Influence of Dietary Inclusion of *Spirulina platensis* for Fishmeal on Growth, Immune Capacity, and Liver of Nile Tilapia (*Oreochromis niloticus*)

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Abstract: Nowadays, fishmeal costs have been increased and the detection of alternative ingredients will allow for the development of the potential growth of aquaculture. The substitution of fishmeal by algae in fish feed is a topic in our study. The present work deals with the evaluation of feeding with different levels of *Spirulina platensis* (0, 5, 20, 35 and 50%) on the growth of Nile tilapia, *Oreochromis niloticus*. At the end of acclimation period, the fish (initial body weight, 1.3±0.06 g) were introduced into the tank (capacity 500 L seawater) at stocking rate of 20 randomly selected fish per tank. The fish was fed with the diets at a rate of 3% body mass twice daily for 90 days. The chemical analysis of algae and fishmeal were estimated. The growth parameters, innate immunity, and histological study of the liver of fish were evaluated. The results revealed that *S. platensis* contained high levels of protein, minerals, vitamins, chlorophylls, phenolic, polyunsaturated fatty acid and antioxidant activity. At the end of feeding trial, the specific growth rate of fish groups fed diets supplemented with *S. platensis* were higher than that of fishmeal control. The results showed that the fish fed 5% *S. platensis* diet had the highest body weight as compared with the control. The lysozyme activity in tilapia was found to increase with increasing *S. platensis* diet with maximum activity (1.79 Units/ml) at the highest showed a highly effective in fish fed 50% *S. platensis* diet. Histologically, the liver tissue showed sever changes in tilapia fed fishmeal control. Meanwhile, the liver showed significant improvement in groups fed the *S. platensis* diet. According to the results, 5% *S. platensis* could be used as an additive in the fishmeal to enhance the growth and immune capacity of fish.
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1. Introduction

In the last decades, the industry of aquaculture has shown fast growth, paying an increase to the national product of most economies in the world. The demand for animal protein, particularly from fish, is continuing to rise with the global population expected to reach nine billion by 2050 (FAO, 2016). At present, about 44% of fish are consumed and this percentage is expected to reach 52% by 2025.

Increasing demands on the use of freshwater for agricultural, industrial and domestic purposes progressively limit freshwater-based aquaculture, particularly in the arid and rain fed regions in the tropics. The efficient uses of seawater environments for aquaculture becomes a vital alternative. Tilapias are the most important aquaculture species in the world today with the possibility to expand their culture into saltwater environments (Bostock *et al*., 2010).

Fish nutritionists continue to search for alternative plant protein sources, to replace fishmeal partially or wholly in aqua-feeds. The substitution of fishmeal by plant proteins in fish feed is a topic in many studies in the area of fish nutrition. Microalgae are promising living cells, which are represented at the aquatic food chain due to their important nutritional value (Brown *et al*., 1997). Indeed, microalgae are unique compared to other living organisms due to their valuable biochemical compounds for aquaculture, human health food and other applications (Halima, 2017). Several microalgae contain in excess of 50 % crude protein with amino acid profile comparable to that of fish meal. In addition, high polyunsaturated fatty acid contents encourage their use in animal feeding and nutrition, particularly in the formulation and processing of aquafeeds (Barone *et al*., 2018). Algae are a good candidate to substitute wholly or partially the fishmeal in aquafeeds (Rama *et al*., 2014). Algae can be used as fresh or handled as meal in the diet of fish. Algae are useful to fish due to their nutritional values, production ratio, and immune-stimulatory effect (Promya and Chitmanat, 2011).

The filamentous blue-green alga, *Spirulina* is a multicellular and have gained considerable popularity as a protein, vitamins, lipids, minerals, carbohydrates,

nucleic acids, enzymes and pigments (Wongputtisin and Sompong, 2015). The nutritive value of a protein is related to the quality of amino acids, digestibility coefficient, as well as by its biological value (Demelash, 2018). It can grow in water, harvested, and processed easily.

The present work aimed to study the chemical constituents of fishmeal and *S. platensis* to investigate the optimal dietary concentration of dried *S. platensis* needed to employ its potential effects on growth performance and immune responses of tilapia. In addition, histological study on the liver of tilapia attained the highest and the lowest growth were examined.

2. Materials and methods

2.1. Algal material

The blue-green alga, *Spirulina platensis* (Cyanophyta) was obtained from the algal Lab, Faculty of Science-Alfaisaliah, University of Jeddah, Jeddah, Saudi Arabia. It was sub-cultured in Zarrouk's medium (1966). The biomass was dried at 60 °C and made into powder and kept in Stoppard bottles at room temperature until use in the diet.

2.2. Chemical analysis of *Spirulina* and fishmeal

The contents of humidity, ash, and fiber of algae were estimated according to AOAC (1995). For extraction of chlorophyll and carotenoids, alga was extracted using water-free methanol as described by McKinney (1941). Phycobiliproteins were extracted according to Bennet and Bogorad (1973). After removal of pigment, the cells were extracted with 1N NaOH as reported by Payne and Stewart (1988). According to Bradford method (1976), protein content was determined. Total carbohydrates were determined according to Kochert (1973) by the method of phenol-sulphuric acid. A mixture of chloroform: methanol (1:2, v/v) was used to extract the lipid and re-extracted with chloroform (Thimmaiah, 2006). The chloroform was evaporated and the total lipid was calculated.

The equation for calculation of N-free extract was represented by AOAC (1984) as follows:

N-free extract % = 100 – (% protein + % lipid + % fiber + % Ash]

The equation for evaluation of gross energy was applied according to Jobiling (1983) as follows:

Gross energy = (Protein × 5.65) + (Carbohydrates × 4.0) + (Fat × 9.45) Kcal/Kg

Total phenolic estimation was carried out with Folin-Ciocalteu reagent (FCR) to Malick and Singh (1980). The standard curve prepared by using a different concentration of Catechol. Vitamins A and C were analyzed according to Neel and Pearson (1963) and Hussain *et al*. (2010). Vitamins were expressed as mg/g. The total contents of elements were measured according to Allen *et al.* (1986). The element contents were estimated in the digestive solution by using ICPS (Inductively Coupled Plasma, Spectrometer, 6000 Series, Thermo Scientific).

2.3. Analysis of antioxidant activity "DPPH radical-scavenging activity"

The scavenging activity of *S. platensis* and fishmeal for DPPH (1,1-diphenyl-2 picrylhydrazyl) radical was monitored as described by Lee *et al*. (2002). The scavenging activity percentage was determined by using the following equation:

The DPPH radical scavenging activity (%) = $[(A_c - A_{s)} / A_c] \times 100$

Where A_c is the absorbance of the control, and A_s is the absorbance of the tested compound. The ascorbic acid was used for comparison.

2.4. Estimation of fatty acid

The fatty acid composition of *S. platensis* and fishmeal was measured by using Gas Chromatograph System (HP 6890) with Mass Selective Detector (HP 5973). The fatty acid was calculated as percentage dry weight

2.5. Experimental fish

Tilapia fish, *Oreochromis niloticus* was obtained from the fish farm at the Faculty of Marine Science, King Abdulaziz University, Jeddah with an average initial body weight 1.3±0.06 g. Fish were acclimated in the aquaria for two weeks before the beginning of the feeding experiments.

2.6. Experimental diet

The fishmeal control was obtained from Maram Feed Mill, Buraidah, Al Qassim, Saudi Arabia. The ingredients of fishmeal control were represented in Table 1. The experimental fish diets were prepared by replacing 0, 5, 20, 35 and 50% of fishmeal control with dry *S. platensis*. The mixture was ground in the mixer of food and boiling water was then added at the ratio of 50% for pelleting. The meat grinder with a 1.5 mm diameter was used for pelleting the diets. The diets were dried at 60 ^{o}C, and then stored in bags at room temperature (Promya and Saetun, 2005).

2.7. Experimental design

Circular plastic fish aquariums were filled with 500 liters seawater coming to the experimental station from Red Sea of Jeddah at the Faculty of Marine Science. Each aquarium was supplied with a continuous system of filtration and aeration and the water was stored for 24 hours before the beginning of the experiment. The fish in all treatments were distributed into the aquaria at the stocking rate of 20 fish per aquarium. Three replicates were prepared for each treatment. Fish reared in the aquariums at 25°C with 14:10 light/dark photoperiod for 90 days experiment. Fish were fed twice daily (at 8:30 AM, and 4:30 PM) at a weekly feeding rate of 3% of body weight. Every two weeks, six fish were sampled from

each group for weighing (g). Before weighing, fish were starved for 24 hours allowing the gut to be empty.

2.8. Calculation of fish growth

The different parameters of growth including weight gain and specific growth rate were calculated using the following equations:

Weight gain (g) = Final body weight - Initial body weight

Specific growth rate (%) = [(ln Final body weight - ln Initial body weight) / number of feeding days] ×100

2.9. Immune capacity by lysozyme activity

Blood of fish was collected using heparinized syringes from the caudal peduncle of fish from each treatment at the end of the experiment. Samples of the blood were centrifuged for 15 minutes and the supernatant was stored at -20 ^{o}C until used for the test of serum bactericidal. The turbidity was used for the assay of lysozyme activity (Parry *et al*., 1965). The blood serum (50 ml) was added to the bacterial suspension of *Micrococcus lysodeikticus* (2 ml). The absorbance was determined at 540 nm every 0.5 minutes for 5 minutes of incubation at 22 ^{o}C. Different dilutions of Chicken egg lysozyme (Sigma) was used as a standard. A unit of lysozyme activity is referred to as the opacity level has decreased 0.001 min^{-1}.

2.10. Histological study

Small slices of *Oreochromis niloticus* liver were prepared and fixed in neutral buffered formalin 10% at the end of the experiment, immediately. Then the fixed samples were handled using the usual histological techniques according to Culling *et al.* (1985). Semi-thin sections of 2-3 microns thick using rotary microtome were stained with hematoxylin and eosin (H and E), respectively. Sections were examined under a light microscope and photographed using Olympus BX51 System Microscope. Ultrathin sections were made for liver tissue according to Woods and Stirling (2002) and scanned by Electron Microscope (CM 100, Philips, Holland).

2.11. Statistical Analysis

All data were the mean of three replicates and expressed as the ±Standard deviation (SD). The data were subjected to One-Way ANOVA by using SPSS program version 16 (SPSS Inc. Chicago, USA).

3. Results

3.1. Chemical composition of diets

The chemical composition of standard fishmeal and *S. platensis* was represented in Table 2. The moisture content in *S. platensis* (12.5±0.16%) was higher than that in the fishmeal (10.2±0.33%). However, the percentage of ash and fiber in fishmeal (12.5±0.31 and 3.1±0.19, respectively) were found to be higher than *S. platensis* (9.3±0.55 and 0.9±0.05, respectively). In fishmeal and *S. platensis* the values of protein (44.19%-44.08%), Vitamin A (6.235 mg/g), Vitamin C (0.312- 0.341 mg/g) and N-free extract (34.71%-34.75%) were approximately within the same range. Whereas, the results of carbohydrates, lipid, phenolic and gross energy in S. platensis (16.52±0.12%, 11.97±0.20 %, 3.91±0.04% and 4282.49±34.01 Kcal/Kg, respectively) were greater than that observed in fishmeal (15.75±0.12%, 5.50±0.30%, 2.12±0.09% and 3646.49±16.07 Kcal/Kg, respectively).

The element composition of fishmeal showed the absence of Na, higher values of Mg (0.41 ±0.03%) and Zn (0.233±0.046 mg/Kg) as compared with *S. platensis* (1.03±0.20%, 0.27±0.02 %, and 0.083±0.021 mg/Kg, respectively) (Table 2). The percentage of K in *S. platensis* (1.47±0.17%) was higher than fishmeal (0.78 ±0.21%).

The results in Table 3 showed the pigment constituents of *S. platensis*. Phycocyanin was the highest pigment content (8.01±0.12 mg/g dry weight) followed by allophycocyanin (3.91±0.09 mg/g dry weight) and phycoerythrin (2.23±0.05 mg/g dry weight). The chlorophyll a and carotenoid contents were represented by 0.87±0.08 and 0.50±0.05 mg/g dry weight, respectively.

3.2. Fatty acid composition

The fatty acid composition is varied considerably for fishmeal and *S. platensis* (Table 4). Palmitic acid (C16:0) was the most abundant SFA in algae (45.95%) and fishmeal (19.95%). As for MUFAs, vaccenic acid (C18:1n7; 22.79%) and palmitoleic acid (C16:1n-7; 6.93%) were reported as major acids in fishmeal and *S. platensis*, respectively. The PUFAs was found to be the maximum in *S. platensis* (34.25%) and the minimum in fishmeal (26.51%). The most abundant PUFAs was the linoleic acid (C18:2n-6) in algae (15.28%) and fishmeal (13.37%). The values of the health-beneficial PUFAs, EPA, and DHA in *S. platensis* (4.46% and 4.69%, respectively) were greater than that observed in fishmeal (1.01% and 3.64%, respectively). However, the GLA and AA in fishmeal (2.16% and 2.89%, respectively) were relatively the highest as compared with *S. platensis* (1.66% and 1.54%, respectively). The ratio of UFAs/SFAs of fishmeal (1.77%) was higher than S. platensis (1.00%). The fishmeal recorded the highest ratio of n6/n3 (2.81%) as compared with *S. platensis* (1.32%). The values of AA/EPA and AA/DHA in *S. platensis* (0.35% and 0.33%, respectively) were lower than that recorded in fishmeal (2.86 and 0.79%, respectively).

3.3. The radical scavenging activity DPPH

As shown from results in Table 5, the values of DPPH radical scavenging of algae and fishmeal were dose-dependent within the range of tested

concentrations. The antioxidant activity exhibited the maximum value by *S. platensis* (12.95±0.09%) and the minimum activity by fishmeal (6.60±0.29%) at the concentration of 40 mg/ml.

3.4. The growth of tilapia

The influence of *S. platensis* diets on the growth performance of tilapia was examined after 90 days of incubation. The results in Figure 1 showed that the replacement of fishmeal with different levels of *S. platensis* could increase the growth of tilapia. Significant differences in growth of fish was found at 5% and 20% *S. platensis* diets. Meanwhile, no significant differences for growth among fish fed 35% and 50% *S. platensis* diets. The body weights of fish fed 5% and 20% *Spirulina* diet increased from 1.3 g to 27.4g and 24.5 g, respectively with a weight gain of 26.1g and 23.2 g, respectively which were greater than the control group (17.8 g). The specific growth rate of fish fed 5%, 20%, 35% and 50% *Spirulina* diets were, 3.39±0.17, 3.27±0.12, 3.16±0.21, and 3.06±0.13, respectively which were higher than control (2.99±0.52).

3.5. Serum lysozyme activity of tilapia

The lysozyme activity in tilapia was found to increase with increasing *S. platensis* levels in the diet after 90 days experiment (Table 6). Significant differences were found in the lysozyme activity of tilapia fed 20%, 35%, and 50% *S. platensis* diet. The maximum activity (1.79±0.17 Units/ml) was observed at 50% *Spirulina* diet as compared with control (0.83±0.01 mg/ml).

3.6. Histological study of the liver

Depend on the maximum and minimum growth, the liver of tilapia fed 5% and 50% *Spirulina* diet, respectively was chosen for the present study. The virtual look of the control liver appeared to be brown in color with hypertrophied in the middle part. Also, sever changes in the liver tissue architecture was observed with severe cytoplasmic vacuolations, pyknotic nuclei, a collapse of blood sinusoids, mild fatty changes, and cellular necrosis (Fig. 2a). However, liver of fish fed 5% *S. platensis* diet showed an improvement with less vacuolated hepatocytes and disintegration in red blood cells (Fig. 2b). On the other hand, the liver tissue showed remarkable improvement, normal size and ruby red color in fish fed 50% *S. platensis* diet (Fig. 2c). A normal hepatocytes structure, extensive pyknotic nuclei, slight vacuoles in some areas, blood vessels congestion and portal vein congestion was also observed in the liver of fish fed 50% *S. platensis*.

Figure (3a-c) clarified the electron microscopy examination of the liver tissue of fish fed fishmeal and *Spirulina* diet. The liver of tilapia fed fishmeal showed the presence of hepatocytes with lipid droplets, karyolysis nucleus, cytoplasmic organelles lysis, autophagic vacuoles, residual bodies, damaged bile canaliculi and hypertrophied mitochondria with electron-dense matrix and destructed cristae (Fig. 3a). In addition, hepatocytes in areas surrounding the central vein appeared severe cytoplasmic vacuolation, necrosis, deformed nuclei, an atrophic endothelial cell with an elongated nucleus and large masses of marginal heterochromatin. Figure (3b) exhibited dilated blood sinusoids, necrotic and degenerated hepatocytes not completely recovered in the liver of tilapia fed 5% *S. platensis* diet. However, the liver tissue appeared recovery effect in tilapia fed 50% *S. platensis* and showed slight lipid droplets with normal forms of the nucleus, nuclear membrane, mitochondria, and rough endoplasmic reticulum (Fig. 3c).

Table 1. Ingredient composition of fishmeal control

Ingredients	g/100g
Soybean	60
Corn Grains	20
Barley	12
Fish Oil	6.3
Minerals	1.0
Amino Acids	0.5
Vitamins	0.2

Table 2. Chemical composition of experimental fishmeal and *S. platensis*. Data are the mean of three replicates ±SD.

Composition	Fishmeal	*S. platensis*
Moisture (%)	10.2±0.33[a]	12.5±0.16[b]
Ash (%)	12.5±0.31[a]	9.3±0.55[b]
Fiber (%)	3.1±0.19[a]	0.9±0.05[b]
Protein (%)	44.19±1.14[a]	44.08±2.36[a]
Carbohydrates (%)	15.75±0.12[a]	16.52±0.12[a]
Lipid (%)	5.50±0.30[a]	11.97±0.20[b]
Phenolic (%)	2.12±0.09[a]	3.91±0.04[b]
Vitamin A (mg/g)	6.235±0.03[a]	6.235±0.02[a]
Vitamin C (mg/g)	0.312±0.01[a]	0.341±0.01[a]
N-free extract (%)	34.71±1.27[a]	33.75±1.23[a]
Gross energy (Kcal/Kg)	3646.49±16.07[a]	4282.49±34.01[b]
Sodium (%)	ND	1.03±0.20
Potassium (%)	0.78±0.21[a]	1.47±0.17[b]
Calcium (%)	0.90±0.27[a]	0.92±0.06[a]
Magnesium (%)	0.41±0.03[a]	0.27±0.02[a]
Zinc (mg/Kg)	0.233±0.046[a]	0.083±0.021[b]
Cupper (mg/Kg)	0.009±0.001[a]	0.003±0.000[a]
Manganese (mg/Kg)	0.083±0.003[a]	0.052 ±0.004[a]
Nickel (mg/Kg)	0.005±0.000[a]	0.008±0.001[a]

ND: Not detected

Means marked with different letters in the same row significantly different

Table 3. The content of photosynthetic pigments in *S. platensis*. Data are the mean of three replicates ±SD.

Pigments	mg/g
Chl. a	0.87±0.08
Carotenoids	0.50±0.05
PC	8.01±0.12
APC	3.91±0.09
PE	2.23±0.05

Table 4. Fatty acid composition (% total fatty acids) of fishmeal and *S. platensis*.

Chemical name	Fishmeal	*S. platensis*
Saturates SFAs		
Myristic acid (C14:0)	1.07[a]	0.12[b]
Palmitic acid (C16:0)	19.95[a]	45.95[b]
Stearic acid (C18:0)	9.75[a]	2.14[b]
Arachidic acid (C20:0)	-	0.15
∑ SFAs	30.77[a]	48.36[b]
Mononsaturates MUFAs		
Palmitoleic acid (C16:1n7)	1.29[a]	6.93[b]
Vaccenic acid (C18:1n7)	22.79[a]	2.10[b]
Oleic acid (C18:1n9)	3.19[a]	4.25[a]
Gondoic acid (C20:1n9)	0.69[a]	1.00[a]
∑ MUFAs	27.96[a]	14.28[b]
Polyunsaturates PUFAs		
Linoleic acid (LA) (C18:2n6)	13.37[a]	15.28[a]
γ-Linolenic acid (GLA) (C18:3n6)	2.16[a]	1.66[a]
α-Linolenic acid (ALA) (C18:3n3)	1.08[a]	1.82[a]
Eicosatrienoic acid (ETE) (C20:3n3)	1.23[a]	2.59[b]
Arachidonic acid (AA) (C20:4n6)	2.89[a]	1.54[b]
Eicosapentaenoic acid (EPA) (C20:5n3)	1.01[a]	4.46[b]
Eicosadienoic acid (EDA) (C20:2n6)	1.13[a]	1.21[a]
Heneicosapentaenoic acid (HPA) (C21:5n3)	-	1.18
Docosatetraenoic acid (DTA) (C22:4n6)	-	1.36
Docosahexaenoic acid (DHA) (C22:6n-3)	3.64[a]	4.69[a]
∑ PUFAs	26.51[a]	34.25[b]
∑ UFAs	54.47[a]	48.53[b]
UFAs/SFAs	1.77[a]	1.00[a]
n6/n3	2.81[a]	1.32[b]
AA/EPA	2.86[a]	0.35[b]
AA/DHA	0.79[a]	0.33[a]

Means marked with different letters in the same row significantly different

Table 5. DPPH radical scavenging activity % of fishmeal and *S. platensis* at different concentrations. Data are the mean of three replicates ±SD.

Concentration (mg/ml)	DPPH radical scavenging activity (%)	
	Fishmeal	*S. platensis*
5	2.44±0.06[a]	3.91±0.05[a]
10	3.66±0.13[a]	5.31±0.05[a]
20	5.37±0.06[a]	7.09±0.04[b]
30	5.86±0.04[a]	9.53±0.06[b]
40	6.60±0.29[a]	12.95±0.09[b]

Means marked with different letters in the same row significantly different

Table 6. Serum lysozyme activity of tilapia fed different *S. platensis* diets after 90 days of growth. Data are the mean of three replicates ±SD.

Treatment	Lysozyme activity (Units/ml)
Fishmeal	0.83±0.019[a]
5%	1.13±0.042[a]
20%	1.33±0.054[b]
35%	1.56±0.071[b]
50%	1.79±0.173[b]

Means marked with different letters in the same row significantly different

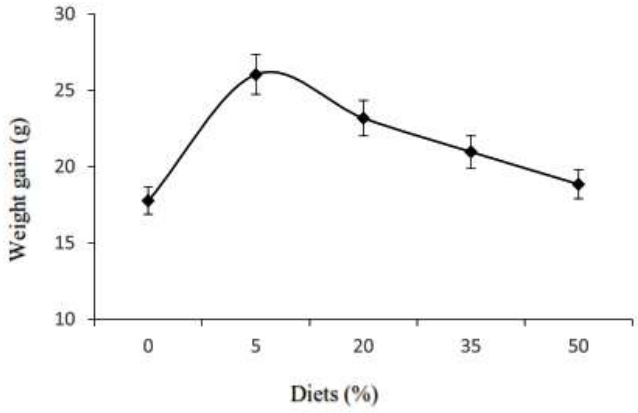

Fig. 1: Weight gain of Nile tilapia fed different *Spirulina platensis* diets.

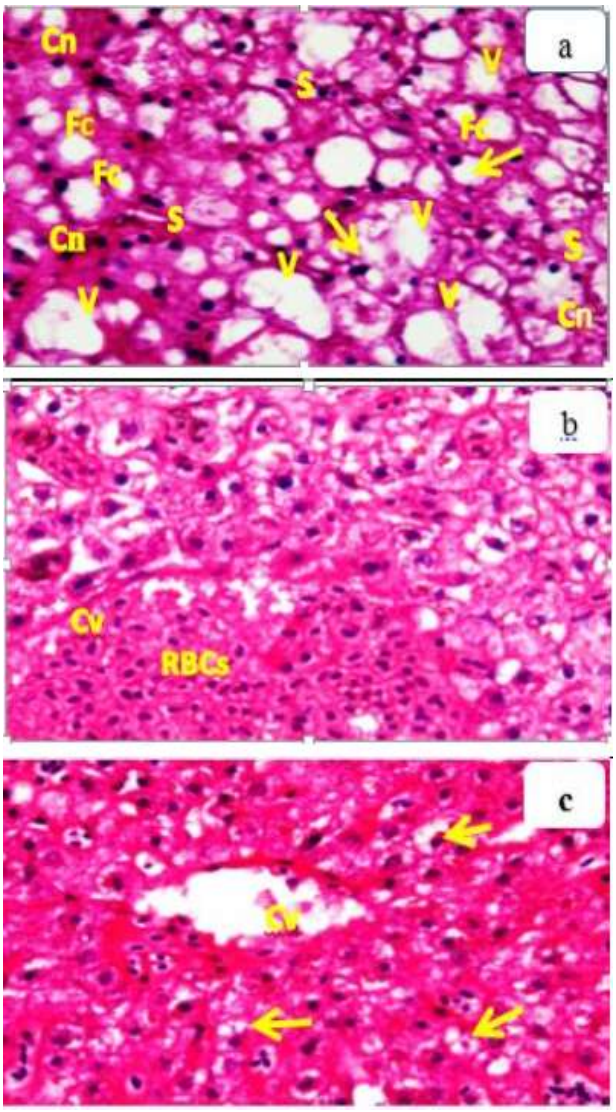

Fig. 2a-c: Histoarchitecture of the liver tissue of Nile tilapia fed fishmeal and *S. platensis* diet under light microscope (x400, H&E). **a:** Tilapia fed fishmeal showed severe cytoplasmic vacuolations (V) with pyknotic nuclei (arrows), and collapse of blood sinusoids (S), mild fatty changes (Fc), and cellular necrosis (Cn). **b:** Tilapia fed 5% *S. platensis* demonstrated marked stasis and disintegrate (arrow) of red blood cells (RBCs) in central veins (Cv). **c:** Tilapia fed 50% *S. platensis* stated nearly normal hepatocytes with some hepatocytes vacuolated (arrows) around central vein area (Cv) and hepatic pancreatic tissue (Hp) with portal vein congestion.

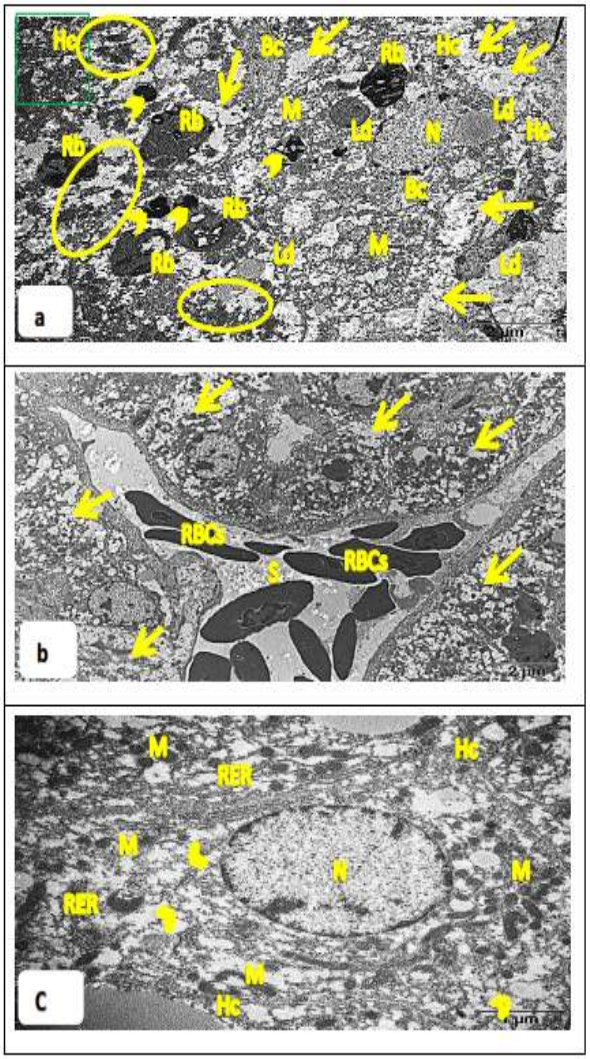

Fig. 3a-c: Electro-microscopic examination (---2µm) of the liver tissue of Nile tilapia fed fishmeal and *S. platensis* diet. **a:** Tilapia fed fishmeal showed cellular damage, lipid droplets (Ld), karyolitic nucleus (N), cytoplasmic organelles lysis (arrows), autophagic vacuoles (arrowheads), residual bodies (Rb), damaged bile canaliculei (Bc), hypertrophied mitochondria (M) with low electron dense matrix and destructed cristae, and atrophied mitochondria with electron dense matrix (Circles), (X18000). **b:** Tilapia fed 5% *S. platensis* appeared dilated of blood sinusoids (S) filling with deformed (RBCs), necrotic and degenerated hepatocytes (arrows), (X3400). **c:** Tilapia fed 50% *S. platensis* exhibited nearly normal hepatocytes (Hc) structure with focal vacuolations (heads arrow), nearly normal mitochondria (M), normal nucleus (N), small and large lipid droplets (arrows), and rough endoplasmic reticulum (RER), (X14000).

4. Discussion

The blue-green algae, *S. platensis* is an ideal bio-resource due to the presence of rich protein, carbohydrates, phycocyanin, chlorophyll a, carotenoids, essential amino acids, polysaccharides, minerals, vitamins, phenolics and provide an excellent source of lipids (Bensehaila *et al*., 2015; Choi and Lee, 2018). Carotenoids are absorbed mainly in the median and terminal part of the fish intestine (Choubert *et al*., 1991) and then transported by lipoproteins (Ando *et al*., 1985). The phycocyanin is the dominant pigment in *Spirulina* and has high anti-arthritic, anti-inflammatory, neuroprotective and hepatoprotective effects, which are closely connected with its antioxidative activity (Cabanero *et al*., 2016). They also found that total phenolic, phycocyanin, carotenoids and chlorophyll-derived present in *Spirulina* might explain their high antioxidant activity. Vitamin C in algae is considered as a promoter for lipid metabolism and may result in an alternative of body composition and nutrient deposition in fish (Ji *et al*., 2003) and surrounding tissues from oxidative damage and protect phagocytic cells (Secombes *et al*., 1988). Minerals play important functions in the living body, including the cell transport and metabolic processes serving as catalytic enzymes cofactors (Misurcova *et al*., 2011).

Regarding fatty acids in S. platensis, the most abundant SFA was palmitic acid as obtained by Gupta *et al*. (2008). The algae have been recognized as sources of polyunsaturated fatty acids ω-3 or ω-6 fatty acids (Suleria *et al*., 2015). Feeding with the PUFA ω-3 together with a low dietary n-6/n-3 ratio are essential to a diet and well-known to be beneficial to health (Ruxton *et al*., 2005). The recommended maximum dietary ratio of n6/n3 PUFAs is 4.0 and the lowest value of the PUFAs/SFAs ratio is 0.45 (Harman, 2001). In this study, these ratios were found to be within the recommended values for both fishmeal and alga.

The present results indicated that the adding of *S. platensis* could significantly increase the growth performance of tilapia and the optimal adding of *Spirulina* was 5%. Güroy *et al*. (2011) reported that the diets supplemented with microalgae have been found to have positive effects on growth performance, feed utilization, lipid metabolism, stress tolerance and disease resistance of fish. The increase in the body weight of fish is proportional to the levels of protein in the diets up to 40% (Teshima *et al*., 1978). The algae species are rich in pigments, protein, biological co-factor and vitamins that might enhance the growth of fish (Kobayashi and Kobayashi, 2001). Yone *et al*. (1986) reported that the algae has the ability to increase the absorption and assimilation of dietary protein. This can clarify the better feed effectiveness in fish fed alga-incorporated diets.

The lysozyme activity is found in a wide range of vertebrates including fish and is one of the defensive factors against invasion by microorganisms (Ibrahem *et al*., 2013). The lysozyme is an important index of the innate immunity of fish (Paulsen *et al*., 2003). According to the present results, feeding tilapia with *S. platensis* diets increased the activity of serum lysozyme and this is benefit to the immune system in fish. In agreement with our results, Promya and Chitmant (2011) found that fingerlings fed *Arthrospira platensis* diets had a higher value of immunity stimulating capacity. Enhancement of phagocytic activity and lysozyme activity have been stated in some shrimps and fish after treatment with carotenoid enriched diets against pathogens (Harikrishnan *et al*., 2011). Lysozymes have digestive tasks (Dobson *et al*., 1984). Several studies indicate that lysozyme may play a role in fish as a defense mechanism against infectious disease (Lindsay, 1986). The increase in the immunity stimulating capacity could be due to the presence of C-phycocyanin in the *Spirulina* alga, which can help build the immunity capacity (Vonshak, 1997). In addition, *Spirulina* contains carotenoids which improve the health of fish and increase the ability to fight off infections (Nakono *et al*., 2003).

Nutritional and physiological status of many fishes has been studied by using liver as an indicator. The fishmeal used in our study had severe effects on the liver of fish. The present result may be returned to the presence of high level of soybean in the fishmeal. In accordance with the present result, Kokou *et al.* (2015) showed growth inhibition and alterations in the morphology of the body organs of several fish species fed soybean meal (SBM). Taddese *et al.* (2014) reported that fish diet may have negative effects on the liver structure, and have no bad effect on the growth. Our results are also consistent with Zhu *et al*. (2016); they found that fed diet containing soybean caused significant endoplasmic reticulum and mitochondrial damage, ultimately resulting in lipids droplets accumulation within hepatocytes. The liver of fish fed soybean meal showed hyperemic central vein, intra-cytoplasmic round circumscribed fat vacuoles, sinusoids with focal extravasations of red cells surrounding the central veins and focal fibrosis (Ismaiel *et al*., 2015). Also, the reduction in the nuclear size of fish may be a mark of malnourishment. A size of a nucleus reveals changes in the liver metabolism of fish fed soybean, such as picnosis, kariolysis of the nucleus or necrosis of the cell (Raskovic *et al*., 2011). They clarified that; these results may be due to the lack of lysine and methionine in soybean meal which cause the

accumulation of triglycerides in the liver. The changes in the liver may be related to some soybean anti-nutritional factors, which were not removed or inactivated by the bioprocessing applied (Kokou *et al*., 2015). The hepatocyte vacuolization in fish may be due to the storage of lipid and glycogen (Rueda-Jassoa, 2004).

In the present study, the less harmful effect of 5% *S. platensis* diet on the liver of tilapia may return to the presence of alga in the fishmeal. However, an improvement was observed in the tissue of tilapia fed 50% *S. platensis* diet due to the presence of high level of *S. platensis* in the diet. Our results were supported by the finding of Jaravata *et al.* (2004) who showed that the incorporation of 50% *S. platensis* in the diet counteracted the disturbed lipid metabolism in tilapia caused by feeding fishmeal. *Spirulina* is a good source of animal feed and its cellular structure is easily digestible and does not contain cellulose (Holman and Malau-Aduli, 2012). Ou *et al*. (2010) reported that phycocyanin averts malondialdehyde formation in the liver, kidney, and pancreas, decrease total cholesterol level and triglycerides level in liver, promote liver glycogen synthesis. The omega-3 fatty acid has an important role in the lowering of cellular inflammation. The eicosanoids are resulting from an arachidonic acid (AA, omega-6 fatty acid) and mediators of cellular inflammation (Liu *et al*., 2016). In addition, the most important omega-3 fatty acids EPA is known to decrease inflammation of cells. The EPA is known to inhibit the delta-5-desaturase (D5D) enzyme that produces AA and the more EPA, the less AA produces. An imbalanced ratio of AA/EPA appears to be damaging (Tocher, 2003). The lowest ratio for both AA/EPA and AA/DHA in S. platensis may explain the good appearance of liver tissues in tilapia fed high concentration of *S. platensis*.

Conclusion

The results from this study provide important information regarding the potential application of the tested alga as a valuable alternative feed ingredient source for Nile tilapia, *Oreochromis niloticus* culture reducing the consumption of fishmeal. The supplementing of tested alga in fish feed has useful approaches to further increase in the nutritional value of fishmeal by improvement growth and liver tissue of tilapia.

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