3% of their biomass (on dry matter basis), repeated every two weeks to readjust the feeding quantity according to National Research Council (NRC) [39] throughout the trial, and 50% of the water was replaced twice a week. Water quality parameters were monitored and maintained throughout the experiment using water quality test kits

Table 2 Ingredients and body composition of basal and experimental isocaloric and isonitrogenous diets (%)

Ingredients (g)	Control	N 5	N 10
Yellow corn	19.5	17	16
Soybean meal	20	20	17
Fish meal	20	17	16
Corn gluten	3	3	3
Gelatin	2	2	2
Sunflower oil	3.5	4	4.16
Wheat bran	30.16	30.16	30
Minerals and vitamins premix	1	1	1
Salt	0.3	0.3	0.3
Vitamin C	0.12	0.12	0.12
Dicalcium phosphate	0.1	0.1	0.1
Methionine	0.32	0.32	0.32
Algae (*N. oculata*)	0	5	10
Proximate analysis (% dry matter basis)			
Crude Protein[a]	32.3	31.4	33.2
Lipid[a]	6.69	6.74	6.86
Ash[a]	7.75	8.49	7.41
Ca[a]	1.17	1.17	1.27
P[a]	0.53	0.53	0.57
DE (Digestable Energy)[b] **(kcal/kg)**	3016	3015	3000

The levels of the micro minerals &vitamins for tilapia are covered by supplementation of trace minerals & vitamins premixes as recommended by NRC (2011). Vitamins premix (IU or mg/kg diet); vit. A 5000, Vit.D3 1000, vit. E 20, vit. k3 2, vit. B1 2, vit. B2 5, vit. B6 1.5, vit. B12 0.02, Pantothenic acid 10, Folic acid 1, Biotin 0.15, Niacin 30. Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2

N. oculataNannochloropsis oculata

[a] analyzed

[b] calculated value

Table 3 Fatty acid composition (percentage of total fatty acid methyl esters (FAMEs) of the experimental diets

[% of FAMEs]		Control	N5	N10
Myristic acid	MAA	1.84	1.43	2.33
Palmitic acid	PA	25.14	20.6	25.16
Stearic acid	SA	3.57	3.06	3.47
Oleic acid	OA	21.3	18.58	18.48
Linoleic acid (LA) (ω-6)	LA	43.52	48.65	42.33
α-Linolenic acid (ALA) (ω-3)	α-LA	2.68	3.2	2.58
EPA; Eicosapentaenoic acid (ω-3)	EPA	1.15	2.54	3.18
(DHA); Docosahexaenoic acid (ω-3)	DHA	0.8	1.9	2.47
∑SFA		30.55	25.09	30.96
∑MUFA		21.3	18.58	18.48
∑n-3		4.63	7.64	8.23
∑n-6		43.52	48.65	42.33
n3/n6		0.11	0.16	0.19

Σ SFA is the sum of saturated fatty acids, Σ MUFA is the sum of monounsaturated fatty acids, Σn-3 is the sum of n-3 polyunsaturated fatty acids, and Σ n-6 is the sum of n-6 polyunsaturated fatty acids

(Aquarium Pharmaceuticals, Inc.) (temperature 25–27 C, dissolved oxygen 6 mg/L, pH 7.5–8, NH_3/NH_4, and nitrite 0.25 mg/L). Daily siphoning of waste material and feces was performed to maintain water quality.

Fish growth performance, sampling, and tissue collection

Each fish was weighed and measured at the beginning and end of the trial to determine the growth indices listed below:

$$\text{Weight gain (g)} = \text{Mean fina lweight (g)} - \text{Mean initial weight (g)}$$

$$\text{Specific growth rate (SGR, \%/day)} = 100 \times [(\text{Ln (mean final body weight)} - \text{Ln (mean initial body weight)}]/\text{culture period (days)}$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed fed (g)}/\text{Weight gain (g)} \times 100$$

Condition factor according to the following formulae: Condition factor (K) = (W/L3) × 100; where: W = weight of fish in grams and L = total length of fish in "cm".

Nine fish were then collected at random from each group after the trial; six were used for sample collection, and the other three were used for proximate composition analyses. Euthanized fish using buffered MS-222 (Tricaine methanesulfonate, Finquel, Argent) were sampled individually. Blood samples were collected via caudal vein puncture and then transferred to a plain tube to clot at room temperature for 4 h before centrifuging at 1198 × g for 10 min to express serum. Serum samples were stored at -20 °C for subsequent lipid profile analysis. Following that, fish were immediately dissected, liver and spleen were removed and weighed for organosomatic indices calculations using the following formulae: Hepatosomatic indices (HSI) = (Liver w) / W × 100, Splenosomatic indices (SSI) = (Spleen w) / W × 100. The liver was then divided into two portions, one of which was fixed in 10% buffered formalin for histopathological analysis. The other was preserved in RNAlater (Invitrogen, USA) solution and kept at—80 °C until gene transcriptome analysis.

Proximate chemical composition analysis of N. oculata, feedstuff, and whole fish body

Proximate composition analysis of *N. oculata*, and diets was determined according to the American Association of Cereal Chemists procedure (AACC) [40]; and for the whole fish body was conducted according to the Association of official analytical chemists procedure (AOAC) [41]. The total nitrogen content (N) in the crude protein (N × 6.25) was determined using the Kjeldahl method (1030-Auto-analyzer; Tecator, Tecator, Sweden). The crude lipid concentration was determined following

ether extraction using the Soxhlet method (Soxtec System HT6; Tecator). Ash content was determined in the samples using a muffle furnace at 550 °C for 6 h.

Fatty acids analyses

Algal extraction and fatty acids analysis was carried out at Central Laboratories Network, National Research Centre, Cairo, Egypt. Briefly, the methylation method described by J Tian, H Ji, H Oku and J Zhou [42], J Folch, M Lees and GH Sloane Stanley [43] was used to extract fatty acids from *N. oculata*, diets, and tissue (whole-body) (based on trichloromethane and methanol). The fatty acid methyl esters (FAMEs) were then determined using an Agilent 7820a Series gas chromatograph (Agilent Technologies) equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A). The GC was equipped with a DB-WAX column (30 m × 250 µm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.9 mL/min at a split ratio of 1:50, injection volume of 1 µL, and the following temperature program: 50 °C for 1 min; rising at 25 °C /min to 200 °C and held for 5 min; rising at 3 °C/min to 220 °C and held for 10 min; rising at 5 °C/min to 240 °C and held for 8 min. The injector and detector were held at 250 °C and 290 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 60–400 and solvent delay of 1 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Lipid profile analyses

Lipoprotein profile, including triglycerides (TG), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and cholesterol, was measured using diagnostic Cobas c pack reagents kits (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions applied on COBAS INTEGRA 400 plus analyzer (Roche Diagnostics GmbH, USA).

Gene transcriptional analysis

According to the manufacturer's instructions, total RNA was isolated from six liver samples of the Nile tilapia at 7 weeks post-feeding using RNAiso reagent (Takara Bio Inc., Japan). A Nanodrop lite spectrophotometer (Thermo Scientific, US) was used to quantify the RNA concentration at OD 260/280 nm. A cDNA was synthesized using SuperScript III First-Strand Synthesis System with Oligo-dT primers (Invitrogen, USA), according to the manufacturer's instructions. The RT-qPCR reaction was carried out using Step One Plus ™ Real-time PCR machine (Applied Biosystems, USA) to quantify stress (*Hsp70, GPx, GST*), immune-related genes *(IL1-β, TNF-α, TGF-β1, IL-10*), apoptotic (*caspase3, PCNA*), and lipid metabolism-related genes (*FAS, PPARα*). *β*-Actin was included as a housekeeping gene. The primer details were previously published [44–46]. Per the manufacturer's procedures, each reaction was conducted in a volume of 20 µl via Thunderbird SYBR qPCR Mix reagents (Toyobo, Japan). The amplification program was 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min with a final dissociation analysis step. After the cycling protocol, the melting curves were obtained to assess the specificity. The mRNA expression data were standardized to the *β*-Actin using the $2^{-\Delta\Delta CT}$ method [47].

Histopathological examination

The intestine, liver, and spleen were dissected from Nile tilapia, then collected and fixed in 10% neutral buffered formalin for 24 h. The dissected organs were processed, embedded in paraffin wax, and sliced at 5 µm. The slices were stained using hematoxylin and eosin [48]. The stained slides were examined under a light microscope (Olympus CX 31).

Statistical analysis

Data were first subjected to normality and homogeneity checks using Kolmogorov–Smirnov and Levene's tests, respectively. The significance between the variables of groups was analyzed by a one-way analysis of variance (ANOVA) using the GraphPad Prism® statistics package version 8.4.2 (GraphPad Software, Inc., USA). Normalized individual fold change values were anchored to the lowest value recorded in each data set, and then Log2 transformed, as described previously. Differences were considered statistically significant when $P < 0.05$, $p < 0.01$, and $p < 0.001$. All data were expressed as mean ± standard error of the mean (SEM).

Results

Growth performance indices

All growth performance parameters are displayed in Table 4. The FW and BWG of Nile tilapia-fed *N. oculata* at 10% were highly increased compared to the N5 and control groups, with no significant between the latter. SGR of the N10 fish group was significantly increased compared to the control group with no statistical changes to the N5 supplemented group. FCR was significantly better in fish-fed *N. oculata* at 10% than in N5 and control groups, without significance between the latter. K factor, HSI, and SSI showed no significance among groups.

Table 4 Growth performance of Nile tilapia fed the experimental diets

Parameter	Feeding Group			*P*-Values		
	Control	N5	N10	N5/Control	N10/Control	N10/N5
IW	34.91 ± 1.47	34.36 ± 1.41	34 ± 1.46	-	-	-
FW	61.45 ± 2.35^{bc}	63.64 ± 2.34^{b}	72.27 ± 2.39^{a}	0.792	0.008**	0.038*
BWG	26.54 ± 2.18^{bc}	29.28 ± 2.32^{b}	38.27 ± 3.03^{a}	0.73	0.007**	0.046*
SGR	1.15 ± 0.091^{b}	1.259 ± 0.09^{ab}	1.55 ± 0.13^{a}	0.742	0.032*	0.149
FCR	2.14 ± 0.26^{a}	1.36 ± 0.13^{a}	0.76 ± 0.09^{b}	0.149	< 0.001***	0.038
K Factor	0.37 ± 0.17^{a}	0.22 ± 0.048^{a}	0.13 ± 0.062^{a}	> 0.999	0.144	0.174
HSI	2.35 ± 0.26^{a}	2.93 ± 0.26^{a}	2.09 ± 0.30^{a}	0.302	0.789	0.094
SSI	0.17 ± 0.027^{a}	0.21 ± 0.023^{a}	0.18 ± 0.021^{a}	0.441	0.935	0.652

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

Values with a different letter superscript within the same row indicate a significant difference between groups ($P < 0.05$)

Table 5 Proximate composition of Nile tilapia (whole fish body) fed the control and experimental diets

	Nile tilapia fed control diet	Nile tilapia fed N5% diet	Nile tilapia fed N10% diet
CP	17.23 ± 0.67a	18.2 ± 0.51ab	20.3 ± 0.47b
CL	12.8 ± 0.15a	11.6 ± 1.08a	12.3 ± 0.34a
Ash	4.8 ± 0.1538a	4.7 ± 0.18a	5.1 ± 0.37a

Values with a different letter superscript within the same row indicate a significant difference between groups ($P < 0.05$)

Proximate composition profiles of *N. oculata*, diets, and fish whole body

The effects of dietary *N. oculata* on the whole-body composition are shown in Table 5. Dietary inclusion was a significant factor, particularly in crude protein (CP) of the fish's whole body, where CP content increased with the increased percent of dietary *N. oculata*. Tilapia fed 5% *N. oculata* supplemented diet showed no significance ($P = 0.48$), but tilapia fed 10% *N. oculata* supplemented diet recorded a statistically increasing value ($P = 0.019$). No statistically significant changes among groups were recorded on the crude lipid (CL) or Ash contents.

Whole-body fatty acids profile

After seven weeks of feeding with *N. oculata*, considerable changes were recorded in fish' fatty acid composition compared to those fed with basal diets (Table 6). Three SFA were found in the fish's whole body, Palmitic (PA), Mystiric (MA), and Stearic acids (SA). In both *N. oculata*-supplemented groups, SA (C18:0) increased ($P < 0.001$), along with the decrease ($P < 0.001$) of MA (C14:0) compared to the control group. A similar significant pattern ($P < 0.001$) of both SA and MA was evident in the N10 group compared to the N5. Nevertheless, the total SFAs demonstrated no statistical difference between groups. Regarding the monounsaturated fatty acids (MUFAs), Oleic acid (OA; C18:1n9) was in the same significant pattern as MA (C14:0). The OA level of the N10 group and the total MUFAs adopt similar patterns to the MA and SFAs, respectively. Furthermore, six polyunsaturated fatty acids (PUFA), three (ω-6) and three (ω-3), were detected in fish bodies. A decrease ($P > 0.05$) was recorded in the level of ω-6 fatty acids of the N10 group compared to the other groups. N5 group recorded the highest significant level of both Arachidonic acid (AA; C20:4n6) ($P < 0.001$) and linoleic acid (LA; C18:2n6) ($P < 0.01$) compared to the other groups, and the N10 group, respectively. The ω-3 fatty acids increased ($P < 0.001$) in both *N. oculata*-supplemented groups in terms of EPA (C20:5n3) and DHA (C22:6n3) levels, being the highest level in the N10 group. Whereas the augmentation of the α-Linolenic acid (ALA; C18:3n3) was higher ($P < 0.01$) only in the N10 than in the control group. Overall, the addition of microalgae significantly influenced the total n-3 PUFAs profiles, mainly in the N10 group and n-3/n-6 ratio at both levels of supplementation.

Fish lipid profile

A noticeable change was observed in the lipid profile of *N. oculata*-supplemented groups, more notably in the N10 group. A significant increase ($P < 0.01$) was observed in the HDL levels, along with a significant decrease ($P < 0.05$) in the LDL levels of both the N5 and N10 groups compared with the control group. No statistical changes were noticed in cholesterol and TG in *N. oculata*-supplemented groups compared to the control one (Fig. 1).

Table 6 Fatty acid composition [percentage of total fatty acid methyl esters (FAMEs)] of Nile tilapia fed the experimental diets

Trivial or (systemic) name	Shorthand name	control	N5%	N10%
Saturated fatty acids (SFA)				
Mystiric acid	C14:0	3.41 ± 0.03^{a}	2.01 ± 0.027^{b}	1.09 ± 0.06^{c}
Palmitic acid	C16:0	27.75 ± 0.42^{a}	27.43 ± 0.42^{a}	28.13 ± 0.19^{a}
Stearic acid	C18:0	8.39 ± 0.20^{a}	10.56 ± 0.20^{b}	15.33 ± 0.19^{c}
Monounsaturated fatty acids (MUFA)				
Palmitoleic acid	C16:1n7	3.75 ± 0.33^{a}	2.33 ± 0.33^{a}	2.63 ± 0.47^{a}
Oleic acid	C18:1n9	22.97 ± 0.58^{a}	13.58 ± 0.19^{b}	11.34 ± 0.20^{c}
Polyunsaturated fatty acids (PUFA)				
Omega-6				
Linoleic acid (LA) (ω-6)	C18:2n6	25.13 ± 0.42^{ab}	27.1 ± 0.42^{a}	12.33 ± 0.19^{b}
Dihomo-γ-linolenic acid (dGLA) (ω-6)	C18:3n6	1.24 ± 0.23^{a}	0.99 ± 0.26^{a}	0.37 ± 0.18^{a}
Arachidonic acid (ω-6)	C20:4n6	0.83 ± 0.05^{bc}	1.3 ± 0.06^{a}	0.84 ± 0.05^{b}
Omega-3				
α-Linolenic acid (ALA) (ω-3)	C18:3n3	2.29 ± 0.32^{a}	4.56 ± 0.19^{ab}	7.21 ± 0.19^{b}
EPA; Eicosapentaenoic acid (ω-3)	C20:5n3	1.47 ± 0.04^{a}	2.35 ± 0.03^{b}	3.45 ± 0.07^{c}
(DHA); Docosahexaenoic acid (ω-3)	C22:6n3	3.75 ± 0.19^{a}	7.35 ± 0.19^{b}	14.63 ± 0.20^{c}
∑n-SFA		13.18 ± 3.72^{a}	13.33 ± 3.74^{a}	14.85 ± 3.91^{a}
∑n-MUFA		13.36 ± 4.30^{a}	7.96 ± 2.52^{a}	6.99 ± 1.96^{a}
∑n-3(ω-3)		2.51 ± 0.35^{a}	4.75 ± 0.73^{ab}	8.43 ± 1.64^{b}
∑n-6(ω-6)		9.07 ± 4.02^{a}	9.79 ± 4.33^{a}	4.51 ± 1.96^{a}
n3/n6		0.28 ± 0.01^{a}	0.48 ± 0.01^{b}	1.87 ± 0.01^{c}

Σ SFA is the sum of saturated fatty acids, Σ MUFA is the sum of monounsaturated fatty acids, Σn-3 is the sum of n-3 polyunsaturated fatty acids, and Σ n-6 is the sum of n-6 polyunsaturated fatty acids

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

Values with a different letter superscript within the same row indicate a significant difference between groups ($P < 0.05$)

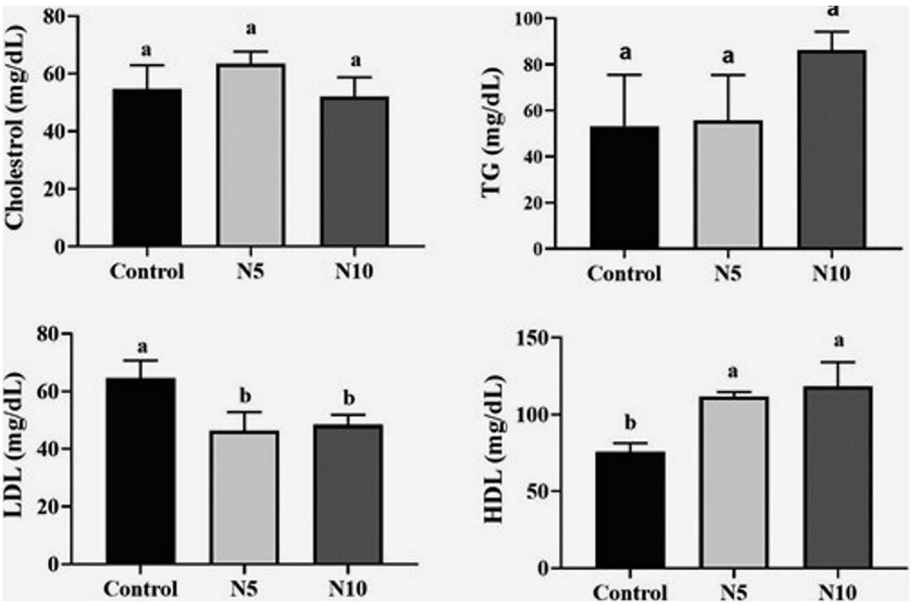


Fig. 1 Effects of *Nannochloropsis oculata (N. oculata)* dietary inclusion on serum lipid profile (cholesterol, TG, LDL, and HDL) in Nile tilapia. The fish were fed with the control diet or diets containing *N. oculata* at 5% or 10% for 7 weeks. Data is expressed as the mean ± SEM of six fish. Values with a different letter superscript are significantly different between groups. Significant levels ($P < 0.05$, 0.01, and 0.001), as determined by One-way ANOVA

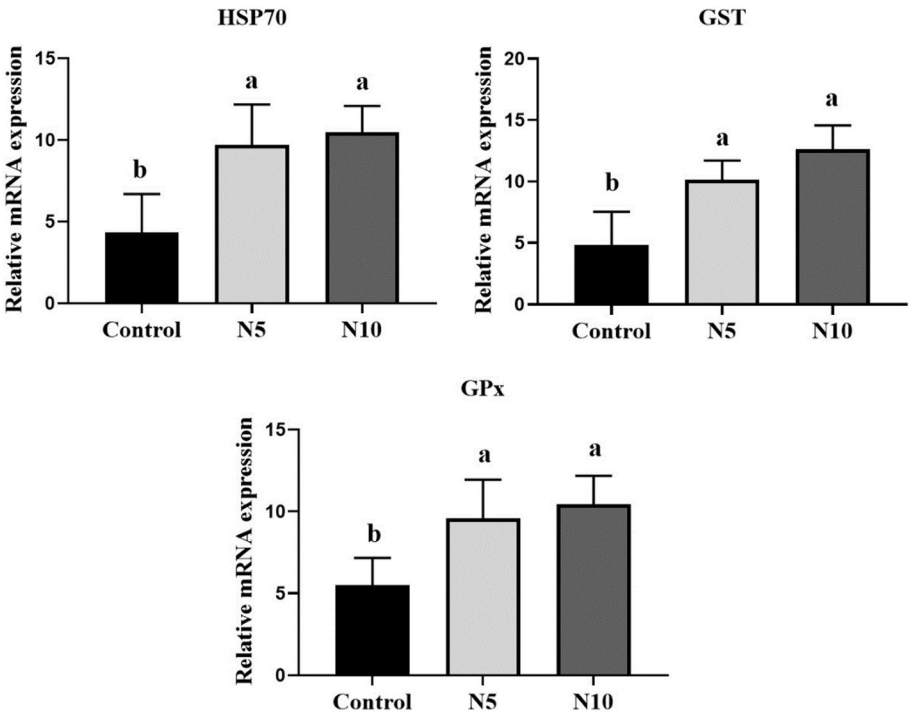


Fig. 2 Effects of *Nannochloropsis oculata (N. oculata*) dietary inclusion on the Nile tilapia hepatic gene expression of *HSP70*, *GST*, and *GPx*. The fish were fed with the control diet or diets containing *N. oculata* at 5% or 10% for 7 weeks. Data is expressed as the mean ± SEM of six fish. Values with a different letter superscript are significantly different between groups. Significant levels ($P < 0.05$, 0.01, and 0.001), as determined by One-way ANOVA

Effect of *N. oculata* supplementation on hepatic genes expression

N. oculata supplementation elicited modulated response in the stress and antioxidant-related genes, where the mRNA expression levels of hepatic *HSP70*, *GPx*, and *GST* showed a significant upregulation at both *N. oculata* supplemented levels compared with the control group ($p < 0.01$; $p < 0.05$) (Fig. 2). The expression pattern of the pro-inflammatory (*IL-1β* and *TNF-α*) and anti-inflammatory cytokines (*TGF-β1* and *IL-10*) involved in the immune response is shown in Fig. 3. *IL-1β* was significantly upregulated in both *N. oculata* incorporated groups [N5% ($p < 0.05$); N10% ($p < 0.01$)], however, a nominal increase was noticed for *TNF-α* and *TGF-β1* at both *N. oculata* levels, and at N10%, respectively, compared to control group. On other hand, the *IL-10* expression level was notably upregulated in the N10 group versus the control one ($p < 0.05$). The expression of hepatic genes involved in lipid metabolic pathways in fish-fed *N. oculata* is presented in Fig. 4. The dietary *N. oculata* at 5 and 10% significantly downregulated *FAS* gene expression levels compared to the control group ($p < 0.01$), while insignificant change was noticed in the expression levels of the *PPARα* gene. Concerning the expression of the apoptotic genes, caspase3 and *PCNA*, no significant changes were exhibited among groups (Fig. 5).

Histopathological findings

Histopathological changes in the intestine, liver, and spleen of Nile tilapia were observed in N5, and N10 supplemented groups. The intestine showed typical histological architecture as the control group (Fig. 5A). Intestinal histomorphology was typical, with minimal intestinal epithelial cell vacuolation detected in N5 and N10 groups (Fig. 5B-D). Also, the liver showed normal histological architecture of the hepatopancreas with minimal vacuolated hepatocytes in the control group (Fig. 6A). The same histological appearance of the control liver was seen in N5 and N10 supplemented groups with a slightly increased vacuolation in the N10 group (Fig. 6B-D). The spleen had a normal histological appearance of mixed red and white pulp with central melanomacrophage aggregations (Fig. 7A). Expanded melanomacrophage aggregations and numerous reactive endothelial blood vessels were highly detected in splenic parenchyma of N5 (Fig. 7B, C) than in N10 groups (Fig. 7D, E).

Discussion

Microalgae were implicated in aquafeed as a good biosourced of the essential FAs for the benefit of the aquaculture industry [49–51]. The microalgal-sourced lipids are preferable as the microalgae are low carbon and renewable sources of biomass and chemical feedstock,

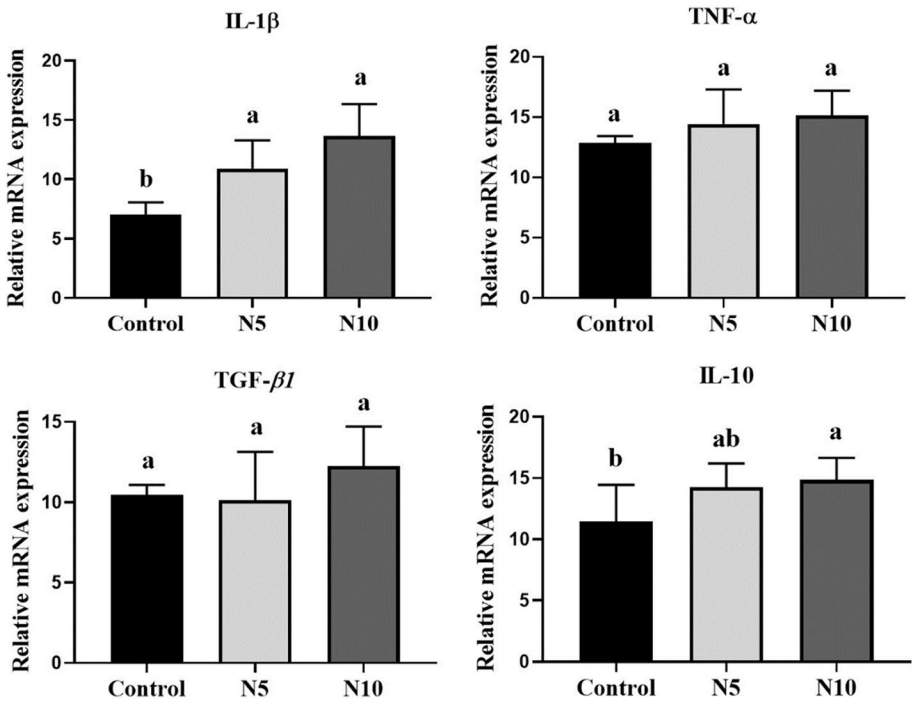


Fig. 3 Effects of *Nannochloropsis oculata (N. oculata*) dietary inclusion on the Nile tilapia hepatic gene expression of *IL-1β, TNF-α, TGF-β1, and IL-10*. The fish were fed with the control diet or diets containing *N. oculata* at 5% or 10% for 7 weeks. Data is expressed as the mean ± SEM of six fish. Values with a different letter superscript are significantly different between groups. Significant levels ($P < 0.05$, 0.01, and 0.001), as determined by One-way ANOVA

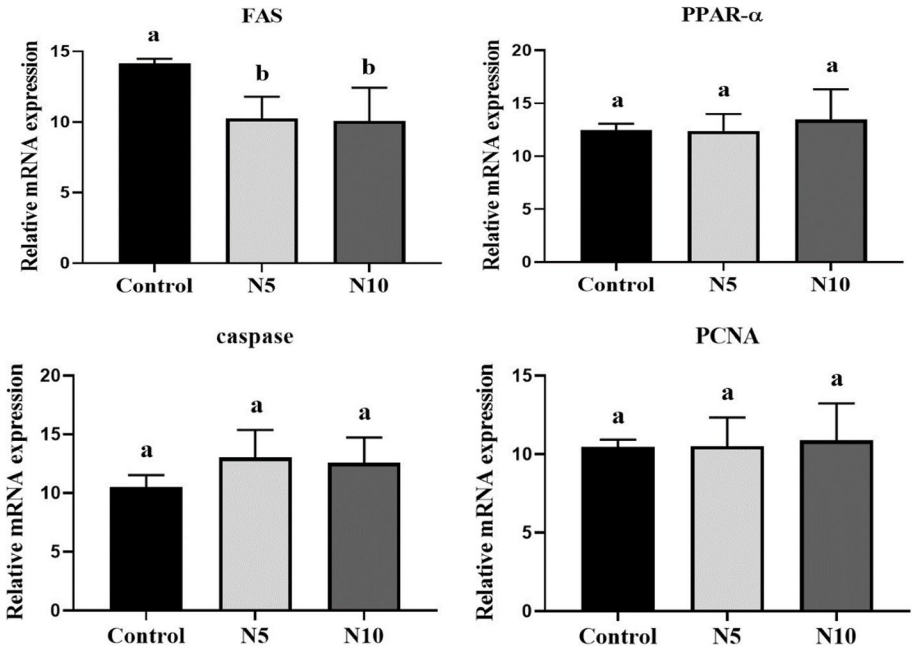


Fig. 4 Effects of *Nannochloropsis oculata (N. oculata*) dietary inclusion on the Nile tilapia hepatic gene expression of *FAS, PPARA, PCNA, caspase3*. The fish were fed with the control diet or diets containing *N. oculata* at 5% or 10% for 7 weeks. Data is expressed as the mean ± SEM of six fish. Values with a different letter superscript are significantly different between groups. Significant levels ($P < 0.05$, 0.01, and 0.001), as determined by One-way ANOVA

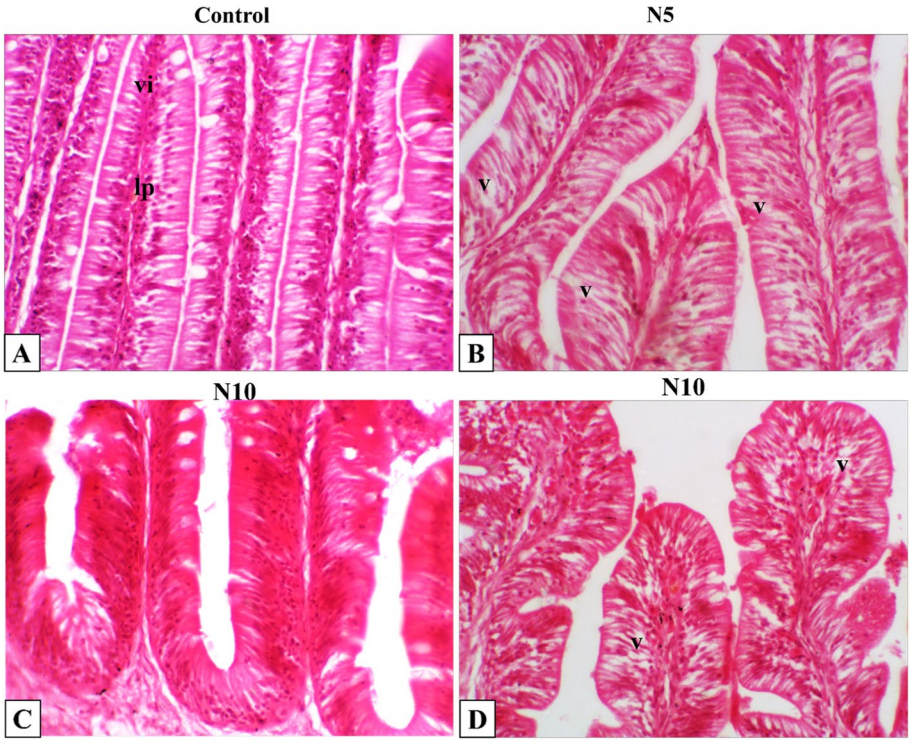


Fig. 5 Representative photomicrograph of Nile tilapia intestine. The fish were fed with the control diet or diets containing N. oculata at 5% or 10% for 7 weeks. **A** Control intestine shows normal histological appearance of intestinal villus (vi) and lamina propria (lp). **B** Intestine of tilapia fed on *N. oculata* at 5% showing normal intestinal architecture with minimal intestinal vacuolation (v). Intestine of tilapia fed on *N. oculata* at 10% shows (**C**) Normal histological intestinal mucosa, and (**D**) Other section with minimal intestinal vacuolation (v). H&E, 400X

which provide high bioenergy to the fish being fed [9]. In addition, *O. niloticus* showed high palatability, acceptability, and digestibility of microalgae, including *Nannochloropsis* spp., in its diets [52], which is consistent with our study, where supplementation of Nile tilapia feed with 5% and 10% *N. oculata* enhanced the fish growth performance indices, including FW, BWG, FCR, and SGR. This enhancement, especially in the FCR, indicates the good acceptability and utilization of the *N. oculata* diet by Nile tilapia. In addition, this reflects the adequacy of *N. oculata* amount in Nile tilapia diets, as the excessive algae meal might negatively affect the aquatic animal growth [53].

In an agreement with our findings, Abdelghany et al. [54] reported an enhancement in the Nile tilapia's growth performance by feeding diets supplemented with 5% *N. oculata* for eight weeks that was better than 15% supplementation. In the same line, 33% replacement of the Nile tilapia meal by *N. oculata* co-product (3%) revealed higher growth performance and body composition [55]. Recently, *N. oculata* replaced soybean at 2.5, 7.5, and 5%; only 2.5% showed the best growth performance [56]. Similarly, dietary inclusion of *Nannochloropsis* sp. (5%) significantly enhanced the antioxidant capacity of the juvenile turbot and (10%) and insignificantly enhanced their weight gain and specific growth rate [57]. On the contrary, this was not the case with Khajepour and Hosseini [58], who reported no improvement in the growth performance of the Atlantic cod supplemented with dietary *Nannochloropsis* spp. up to 30%. Noteworthy, fish species and the morphological structure of its stomach and intestine; the composition of the used *Nannochloropsis* sp., and the basal feed are the main factors affecting the fish's feed utilization [54]. In addition, the big-sized fish are known for their lower utilization and sensitivity to the nutritional additives than the smaller-sized fish [26].

In the present study, CP was increased in Nile tilapia whole body fed *N. oculata* at 10% compared to other groups. These results reflect the positive influence of the high protein content of *N. oculata* 46.3% supplemented diets. Our result is consistent with previous works that reported growth improvement upon dietary CP-rich microalgae supplementation in juvenile Nile tilapia [55, 59], hybrid red tilapia [60], and channel catfish [61]. The organosomatic indices were similar between fish fed with or without supplemented diets. Stable lipid content of the whole body and HSI suggested no adverse effects of

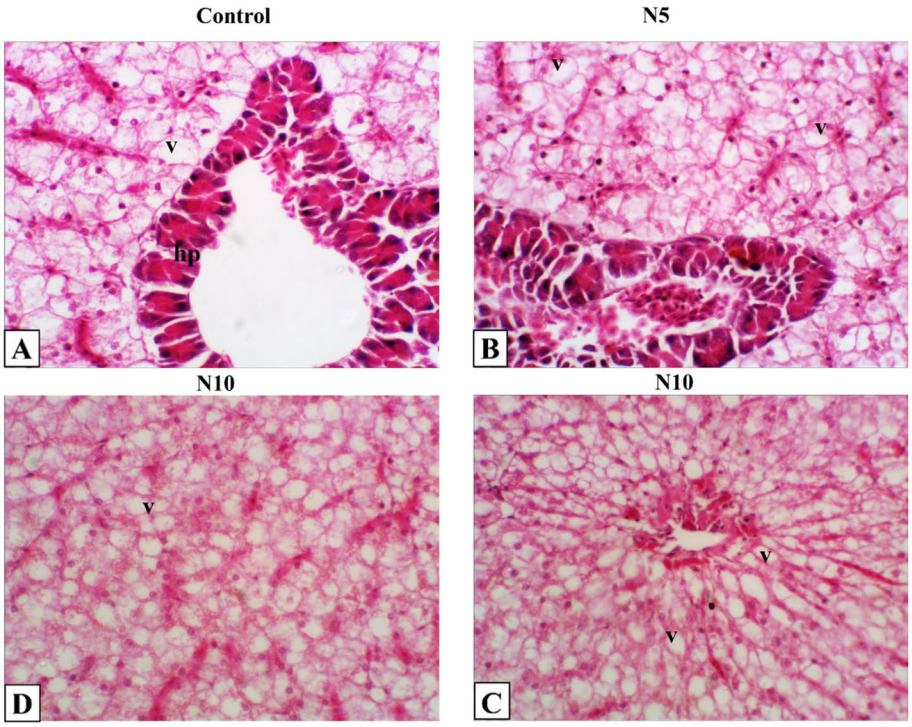


Fig. 6 Representative photomicrograph of Nile tilapia liver. The fish were fed with the control diet or diets containing N. oculata at 5% or 10% for 7 weeks. **A** Control liver shows normal histological appearance of hepatopancrease (hp) with vacuolated hepatocyte (v). **B** Liver of tilapia fed on *N. oculata* at 5% shows minimal hepatic vacuolation (v). **C, D** Liver of tilapia fed on *N. oculata* at 10% shows diffuse moderate to severe hepatic vacuolation (v). H&E, 400X

diets on lipid deposition [62]. Since the fatty acid composition of the fish body resembles their feed's fatty acid composition, fish feed supplementation with a source of essential fatty acids (EFAs) stimulates their growth as EFAs deficiency retards the growth [63]. The current study obtained promising findings of *N. oculata* as an ω-3 PUFA source in the Nile tilapia diets, where the inclusion of *N. oculata* in the Nile tilapia feed was a key feature affecting the ω-3 composition in the fish's whole body, besides a significant increasing ratio of ω-3/ω-6; this might explain the improvement of fish growth performance, where an augmentation was observed on the fish FW, BWG, and SGR values and on their body content of PUFA.

Our study revealed a marked decrease in LDL levels with a notable increase in HDL levels in the *N. oculata*-supplemented groups and insignificant changes in the TG and cholesterol levels. These results infer that *N. oculata* had a hypolipidemic effect on Nile tilapia via significant contribution of algal EPA and DHA combined with soluble and insoluble fibers as well as phytosterols. The long-chain ω-3 PUFAs were the most predominant in the lipid profile of fish whole-body fed with *N. oculata*. Mainly, microalgae are rich in LCPUFAs [12], which improves their fish body profile level. Noteworthy that the rate of EPA production in the fish body is reflected by the composition of ω-3 PUFA, mainly EPA, in their diets [52]. Elongase and desaturase activities convert EPA to DHA, providing the partial DHA requirements in different species [25], explaining the augmentation of DHA at the expense of EPA levels of N5 and N10 groups. Noteworthy, as DHA is known to decrease the fat [64], its richness in the whole fish body might explain the normal lipid profile and similar HSI [65]. LA and DHA FAs are implicated in methanogenesis suppression and activate nutrient degradability [66], which could explain the enhancement of the fish health and growth performance while keeping their organs' normal performance. Similar organo-somatic indices (HSI and SSI) and serum lipoproteins (TG and Cholesterol) in *N. oculata*-supplemented groups compared to the control group indicate the normal functional performance of the body organs.

Microalgae contain bio-active micro-minerals, enzyme cofactors implicated in protein synthesis, antioxidation defenses, inflammation amelioration, and multiple physiological functions [66]. In general, diets supplemented with microalgae modulate the fish's lipid metabolism and stimulate lipase activity [67, 68]. As diets rich in PUFAs decrease liver lipogenesis by suppressing the expression of fatty acid synthase-relevant genes, this is opposed to

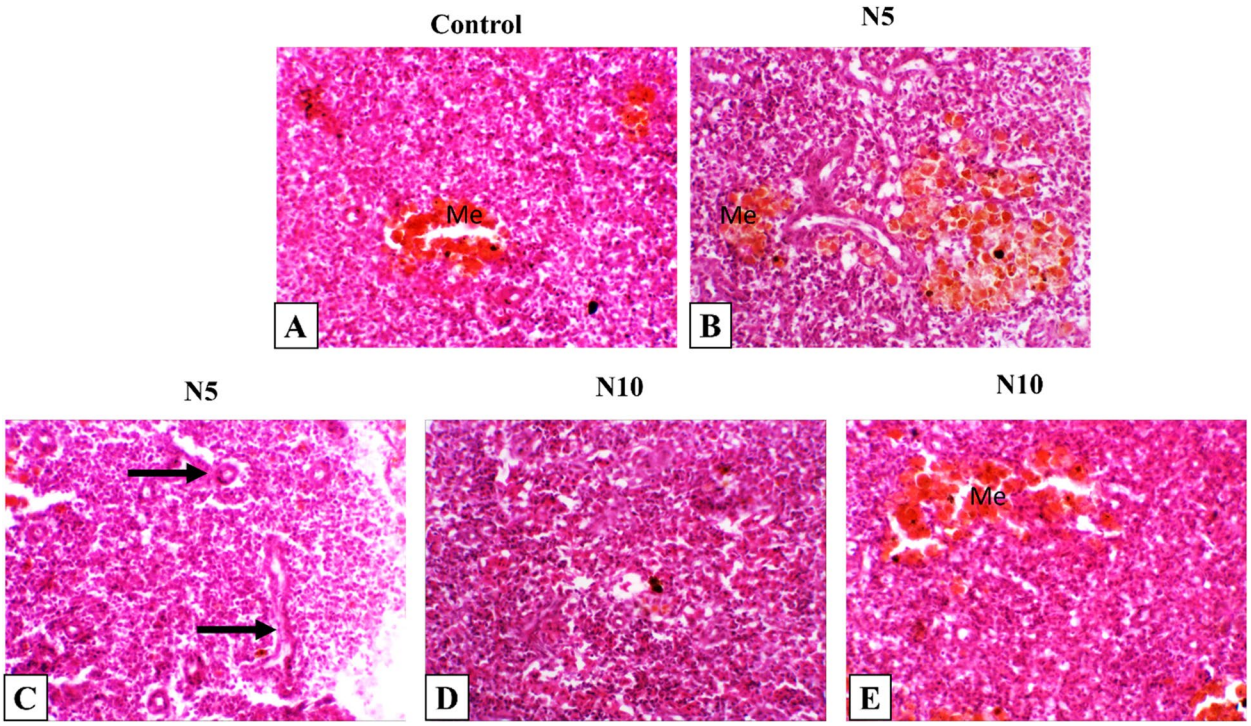


Fig. 7 Representative photomicrograph of Nile tilapia spleen. The fish were fed with the control diet or diets containing N. oculata at 5% or 10% for 7 weeks. **A** Control spleen shows mixed red and white pulp and melanomacrophage aggregation, H&E, 400X. Spleen of tilapia fed on *N. oculata* at 5% (**B**) shows extensive, activated melanomacrophage, and (**C**) many reactive endothelial blood vessels (thin arrows). **D, E** Spleen of tilapia fed on *N. oculata* at 10% shows normal histological appearance with minimal lymphoid depletion (thin arrow), other section showing normal architecture with activated melanomacrophages aggregates. H&E, 400X

the diets rich in carbohydrates that stimulate lipogenesis, leading to the elevation of TG levels [1]. To the best of our knowledge, the lipid-lowering effect of *N. oculata* has not been extensively investigated in fish, so we will cite *Nannochloropsis* research studies with other species. *N. oculata* dietary inclusion plays a vital role in the improvement of lipid profile in diabetic rats administered 10 and 20 mg/ kg BW of *N. oculata* for three weeks, where a notable decrease in plasma lipid profile excluding the HDL-C levels was detected; however, in healthy rats, no significant changes in lipid profile were noticed [69]. Microalgae such as *Chlorella vulgaris* (*C. vulgaris*) and *Spirulina platensis* have a lipid-lowering effect, showing an increased HDL and decreased LDL levels upon diet supplementation of African catfish for 12 weeks [70]. In support, a marked decrease in total cholesterol, triglyceride, and LDL levels was revealed in *C. vulgaris*-supplemented Nile tilapia (5%) for one month [71]. Similar effects were observed in common carp-fed *Spirulina* microalgae (3% & 5%) in the diet for 42 days [72].

The liver is one of the main organs involved in fat accumulation, such as PUFAs, which are essential for membrane function in fish, and the liver is generally regarded as the essential tissue for lipogenesis and β-oxidation in fish [73]. The expression of hepatic genes related to the antioxidant defense system was analyzed to investigate further the molecular mechanisms of improved antioxidant responses due to *N. oculata*. Results showed that all tested genes encoding antioxidant and immunity (*Hsp70*, *GST*, and *GPx)* responses in the liver were significantly upregulated in Nile tilapia fed with *N. oculata* after 7 weeks. *Nannochloropsis* sp. diminished the lipid peroxidation and enhanced the antioxidant capacity in turbot [57], Atlantic salmon [74], and Nile tilapia [75]. The vital ROS buffering systems, including GPx and GST enzymes, are mainly involved in quenching lipid-peroxidizing chain reactions, reducing H_2O_2, and lipid peroxides as GSH is oxidized to GSSG; this elucidates the ability to protect cells from oxidative damage [76]. Molecular chaperone, *Hsp70*, a biomarker of stress, plays a significant role in the fish's survival against critical stress response, and thus it is employed as a sensor of cellular redox changes as they have been believed to activate ROS scavengers such as antioxidant enzymes [77]. Besides, it stimulates immune responses [78]. An increase in liver and serum GPx and SOD enzyme activities of juvenile turbot (*Scophthalmus maximus* L.) fed a diet with 5% *N. oceania*-derived defatted meal for

10 weeks, compared to that fish fed on an algae-devoid diet [57]. Besides, in our recent study dietary supplementation of Nile tilapia on *N. oculata* at 5% under naïve conditions for 4 weeks, significantly increased hepatic and intestinal *GST* and intestinal *GPx* genes [75]. In another study, serum SOD and GPx activities recorded the highest values in Nile tilapia fed on a diet partially replaced with *N. oculata* at 5% for 60 days compared to the control fish [56]. Such *GPx* upregulation effect was not evident in the liver of whole *N. oculata*-fed Atlantic Salmon (*Salmo salar*) at 30% for 60 days [79]. Similar induction in the expression of *Hsp70* was evident in the liver of whole *N. oculata* at 10% in Nile tilapia under naïve conditions for 4 weeks [75]. Dietary microalgal *Tribonema* sp. at 5% for 6 weeks notably upregulated mRNA level of *Hsp70* in golden pompano [80]. It has been well documented that natural bioactive compounds in microalgae induced *Hsps* expression in the aquatic organism [81, 82]. Thus, we hypothesized that the potential role of *N. oculata* as an immune response stimulator in Nile tilapia might improve the tolerance of Nile tilapia to various stresses. These results also indicated that the *N. oculata* possessed the highest antioxidant capacities, targeting a prospective new source of natural antioxidants [83]. These results are linked to the increased level of ω-3 PUFAs (α-linolenic, ALA, C18:3 ω3) and eicosapentaenoic (EPA, C20:5 ω3) in the present study, suggesting that ω-3 PUFAs might significantly contribute to *N. oculata* antioxidant activity. Additionally, carotenoids of *N. oculata* have similar antioxidant activity [84]. Thus, the higher gene expression of antioxidant-related genes in the liver of *N. oculata*-fed fish could indicate an improved response to oxidative stress, indicating the importance of microalgal DHA in enhancing the antioxidative enzyme activities to maintain the redox balance [85–87].

Cytokines play an important role in mediating the immune responses interceded by inflammation. Among the cytokines, *IL-1β* and *TNF-α* are potent inflammatory mediators that can fortify secure reactions by mobilizing lymphocytes or by motivating the arrival of a variety of cytokines that initiate macrophages, Natural killers (NK) cells, and lymphocytes to promote the immune response of fish [88]. In the present study, the higher expression of *IL-1β* ($p < 0.05$, $p < 0.01$) and *TNF-α* ($p > 0.05$) was found in the liver when supplemented with 5 and 10% dietary *N. oculata,* respectively, for 7 weeks. Thus, it could be speculated that *TNF-α* was less sensitive than *IL-1β* to dietary *N. oculata*. Similarly, significant upregulation of *IL-1β* and *TNF-α* has been demonstrated in Nile tilapia fed the diet with *N. oculata* (5, 10, 15%) for eight weeks [54]. The expression of *IL-1β* and TNF-α genes also increased in Nile tilapia fed with 10% dietary microalgal *Spirulina*, *Arthrospira platensis* for eighty-three days [89]. Furthermore, different fish species and diet formulations could also account for the inconsistent results.

Nannochloropsis species are considered a valuable source of n-6 LCPUFAs associated with metabolic pathways related to immune response and inflammation via eicosanoid signaling. Generally, n-6-PUFAs are well known to activate the signaling molecules of nuclear transcription factor-κB (NF-κB), a major transcription factor that promotes pro-inflammatory cytokines genes transcription [90, 91]. Thus, these facts could propose the induction of pro-inflammatory genes in our study after *N. oculata* supplementation, proposing its ability to maintain an active local immune system after the feeding trial. The anti-inflammatory cytokines, *IL-10* and *TGF-β1*, quell the inflammatory response by downregulating the transcriptional levels of cytokine expression [92, 93]. As observed herein, hepatic *IL-10* was upregulated in the N10% group compared to the control group; however, changes were not evident in the hepatic *TGF-β1* gene in both *N. oculata* supplemented groups. A similar expression pattern of hepatic *TGF-β1* has also been reported in Nile tilapia fed the same dose regimen of dietary *N. oculata* under naïve conditions for 4 weeks [75]. These data strengthened the role of *N. oculata* in enhancing the immune response while maintaining its anti-inflammatory prosperities.

To further investigate how *N. oculata* regulated lipid metabolism, the expression of genes related to lipid metabolism in the liver was analyzed. Fatty acid metabolism involves anabolic and catabolic processes catalyzed by key enzymes relevant to its transcriptional factors [94]. Fatty acid synthase (*FAS*) catalyzes the de novo long-chain fatty acid synthesis by converting acetyl-CoA and malonyl-CoA to stearic acid, which is further transformed into monounsaturated fatty acids [95, 96]. Peroxisome proliferator-activated receptor alpha (*PPARα*) has a pivotal role in reducing lipid accumulation by inducing β-oxidation and lipolysis of fatty acids by modulation of gene expression encoding peroxisomal fatty acid-catabolizing enzymes as a transcription factor [97].

No information has been published about evaluating the effects of dietary *N. oculata* levels on mRNA expression of lipid-related genes in fish. *FAS* expression was downregulated in *N. oculata* supplemented groups compared with the control group, while there are no remarkable differences in the *PPARα* expression levels in the same groups. In the same context, hepatic *FAS* gene expression was also diminished in juvenile black seabream fed the higher DHA/EPA ratios (1.60) [25], although the expression of PPAR-*α* was markedly decreased. Similar results were reported in Siberian sturgeon following feeding with high dietary LC n-3HUFA [98]. The mRNA levels of hepatic *FAS* and *PPARα* were not significantly changed in

golden pompano fed either microalgal *Tribonema* sp. (1% and 5%) [80] or microalga *Porphyridium* sp. (10% and 50%) for 6 weeks [99].

Dietary PUFAs, especially EPA and DHA, inhibit the transcription of the *FAS* gene and so the hepatic lipogenesis in teleost [73, 100]. Nevertheless, *PPARs* are known to be fatty acid sensors responding to increased cellular fatty acid levels arising from changes in nutritional lipid status [101, 102]. Thus, our data investigated the roles of *N. oculata* supplementation in regulating fatty acid homeostasis via decreasing the *FAS* expression and unchanged *PPARs* genes, eventually leading to less lipid accumulation with subsequent no adverse effect on hepatic β-oxidation of Nile tilapia.

The PCNA, a ring-like protein, provides a regulatory role in DNA replication and cellular metabolism as it is remarkably conserved among eukaryotes [103, 104]. The PCNA has been used as a widespread model in cancer studies and toxicity bioassays in teleost [105, 106]. Besides, caspases are a family of proteins that are one of the main effectors of apoptosis, breaking double-strand DNA [103]. In our study, no marked change in the transcriptional levels of *PCNA* and *caspase*3. After 8 weeks of two different microalgal extracts of 21 and 37% *Phaeodactylum tricornutum*-derived β-glucans, intestinal *PCNA* gene expression was not changed in gilthead seabream juveniles [107]. Results from this study concur with a previous study that demonstrated the transcription level of intestinal *PCNA* was not changed, and lower expression levels of the caspase gene were evident in Atlantic salmon fed a 100% plant oil-based diet, demonstrating that there were no abnormal changes in intestinal cells renewal [103]. Accumulating evidences support that dietary microalgae *Porphyridium* sp. for 6 weeks can diminish caspases mRNA expressions, inhibiting the apoptosis of hepatocytes to protect the liver of juvenile golden pompano [99]. Also, mRNA levels of hepatic *caspase*3, *caspase*6 and *caspase*9 were not significantly affected in golden pompano fed microalgal *Tribonema* sp. (1% & 5%) for 6 weeks [80]. Based on our results, there is no abnormal cell proliferation and apoptotic changes in hepatic tissue of the *N. oculata* groups, suggesting its ability to maintain the hepatic health.

The histological analyses of *N. oculata* dietary supplementation on liver, intestine, and spleen samples did not reveal any considerable negative effect caused by the dietary supplement. The visible vacuoles were seen in all groups. The minor changes in the intestine indicate that *N. oculata* keeps the intestine's healthy architecture, reflecting its positive effects on the absorption and digestibility capability and thus the nutrient uptake; this also coincided with our finding of the growth performances. The presence of vacuoles in all groups could be attributed to the natural deposition of fat in the liver of Nile tilapia or the high content of fatty acids in *Nannochloropsis* incorporated groups [108]. Furthermore, the spleen shows melanomacrophage activations at 5%and 10% *N. oculata* supplementary levels, indicating immune response enhancement. The absence of inflammatory changes in the liver, intestine, and spleen could be attributed to the significant increase of eicosapentaenoic acid and docosahexaenoic acid (DHA) in *Nannochloropsis* incorporated groups, which play an important role in ameliorating the increase of pro-inflammatory cytokines. Thus, the enhancement of the growth performances, antioxidant activities, and immune response reported in the present study were all histopathologically evident in the intestine, liver, and spleen; and are consistent with previous findings in Nile tilapia and European sea bass [54, 75, 109, 110] emphasizing on the benefits and safety effects of *N. oculata* dietary inclusion on health performance and immunity of Nile tilapia.

Aside from the strengths of our study already mentioned, the CRD design was appropriate for examining the effects of dietary supplements and establishing causality. Additionally, this research helped close a knowledge gap regarding the use of *N. oculata* feed additives as a source of n3-long chain amino acids (LCPUFAs). This study may have some potential limitations, including the inability to generalize the findings to all fish types and the paucity of data on the effects of *N. oculata* feed additives on fish FAs analysis and FAs-related genes. Additional dosing rates of *N. oculata* in feed may also be important to observe a dose-dependent effect and the need to investigate the treatment's effects after a fish pathogen challenge. Therefore, additional research is required to clarify these issues.

Conclusions

The current study sheds light on the beneficial effects of *N. oculata* dietary inclusion as a protein and lipid source on Nile tilapia growth performance and fatty acid profile. It is a high-quality microalga and a fish oil-saving feed additive. Notably, *N. oculata* incorporation had a positive effect on the expression patterns of antioxidant, cytokine, lipid, and apoptotic-related genes. Thus, aquafeed fortified with *N. oculata* has the potential to improve fish health and increase aquaculture sustainability.

Abbreviations

N. oculata	*Nannochloropsis oculata*
ω-3 PUFAs	Omega-3 polyunsaturated fatty acid
HDL	High-density lipoproteins
LDL	Low-density lipoproteins ()
TG	Triglycerides
IL-1β	Interleukin-1β
FAS	Fatty acid synthase

PPARα	Peroxisome proliferator-activated receptor alpha.
TNF-α	Tumor necrosis factor-*α*
TGF-β1	Transforming growth factor-β1
PCNA	Proliferating cell nuclear antigen
FAs	Fatty acids
ROS	Reactive oxygen species
PUFAs	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
NRC	National Research Council
SGR	Specific growth rate
K	Condition factor
HIS	Hepatosomatic indices
SSI	Splenosomatic indices
MS-222	Tricaine methanesulfonate
AACC	American Association of Cereal Chemists
AOAC	Association of official analytical chemists
FAMEs	Fatty acid methyl esters
CP	Crude protein
CL	Crude lipid
EFAs	Essential fatty acids
NK	Natural killers
NF-κB	Nuclear transcription factor-κB

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Authors' contributions
EZ: conceptualization, investigation, methodology, validation supervision, reviewing and editing. SE: Methodology, investigation, validation, contribute to writing the original draft. FA: investigation, contribute to writing the original draft. II: Methodology, investigation, contribute to writing the original draft. AK: investigation, validation, resources. EE: investigation, validation, resources. All authors read and approved the final manuscript.

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Availability of data and materials
The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All fish in the experimental protocols were reared and handled in accordance with the guidelines of the local Administrative Panel on Laboratory Animal Care and Committee of Mansoura University with the code number (R/108), which specifically approved this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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