POTENTIAL APPLICATION OF THE BLUE-GREEN ALGA (*SPIRULINA PLATENSIS*) AS A SUPPLEMENT IN THE DIET OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract. Nowadays the need to find alternate feedstuff constituents able to supply similar nutritional value at low cost is becoming increasingly evident. To assess the inclusion of *Spirulina platensis* biomass in the diet of Nile tilapia, *Oreochromis niloticus*, an experiment in a random arrangement design was established with five treatments (0, 5%, 20%, 35%, and 50% *S. platensis* diets). The experiment lasted for 90 days. The chemical constituents of diets were estimated. The growth performance, biochemical constituents, and histological study of the kidney of tilapia were investigated at the end of experiments. The results showed that the different levels of *S. platensis* diets enhanced the growth performance and body constituents of tilapia. The maximum increments were observed in tilapia fed by 5% *S. platensis* diet compared to algae-free control diet. The tissues of tilapia had better profiles of fatty acid (high n-3, low n-6/n-3) with 5% *S. platensis* diet than those of the control. The diet of *S. platensis* induced an improvement in the histoarchitecture of the kidney in tilapia. Meanwhile, the control group exhibited some alterations in the cellular architecture of the kidney. As clear from the present results, supplementing 5% *S. platensis* in the diet has been found to increase the nutritional value of diet, and improve growth and tissue characteristic of tilapia.
Keywords: *microalgae, fish, growth performance, body composition, histology of kidney*

Introduction

Aquaculture is the fastest-growing food production system globally, with an increase in the production of fisheries crops. The growth in aquaculture has been based on an availability of fishmeal or any other protein source. Aquaculture diets need to increase average daily gain. Fishmeal is considered to be the main bases of protein, minerals, vitamins, and unsaturated fatty acids in the aquatic feedstuffs (Naylor et al., 2009). As a result of the decline in fishery resources and the increase in the price of fishmeal, there is awareness to find alternatives to this limited component. Quality of aquafeed is one of the most important criteria for the success of aquaculture.

The use of algae as an additive feed in aquaculture has received a lot of attention due to the positive effect on weight gain (Ophilia and Ramanujam, 2017). The popularity of microalgae as fish feed is increasing rapidly as the suitable alternative source in modern aquaculture industry (Becker, 2007). Various species of microalgae have been assessed

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for their nutritional value and incorporated into fish feed formulation for higher fish production (Sen Roy et al, 2015; Sirakov et al., 2015).

Microalgae have low fiber and high lipid content (Suganya et al., 2016). Recently, microalgae were effectively used in the diets of fish to improve the growth and fatty acid profile in tilapia (Sarker et al., 2016). Among the microalgae, *Spirulina platensis* is a filamentous blue-green alga and has an attention in aquaculture feeds in current years (Guroy et al., 2012). *Spirulina* becomes one of the most commonly used microalgae in aquafeeds as it contains high quantities of pigments such as carotenoids, proteins, essential amino acids, vitamins, essential fatty acids, and minerals (Habib et al., 2008).

Nile tilapia (*Oreochromis niloticus*) are cichlid fishes that were presented into many regions of the world in the second half of the 20th century. They are among the greatest significant farmed fishes in the world and are the second cultivated fish worldwide (El-Sayed, 2006). Tilapia is a good food special for users because it has a high content of proteins and minerals and a low-fat fish (Stoneham et al., 2018). There is a prospect for manufacturers to develop the value of nutrients in tilapia.

The present study planned to estimate the optimum level of inclusion of *Spirulina platensis* in the fish diet and its effect on the growth performance, biochemical constituents as well as the kidney histoarchitectures of Nile tilapia (*Oreochromis niloticus*).

Materials and methods

Spirulina platensis

The *Spirulina platensis* (Blue-green algae) was attained from algal Lab, Faculty of Science, University of Jeddah, Saudi Arabia. It was sub-cultured in Zarrouk's medium (1966). The algal cells were thoroughly washed with fresh water followed by distilled water and then dried at 60°C. The dry algal biomass was fine-milled with a laboratory blender, passed through a 0.6 mm diameter hole-sieve, and then kept in Stoppard bottles at room temperature until use in the diet.

Experimental diet

In the present study, a commercial diet (control) was obtained from Maram Feed Mill, Buraidah, Al Qassim, Saudi Arabia. The ingredients of the control diet (g/100 g) were soybean (60), barley (12), fish oil (6.3), corn grains (20), amino acids (0.5), vitamins (0.2) and minerals (1.0) (*Table 1*).

Table 1. *Ingredient composition of control diet*

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

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The total protein of the control diet according to our analysis was 44.19%. Diets were formulated to replace 5, 20, 35 and 50% in the commercial diet by *Spirulina platensis.* The mixture was homogenized in a food mixer (Braun, Germany). Boiling water was then blended into the mixture at the ratio of 50% for pelleting. The diets were pelleted using meat grinder (Braun, Germany) with 1.5 mm diameter and then dried at 60°C. The diets were stored in bags at room temperature (Promya and Saetun, 2005).

Experimental fish

Experiments were carried out in the aquarium at the Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia. Nile tilapia, *Oreochromis niloticus* was obtained from the fish farm with an average initial body weight of 1.3±0.06 g. Fish were adapted in the aquaria for two weeks before the beginning of the feeding experiments. Throughout the experimental period, the quality of water in the culture was checked daily for temperature, pH, oxygen, nitrite, nitrate, phosphate, total alkalinity and salinity in all aquariums.

Experimental design

The present experimental study was carried out at the experimental station of Faculty of Marine Science, King Abdulaziz University, Jeddah, Saudi Arabia. Circular plastic fish aquariums were filled with 500 liters of the Red Sea water. Each aquarium was supplied with a continuous system of filtration and aeration (Reef Octopus Classic 202-S Space Save Protein Skimmer, Florida, United States). Before the beginning of the experiment, the water was stored for 24 hours. The fish were distributed into the aquaria in all treatments 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 every day at 8:30 AM, and 4:30 PM at a weekly feeding rate of 5% of body weight. Every two weeks, six fish were sampled from each group for weighing (g). Fish were starved for 24 hours before weighing to allow the gut to be empty.

At the end of the experiment, fish groups were subjected to air exposure test for 5 minutes and then returned back to the water to test the stress response. The survival rate for each group was estimated as follows:

$$\text{Survival rate (\%)} = (\text{Surviving fish number} / \text{Initial number of fish}) \times 100 \quad \text{(Eq.1)}$$

Physico-chemical analysis of water in the aquarium

During the experimental period, the quality of water was monitored daily in the culture systems for temperature (°C, H198311Temperature Tester, Hanna Instruments), pH (pH Meter HANNA HI98128), dissolved oxygen (mg/l, Dissolved Oxygen Meter YSI 58), and salinity (‰, Conductivity/Salinity Meter ATAGO 1976). However, nitrite (ppm), nitrate (ppm), phosphate (ppm), and total alkalinity (ppm) were estimated by using Hanna's Chemical Test Kits, Michigan, United State.

Chemical analysis

The chemical constituents of diets and tilapia body were analyzed as follows:

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Estimation of total soluble protein

After the removal of pigment, cells were extracted as described by Payne and Stewart (1988) to measure the total soluble protein as % dry weight.

Estimation of total soluble carbohydrate

The total carbohydrate was quantitatively estimated by the method of phenol-sulphuric acid according to Kochert (1973). The absorbance was measured at 490 nm by using Spectrophotometer (Shimadzu, UV-1800, USA). Glucose was used as the standard. The total soluble carbohydrate was estimated as % dry weight.

Lipid extraction

The chloroform-methanol mixture (1:2, v/v) was used for extraction of lipid following the method of Thimmaiah (2006). The layer of chloroform was dried by evaporation, weighed and the total lipid was estimated.

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

$$\text{Gross energy} = (\text{Protein}\times 5.65) + (\text{Carbohydrates}\times 4.0) + (\text{Fat}\times 9.45) \ \text{Kcal/Kg} \quad \text{(Eq.2)}$$

Estimation of total phenolic

Total phenolic estimation was carried out with Folin-Ciocalteu reagent (FCR) to Mallick and Singh (1980). The total phenolic was expressed as a dry weight percentage.

Estimation of total carotenoids in fish

The fish meat was dried for 12 hours at 60°C and then pounded into fine powder. The carotenoids of the dry tissue were extracted for 30 minutes in acetone (85%) as described by Saleh et al. (2011). The absorbance of pigment was read at 450 nm and the concentration of carotenoids was estimated as μg/ml by application of the following equation and the result was expressed as mg/g dry weight.

$$\text{Carotenoids} = (\text{OD}\times V\times \text{DF}) / (2500\times 100) \ \mu\text{g/ml} \quad \text{(Eq.3)}$$

where OD is the optical density at 450 nm, V is the volume of extract, DF is the dilution factor, and 2500 is the average extinction coefficient of carotenoids.

Determination of fatty acid

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used for the estimation of the fatty acid of algae and fish tissue. The Gas Chromatograph System of 6890 HP with Mass Selective Detector of 5973 HP at the Chemical Lab. FARP, Faculty of Agriculture, Cairo University, Egypt was used.

Growth parameters

The effect of different algae diet on tilapia performance was estimated according to the following equations as described by Promya and Chitmanat (2011):

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$$\text{Weight gain} = \text{Final weight} - \text{Initial weight} \quad \text{g/fish} \qquad \text{(Eq.4)}$$

$$\text{Average daily gain} = (\text{Final weight} - \text{Initial weight}) / \text{days} \quad \text{g/fish/day} \qquad \text{(Eq.5)}$$

$$\text{Relative gain rate} = (\text{Weight gain} / \text{Initial weight}) \times 100 \qquad \text{(Eq.6)}$$

$$\text{Specific growth rate} = [(\ln \text{Final weight} - \ln \text{Initial weight}) / \text{days}] \times 100 \qquad \text{(Eq.7)}$$

$$\text{Feed conversion rate} = \text{Weight of feed} / \text{Weight gain} \quad \text{g/fish} \qquad \text{(Eq.8)}$$

$$\text{Feed efficiency} = (\text{Weight gain} / \text{Feed intake in dry mass}) \times 100 \qquad \text{(Eq.9)}$$

Histology of kidney

Small slices of kidney were taken and fixed directly in 10% neutral buffered formalin at the end of the experiment. The usual histological techniques were used for proceeding the fixed specimens as described by 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 an Olympus BX51 System Microscope. Ultrathin sections were made for kidney tissue according to Woods and Stirling (2002) and examined by Electron Microscope (CM 100, Philips, Holland).

Statistical analysis

Results were expressed as means ±SD (Standard Deviation). Data were statistically analyzed by using analysis of variance to detect differences between individuals. All statistical analysis was achieved by using SPSS program version 16 (SPSS Inc. Chicago, USA).

Result

The diets compositions

The results in *Figure 1* investigated the chemical composition of *S. platensis* diet at different levels (0, 5, 20, 35 and 50%). The percentage of total soluble protein of different diets was ranged between 44.14% to 44.19% dry weight. The carbohydrate contents showed variations according to the concentration of algae replaced diet. The high values of carbohydrates (16.03% and 16.14% dry weight) were found in 35% and 50% *S. platensis* replaced diet, respectively. The maximum lipid content (8.74% dry weight) was observed in 50% *S. platensis* diet meanwhile, the minimum value was recorded by control diet (5.5% dry weight). Among the studied *S. platensis* diets, the level of 50% was found to have the highest phenolic content (3.02% dry weight). The least phenolic content was observed with the control diet (2.12% dry weight).

Water quality parameters of the aquarium

The water quality parameters of the aquarium were represented in *Table 2*. The results showed that the temperature was 28±0.1ºC, pH was 7.8±0.4, dissolved oxygen

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was 4.5±0.9 mg/l, phosphate was 0.25±0.06 ppm, total alkalinity was 152±5.3 ppm and salinity was 39‰. The levels of total ammonia, nitrate, and nitrite were 0.0 ppm.

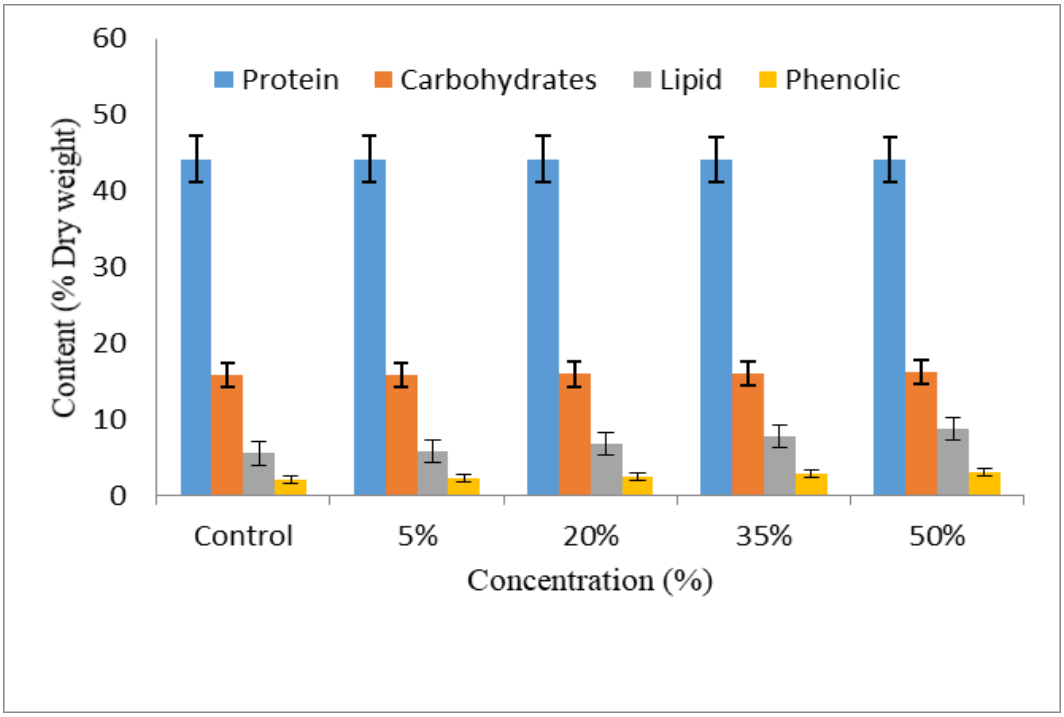

Figure 1. *Chemical composition of different concentrations of S. platensis diets. Each value is presented as mean ±SD (n = 3)*

Table 2. *Water quality parameters of the aquarium*

Parameters	Value
Temp. (°C)	28±0.1
pH	7.8±0.4
Dissolved oxygen (mg/l)	4.5±0.9
PO_4 (ppm)	0.25±0.06
Total alkalinity (ppm)	152±5.3
Salinity (‰)	39.0
NO_2 (ppm)	0.0
NO_3 (ppm)	0.0

Growth performance

The growth performance of tilapia fed different levels of *S. platensis* diets rearing 90 days were illustrated in *Table 3*. The replacing of the fish diet by *S. platensis* improved the growth performance of tilapia including weight gain, average daily gain, specific growth rate, and feed efficiency as compared with control. The growth performance showed significant differences at the level of 5% *S. platensis* diet for all variables. The maximum relative gain rate (20.1%) and specific growth rate (3.06%) were observed in tilapia fed 5% *Spirulina* diet compared with control (13.6% and 2.71%, respectively).

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The Feed conversion ratio was decreased in all treatments of *S. platensis* (0.78-0.80 g/fish) as compared with control (0.81 g/fish). Compared with the feed efficiency of control (124%), tilapia fed 5% *Spirulina* diet showed the highest value (126.7%). The survival rate of tilapia showed no significant differences among treatments, which was above 90% in all groups.

Table 3. *Growth performance of Nile tilapia fed different concentrations of S. platensis diets after 90 days of growth. Each value is presented as mean ±SD (n = 3)*

Growth parameters	Control	5%	20%	35%	50%
Initial body weight (g)	1.3±0.06	1.3±0.06	1.3±0.06	1.3±0.06	1.3±0.06
Final body weight (g)	19.1±1.58^{a}	27.4±1.83^{b}	24.5±1.78^{b}	22.3±1.68^{a}	20.2±1.43^{a}
Weight gain (g)	17.8±0.81^{a}	26.1±0.93^{b}	23.2±0.91^{b}	21.0±0.85^{b}	18.9±0.73^{a}
Average daily gain (g/fish/day)	0.15±0.02^{a}	0.22±0.01^{b}	0.20±0.03^{b}	0.18±0.02^{b}	0.16±0.01^{a}
Relative gain rate (%)	13.6±1.23^{a}	20.1±1.19^{b}	17.8±1.52^{c}	16.1±1.41^{c}	14.5±1.26^{a}
Specific growth rate (%)	2.71±0.42^{a}	3.06±0.18^{b}	2.94±0.67^{a}	2.83±0.55^{a}	2.77±0.63^{a}
Feed conversion ratio (g/fish)	0.81±0.19^{a}	0.78±0.11^{b}	0.79±0.13^{b}	0.80±0.17^{a}	0.80±0.19^{a}
Feed efficiency (%)	124.0±1.71^{a}	126.7±1.83^{a}	125.8±1.82^{a}	124.9±1.62^{a}	124.4±1.50^{a}
Survival rate (%)	92±1.02^{a}	100±0.00^{b}	100±0.00^{b}	93±1.11^{a}	93±1.01^{a}

Means marked with different letters in the same row significantly different

Body composition of fish

The results in *Figure 2* illustrated the biochemical constituents of tilapia fed *S. platensis* diets. The higher contents of protein (23.95% and 23.11%), carbohydrates (19.85% and 19.07%), and lipid (4.76% and 4.57%) were detected in the muscle of tilapia fed the diets with 5% and 20% *S. platensis*, respectively as compared with control (16.28%, 14.75% and 2.99%, respectively). In the muscle of tilapia fed the different levels of *Spirulina* diets, phenolic (ranged between 2.33% and 2.53%) and carotenoid contents (ranged between 4.3% and 4.7%) were significantly higher than control levels (0.67% and 2.11%, respectively).

The fatty acid composition of fish

According to the result of growth performance, the diet of 5% *S. platensis* was found to induce the maximum growth of tilapia. Consequently, tilapia fed 5% *S. platensis* was chosen for studying the fatty acid composition. The results in *Table 4* showed a lower level of saturated fatty acids (SFAs, 35.05%) in tilapia fed 5% *S. platensis* as compared with control (45.86%). The highest value of monounsaturated fatty acids (MUFAs) was observed with oleic acid (C18:1 n-9) in both tilapia fed *Spirulina* diet (23.96%) and control (28.09%).

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The concentration of polyunsaturated fatty acids (PUFAs) of tilapia fed 5% *S. platensis* (34.15%) was found to be higher than control (23.59%). The results showed differences in the PUFAs, Alpha-Linolenic acid (ALA: 2.31%), Docosahexaenoic acid (DHA: 8.39%), ∑ n-3 (14%), and n-3/n-6 (0.69%), of tilapia muscle fed 5% *S. platensis* as compared with control group (0.41%, 1.13%, 2.55%, 0.12%, respectively).

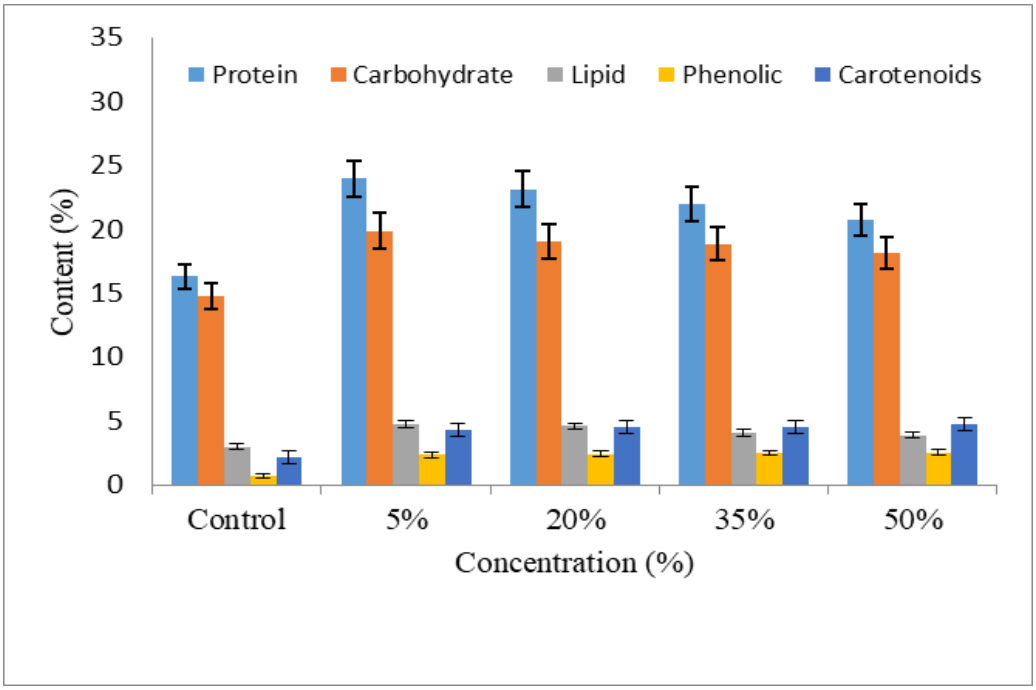

Figure 2. *Body composition of Nile tilapia fed different concentrations of S. platensis diets after 90 days of growth. Each value is presented as mean ±SD (n = 3)*

Histological study of kidney

Depend on the maximum and the minimum growth, the kidney of tilapia fed 5% and 50% *S. platensis* diet, respectively was chosen for the present study. *Figure 3 (a-c)* showed the histoarchitecture of the kidney tissue in tilapia under light microscope, The results clarified severe disorganization of renal tissue fibrosis, vacuolation of proximal tubule (I and II), which led to hyaline degeneration, marked tubular disintegration with necrosis, decreased of hematopoietic tissue, glomerular shrinkage with disintegration and the widened the Bowman's space, was observed in tilapia fed control diet (*Figure 3a*). In tilapia fed 5% *Spirulina* diet, a limited damage (vacuolation, necrosis, and congestion) was obtained almost in the proximal tubule (II) (*Figure 3b*). A slight glomeruli congestion, tubular necrosis, leukocytes infiltration and mild heteropoietic tissue degeneration have been demonstrated in tilapia fed 50% *Spirulina* diet (*Figure 3c*).

The results in *Figure 4 (a-c)* showed the electron micrographs of the kidney tissue in tilapia. The proximal tubules in the kidney of tilapia fed control diet showed disturbed brush border brush border of proximal tubule II, and tubular lumen filled with cellular debris, deformed desmosomes (*Figure 4a*). However, an irregular and destructed membrane, basal enfolding, swollen mitochondria with disoriented cristae, vesiculated rough endoplasmic reticulum, and Golgi bodies atrophy were obtained in

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tilapia fed 5% *S. platensis* (*Figure 4b*). The kidney tissue of tilapia fed 50% *S. platensis* exhibited nearly normal structures with mild interstitial tissue degeneration (*Figure 4c*).

Table 4. *Fatty acid composition (% dry body weight) of Nile tilapia fed 5% S. platensis diet after 90 days of growth*

Chemical name	Lipid name	Control	5% *S. platensis*
Saturates SFAs			
Myristic acid	C14:0	1.49[a]	1.31[a]
Palmitic acid	C16:0	10.19[a]	1.62[b]
Margaric acid	C17:0	5.23[a]	19.03[b]
Stearic acid	C18:0	19.29[a]	0.87[b]
Nonadecylic acid	C19:0	2.64[a]	10.09[b]
Arachidic acid	C20:0	4.89[a]	0.97[b]
Heneicosylic acid	C21:0	2.13[a]	1.16[b]
∑ SFAs		45.86[a]	35.05[b]
Mononsaturates MUFAs			
Palmitoleic acid	C16:1 n-7	0.71[a]	0.99[b]
Vaccenic acid	C18:1 n-7	0.42[a]	5.35[b]
Oleic acid	C18:1 n-9	28.09[a]	23.96[b]
Eicosenoic acid	C20:1 n-9	0.11[a]	0.26[b]
∑MUFAs		29.33[a]	30.56[b]
Polyunsaturates PUFAs			
Linoleic acid	C18:2 n-6	5.13[a]	14.20[b]
Alpha-Linolenic acid (ALA)	C18:3 n-3	0.41[a]	2.31[b]
Gamma-Linolenic acid (GLA)	C18:3 n-6	0.12[a]	0.88[b]
Eicosadienoic acid	C20:2 n-6	15.27[a]	2.36[b]
Eicosatrienoic acid	C20:3 n-3	0.32[a]	1.39[b]
Arachidonic acid (AA)	C20:4 n-6	0.36[a]	1.42[b]
Eicosapentaenoic acid (EPA)	C20:5 n-3	0.47[a]	0.89[b]
Heneicosapentaenoic acid (HPA)	C21:5 n-3	0.22[a]	1.02[b]
Docosatetraenoic acid (DSA)	C22:4 n-6	0.16[a]	1.29[b]
Docosahexaenoic acid (DHA)	C22:6 n-3	1.13[a]	8.39[b]
∑ PUFAs		23.59[a]	34.15[b]
∑ UFAs		52.92[a]	64.71[b]
Total Fats		98.78[a]	99.76[a]
UFAs/SFAs		1.15[a]	1.85[a]
∑ n-3		2.55[a]	14.00[b]
∑ n-6		21.04[a]	20.15[a]
n-3/n-6		0.12[a]	0.69[b]

Means marked with different letters in the same row significantly different

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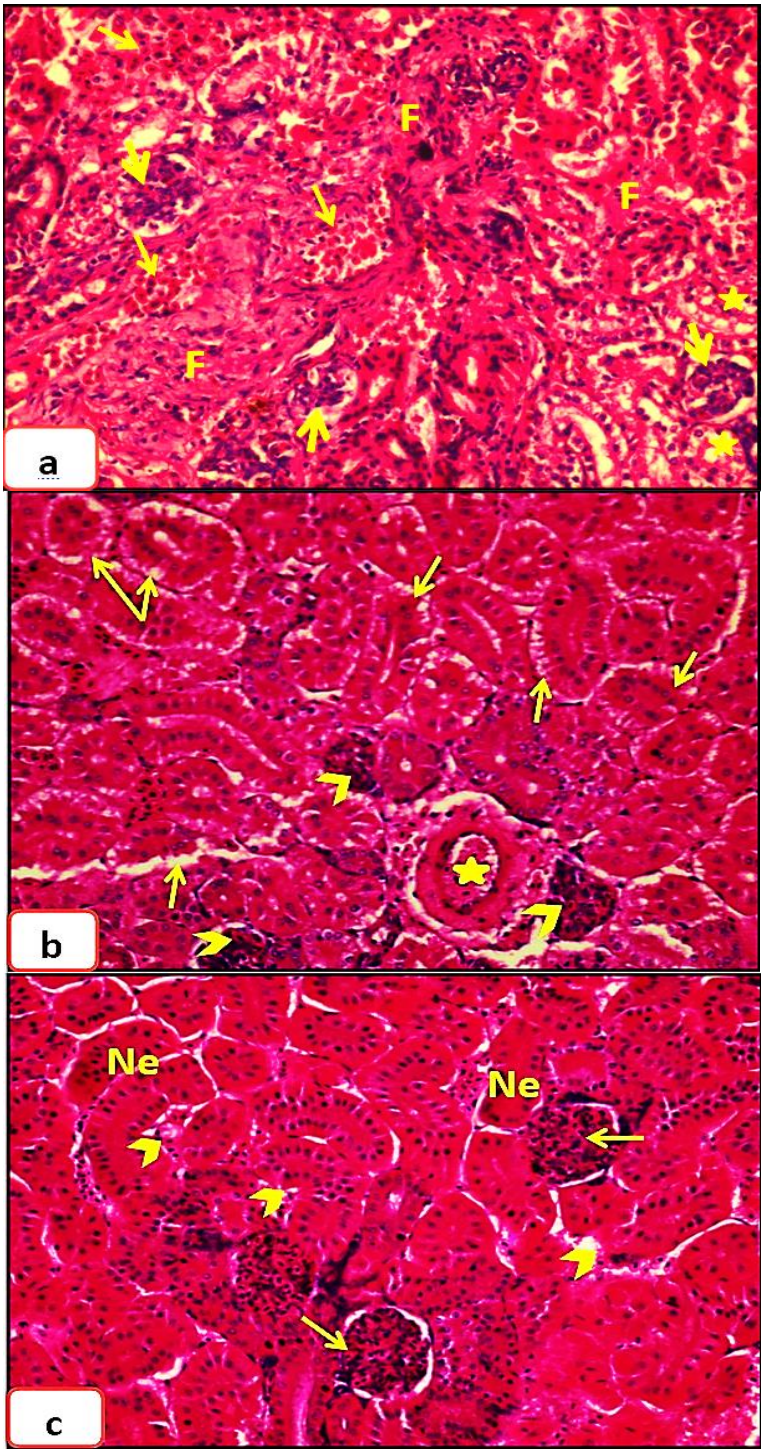

Figure 3(a-c). *Histoarchitecture of the kidney tissue of Nile tilapia fed control, and 5% and 50% S. platensis diets under light microscope (x400, H&E);* ***a:*** *Tilapia fed control diet showed severe disorganization of renal tissue with fibrosis (F), proximal tubule I cells vacuolations (*), glomerular cells lysis (thick arrows), and hematopoietic tissue increased (thin arrows);* ***b:*** *Tilapia fed 5% S. platensis diet demonstrated epithelial vacuolations of almost the proximal tubule II (arrows), glomerular enlargement (arrowheads) and blood vessel damaged (*);* ***c:*** *Tilapia fed 50% S. platensis diet stated slight glomeruli congestion and tubular necrosis (Ne) and mild hematopoietic tissue degeneration (arrowheads), and leukocytes infiltration (arrows)*

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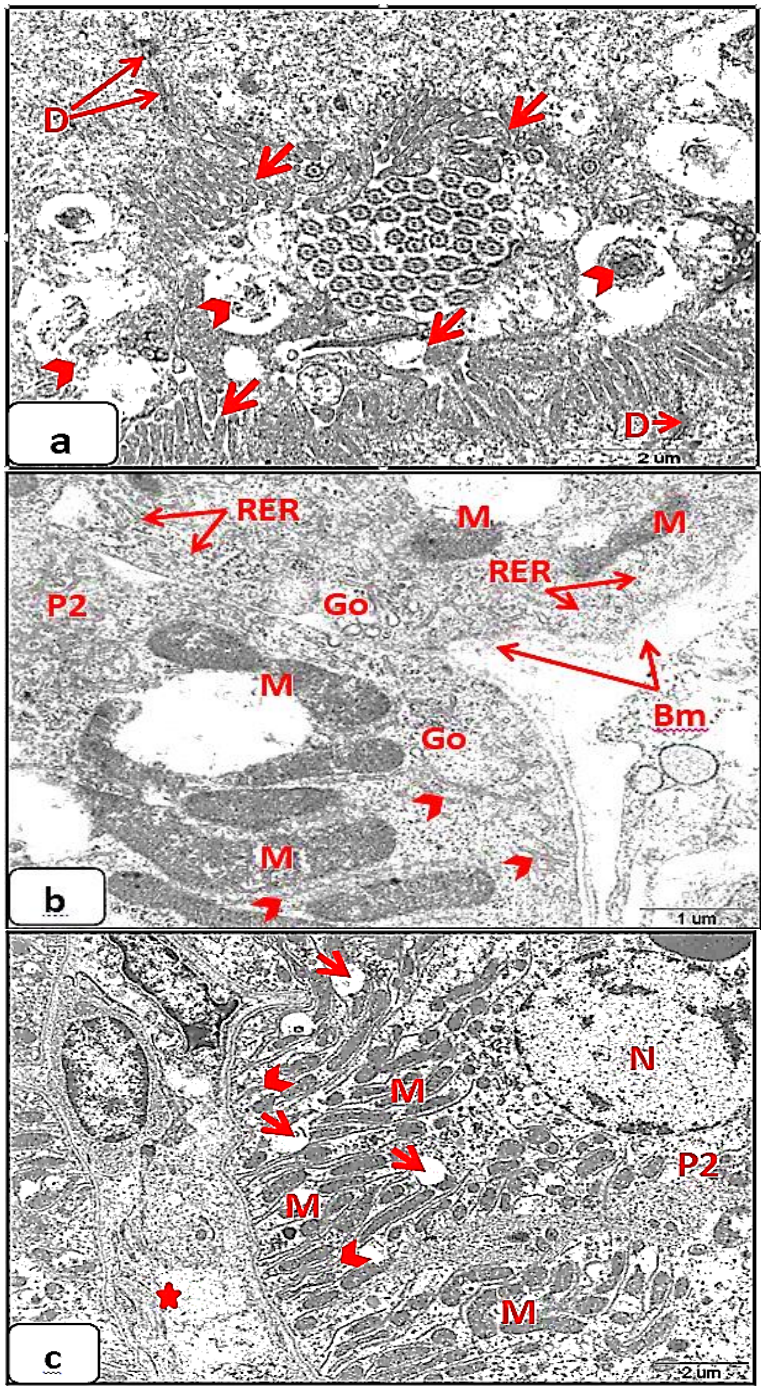

Figure 4(a-c). *Electromicroscopic examination of epithelial cell of a proximal tubule in the kidney tissue of Nile tilapia fed control, and 5% and 50% S. platensis diets;* ***a*** *(2 µm): Tilapia fed control diet showed short and deteriorated brush border (arrows) and tubular lumen filled with cellular debris (arrowheads), deformed desmosomes (D), (10500X);* ***b*** *(1 µm): Tilapia fed 5% S. platensis diet showed irregular and destructed basement membrane (Bm), short and damage basal enfolding (arrowheads), swollen mitochondria (M) with dense electron density and disoriented cristae, shorted and vesiculated RER, and Golgi apparatus atrophy (Go), (13500X);* ***c*** *(2 µm): Tilapia fed 50% S. platensis diet exhibited normal basal enfolding (arrowheads), nucleus (N) with intact nuclear envelope, normal elongated mitochondria (M). Note also few empty vacuoles (arrows), and mild interstitial tissue degeneration (*), (5800X)*

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Discussion

The commercial diet is an important dietary protein source for fish (Muzinic et al., 2006). Fish nutritionists continue to search for substituted plant protein sources, to replace fish diet partially or wholly in aquafeeds. Algae are a good candidate to replace wholly or partially the fish diet in aquafeeds (Rama et al., 2014). Algae are beneficial due to their nutritive value (protein, carbohydrate, fats, and minerals) and immune-stimulatory effect (Promya and Chitmanat, 2011).

The *Spirulina* powder has a high quality of protein content (60%) which is greater than other sources such as soybeans and peanuts (FAO, 2008). *S. platensis* has essential amino acids, fatty acids, polysaccharides, vitamins, mineral substances, beta carotene, and other pigments (Blinkova et al., 2001). *Spirulina* is characterized by the absence of cellulose in its cell walls, so it has a special value for fish (Sasson, 1997).

The microalgae have biologically active compounds such as phenolic (Abd El Baky et al., 2008). Phenolic compounds have many biological activities such as anti-carcinogenic, anti-inflammatory, and anti-atherosclerotic activities (Chung et al., 1998). According to Abd El-Baky et al. (2009), the phenolic content of *S. platensis* was recorded as 1.29% of dry weight. However, the total phenolic contents of *Spirulina* sp. was 15.4 mg/g dry algae matter according to Miranda et al. (1998) and 0.95 mg/g as found by Pumas and Pumas (2014).

The success of aquaculture to optimize fish health and growth rates depends on the ability to keep the aquatic environment and water quality parameters. These critical parameters are dissolved oxygen, pH, temperature, ammonia, nitrite, alkalinity, and CO_2 (Ebeling, 2016). The water quality parameters in the present study were normal during the experimental period. These results showed that the experimental diets have no harmful effect on the water where the experimental tilapia had been stocked. In accordance with the present results, Bindu and Sobha (2005) showed that the parameters of the water quality in the experimental cisterns were within the optimum range for growth of fish fed algae diets.

The present study indicated that the growth performance of tilapia was influenced significantly by different levels of *Spirulina* in the diet. Nakagawa and Gomez-Diaz (1975) used 5-20% *Spirulina* as a feed for the giant freshwater prawn, and found an improvement in the growth, survival, and feed utilization of prawn. The lower value of the feed conversion ratio of tilapia was obtained by 5% *Spirulina* diet, while the higher value was represented by the control. Our data are in agreement with Khalafalla and El-Hais (2015) who found that the feed conversion ratio for *Oreochromis niloticus* using 5% algae dietary supplementation showed lower values than control. The growth of tilapia is significantly affected by the source of protein in the diet (Maina et al., 2002). Kim et al. (2012) found that the presence of 30% protein in the fish diet caused more weight gain and lower feed conversion ratio. Teshima et al. (1978) showed that the diets containing 35-40% protein increased the body weight gain and gave a high feed efficiency for *Tilapia zillii.* Patnaik et al. (2014) reported that the composition of feed has a direct effect on the growth of fish and the deficiency of protein in the fish feed leads to the inhibition of growth.

In agreement with our results, Nakagawa et al. (1993) showed that the supplementation of the green algae at the level of 2.5–5.0% diet caused an increase in the feed efficiency of black sea bream. Ergun et al. (2008) reported that tilapia fed 5% green algae meal showed an increase in growth performance as compared with the control diet. It has been recorded that algae can induce the absorption and assimilation

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of dietary protein (Yone et al., 1986). This can explain the better feed efficiency in fish fed alga-incorporated diets.

Several studies showed that the presence of small amount of algae in aquafeeds induced growth performance, fish quality, stress response, the efficiency of feed utilization, intestinal microbiota, disease resistance, and physiological activity (Valente et al., 2006; Ergun et al., 2008; Batista et al., 2016). Similarly, some previous works showed that low level of algae in fish diet attained a beneficial effect on growth performance. Hashim and Mat Saat (1992) found that the growth rate, feed efficiency and feed consumption of Snakehead fry increased with the incorporation of 5% *Ulva* sp. in the diet. On the other hand, the high inclusion of algae reduces fish growth and feed efficiency as reported by Oliveira et al. (2009). They concluded that the growth of fish in the presence of algae appeared to be dependent on species and dose.

Spirulina-containing feed was found to improve growth performance and reduce the survival rate. Our findings are similar to that of El-Tawil (2010) who showed that the survival rate of tilapia fed algae was ranged between 93 and 100% among treatments with 5-25% alga diet. In addition, Khalafalla and El-Hais (2015) reported that fish fed diet supplemented with 5% green algae had 100% survival rate. Zhu et al. (2016) found that the survival rate for juvenile white-spotted snapper using the 5% and 10% algae diets was higher than 15% and 20% groups. The nutritional value of algae additions is commonly assessed in terms of growth and survival (Nakagawa et al., 1985). The algae species are rich in many contents which enhance the growth and survival of fish such as carotenoid pigments, vitamins, and biological co-factors (Kobayashi and Kobayashi, 2001). The low palatability of the algae may cause a reduction in the survival rates of fish (Mustafa and Nakagawa, 1995).

In the present study, soybean was the protein source in the control diet. The growth of tilapia fed the control diet was the lowest as compared with tilapia fed *Spirulina* diet. The lowest growth of tilapia in response to control diet may be returned to the effect of soybean. Although soybean protein has been widely used in aquafeeds, alterations in the morphology and growth inhibition have been detected in fish (Sahlmann et al., 2013). Those changes have been qualified to the antinutritional factors of soybean (Fuentes-Appelgren et al., 2014). In comparison with soybean, algae have a significant benefit as a source of protein or fishmeal replacements because they contain low levels of anti-nutrient factors (ANF) such as tannins, phytic acid, and trypsin inhibitor (Kokou et al., 2015).

In the present study, the proximate analysis of protein, lipid, carbohydrates, phenolic and carotenoid contents of tilapia muscle was influenced by dietary algae level. The different levels of *Spirulina* diets (5-50%) induced these contents in tilapia muscle. In agreement with our results, Rama et al. (2014) found that *Ulva reticulata* improved the growth parameters and coloration in goldfish. Also, the present results are in agreement with the finding of Yildirim et al. (2009) who reported that the protein content of *Oncorhynchus mykiss* increased by feeding with *Enteromorpha linza*. Abdel-Warith et al. (2015) showed that increasing the amounts of *Ulva* diet (30%) induced a slight increase in protein muscle of African catfish *Clarias gariepinus.* In disagreement, Soler-Vila et al. (2009) found that 15% *Porphyra* in the diet increase protein deposition in the muscle of rainbow trout and the diet level-up to 10% would not have an effect. However, Menghe et al. (2009) suggested that the body composition or feed utilization of channel catfish feeding did not affect with 2% algae diet. The body lipid in tilapia was found to be enhanced with the feeding of algae. It was reported that the body lipid

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concentration of fish is positively related to the level of dietary lipid and carbohydrates (Mustafa et al., 1995).

Mustafa and Nakagawa (1995) showed that the supplementation of algae in a small amount in the diet caused an enhancement in the growth, the digestive efficiency of feed, and significant enhancement in the physiological condition of the carcass. Differences among results of suitable levels of algae may be varied depending on its incorporation level, the habits of feeding, the species and age of fish and algae (El-Tawil, 2010). The body composition of fish is primarily influenced by diet composition, feeding practices, fish size, and can be controlled through nutrition (Cabanero et al., 2016). The metabolism of carbohydrate, fats, and protein in fish can be enhanced due to the presence of large amounts of natural chlorophyll, β-carotene, B-group vitamin, vitamin E, iron, and potassium in *Spirulina* contains, (Islam, 2016).

Pigmentation is an important quality characteristic of the fish for consumer acceptability. There is a big request for the presence of natural carotenoids in aquafeed to attain bright coloration in fish (Gupta et al., 2007). The results of Rama et al. (2014) are in conformity with our results, they showed that the carotenoids content of fish fed algae diet were increased and the carotene content between treatments differed significantly. James et al. (2006) reported that the supplementation of dietary feed induced an increase in the carotenoid content of the fin, skin, and muscle of *Xiphophorus helleri*. Similarly, Guroy et al. (2012) confirmed an enrichment in yellow cichlid fed the diet containing *Spirulina*. Carotenoids are known to have a confident role in the metabolism of fish by increasing the nutrient utilization, growth and survival rate (Bindu and Sobha, 2006). Soler-Vila et al. (2009) reported that the bioactive pigments found in algae clearly displays an important role in the formation of polyunsaturated fatty acid.

The diet of 5% *Spirulina* altered tilapia fatty acid composition, this may be returned to the high levels of SFAs and UFAs present in algae diet as reported by Trushenski et al. (2013). García-Ortega et al. (2016) found similar results with giant *Epinephelus lanceolatus* fed diets containing algae species. The fish fed *Spirulina* supplements containing more Gamma-linolenic acid (GLA), linoleic acid, eicosatrienoic acid, docosapentaenoic acid and eicosatetraenoic acid showed significant differences in the fatty acid profile (Vonshak, 1997). In particular, *Spirulina* is rich in GLA which is an essential fatty acid rarely available in ingredients or diet (Tanticharoen et al., 1994). The GLA and other unsaturated fatty acids are essential for the growth of cell as structural elements of cell membranes.

Microalgae was positively used in fish diets to increase the fatty acid profile and the production characteristics of tilapia (Sarker et al., 2016). *Spirulina* is rich in α-linolenic acid (18:3 n-3) and has been recognized to increase the concentration of α -linolenic acid in tilapia (Stoneham et al., 2018). Teoh et al. (2011) reported that tilapia are restricted in their capability to elongate and desaturate the fatty acids C18:3 n-3 and C18:3 n-6 into polyunsaturated fatty acids of longer chain (C20:4 n-6, C20:5 n-3, C22:5 n-3, C22:6 n-3). Therefore, one or both of these fatty acids must be supplied in the diet, depending on the essential fatty acid requirements. The algae are rich sources of polyunsaturated fatty acids, n-3 or n-6 (Suleria et al., 2015).

The results showed a negative effect on the kidney of tilapia fed the commercial control diet which may be returned to the presence of soybean in the diet. Soybean contains many kinds of anti-nutritional factors affects the nutritional value, utilization, and digestibility of soybean protein (Herkelman et al., 1992), causing digestive and

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metabolic diseases of animals (Sun et al., 2005). Protease inhibitors are found in soybean and capable to discourage the activity of the enzyme that has the ability to proteolysis. Amino compounds resulting from breaking protein in control diet may be turned into ammonia, which the liver converting to urea if the rate of urea increased in the blood that led to an acute tubular necrosis causing sudden deterioration in the kidney function (Schrier et al., 2004). However, the positive effect of *S. platensis* diet on the kidney tissue of tilapia may be returned to the presence of different contents in alga responsible for the improvement of the inflammation. Spirulina is characterized by a high proportion of phycocyanin, minerals, vitamins, and antioxidant activity (Estrada et al., 2001). The dominance of these contents lead to powerful anti-inflammatory, anti-arthritic, neuroprotective, hepatoprotective effects and its therapeutic efficiency has been verified against various conditions (Rimbau et al., 1999). Ou et al. (2010) showed that phycocyanin has the ability to increase the expression of essential enzymes and the chemicals related to the balanced function of the kidney which leads to the detoxification. The phycocyanin keeps the antioxidative ability, prevent malondialdehyde formation in the pancreas, kidney, and liver (Ou et al., 2012). The phycocyanin is thought to protect against renal kidney failure (Iijima et al., 1982). The presence of these active metabolites in *Spirulina* play important roles in maintenance tilapia healthy.

Conclusion

The present study illuminated that high growth performance, better biochemical contents and promise beneficial n-3 content in Nile tilapia, *Oreochromis niloticus* fillets were detected in group feed 5% *S. platensis* diet. It can be confirmed the usefulness of algae to partially replace fishmeal in diets for tilapia and can be applied to improve the commercial production of tilapia at dietary levels up to 5 % with no adversative effects on growth performance. In the future, we need to improve processing methods, minimize antinutritional factors, improve the palatability and flavor, and develop new products that will be suitable for fish consumption.

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