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RESEARCH PAPER

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Improvement of Growth and Viability of *Oreochromis niloticus* in a Biofloc System Using *Chlorella vulgaris*

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Abstract

This study aimed to enhance Nile Tilapia growth using *Chlorella vulgaris* as a food additive in the biofloc system. Different concentrations of *C. vulgaris* were tested in four different treatments compared to control. The growth rate of Nile tilapia was parallel with *C. vulgaris* addition to the treatments. The best productive value (growth performance) for Nile Tilapia was recorded in T1 that was distinctly superior to the other treatments. The use of *C. vulgaris* in the biofloc system decreased feed conversion ratio (FCR) values; whereas the most significant value was observed at T1. Phytoplankton structure in Nile Tilapia gut was predominated with *C. vulgaris* representing 67.7% of the total phytoplankton crop. Statistical analysis also approved that the most important factor affecting Nile Tilapia growth was *C. vulgaris* addition, and some other chemical variables that affect phytoplankton's growth such as PO_4. In addition, muscle protein ratio of Nile Tilapia increased with increasing *C. vulgaris* concentrations. Our data concluded that increasing *C. vulgaris* concentration improved the growth performance of Nile Tilapia under the biofloc condition.

Introduction

One of the most important fish species worldwide is *Oreochromis niloticus* (Nile tilapia) with about 4.2 million tons, representing 8% of fish aquaculture. World production of aquaculture outside aquatic plants reached 80 million tons in 2016, with inland fish aquaculture production representing 59.3% (FAO, 2018).

Aquaculture is also of great significance because its production accounts for approximately 60% of the total aquatic protein used for human consumption. Still, as a rapidly growing market, it puts immense pressure on the aquaculture industry to find healthy and cost-effective ingredients in fish foods (Salin et al., 2018; Goda et al., 2020).

Biofloc technology (BFT) is an eco-friendly technique, contains whole essential nutrients, plays a vital role in water quality control and nutrient cycling in the culturing cell, in addition to improving water quality by converting ammonia to nitrate, reducing required dietary protein, reducing feed conversion ratio (FCR) and feed costs and improving fish health by competition with pathogens (Liñán-Cabello et al., 2002; Ju et al., 2008a; Ballester et al., 2010; Ray et al., 2011; Emerenciano et al., 2012a and b; Nimrat et al., 2013; Avnimelech, 2015; Emerenciano et al., 2017).

Microalgae is one of the most significant biotic factors shaping the thriving culture of Nile tilapia in semi-intensive ponds (Mbonde et al., 2017); hence phytoplankton is a source of natural feed for fish farming in the pond (Arifin et al., 2018). Phytoplankton can be used in fish feed to enhance fillet quality by deposition of n-3 polyunsaturated fatty acids and is considered a substitute for fish meal and fish oil in aquatic feeds (Sarker et al., 2016).

One of the most frequently exploited phytoplankton species owing to its high protein content (about 51%–58% of its dry weight), is *C. vulgaris*. It contains essential amino acids (Becker, 2007), along with many other beneficial substances (Rodriguez-Garcia & Guil-Guerrero, 2008), and can be used as a good protein source for African catfish and a substitute for fishmeal in catfish diets (Enyidi, 2017).

Therefore, using algae as an unconventional feed ingredient and feed additives in replacement of high-cost feed materials such as fishmeal has been potentially increased (Badwy et al., 2008). Some microalgae in fish feeding experiments resulted in increased growth, physiological activity, and disease resistance (Roy & Pal, 2015).

However, using *C. vulgaris* in the biofloc system still needs further study. Therefore, this study was designed to determine the efficacy of *C. vulgaris* in improving the growth and viability of Nile tilapia in the biofloc system and the sensitivity of fish growth to other factors.

Material and Methods

Experimental Conditions

This study was conducted in Fish Nutrition wet Lab, at fish research station, National Institute of Oceanography and Fisheries (NIOF), El- Kanater El khayria, Egypt, in plastic tanks (125L capacity filled to 100L with water). The experiment was designed in triplicates, with four different treatments besides the control. The source of water was originally from a freshwater well. The tanks were operated with zero water exchange; however, water was added as needed to replace evaporated losses. The tanks were aerated by aquarium air pumps to maintain the proper oxygen level.

Experimental Fish

Nile Tilapia (*Oreochromis niloticus*), mono sex, was purchased from a commercial hatchery in Fayom Governorate (Egypt). Fish were acclimated to the experimental conditions for two weeks before the feeding trial. Fish initial body weights (IBW) were ranged from 6.05 to 6.68g. Fish were randomly assigned to 15 tanks (20 fish each). The treatments were designed in triplicate replication. Fish were weighted and their length was measured every two weeks through the experimental period (75 days). Fish were not fed at the weight day.

Preparation of the Experimental Alga

C. vulgaris was cultured in a Hydrobiology Lab in National Institute of Oceanography and Fisheries (NIOF), El- Kanater El khayria, Egypt. BG11 culture medium was used to grow *C. vulgaris* (Ilavarasi et al., 2011). Inoculation of *C. vulgaris* (10^3 cells/ ml) was done, through different interval times, in several 1500 mL flasks filled to 500 mL medium and under controlled conditions supported by a continuous air pump for aeration, temperature 24°C±2°C, pH 7.3 and light intensity ~ 2000 lux. (Measured at water surface) and a fluorescent lamp for 24-hour lighting.

Diet and Feeding Protocol

One practical diet was formulated as isonitrogenous (30% crude protein) and isocaloric (20 kj/g diet) for all treatments. Four different treatments (namely; T1, T2, T3, and T4) comprising four different volumes (80, 40, 20, and 10 ml, respectively) of *C. vulgaris* culture (8 days incubation, 5×10^5 cell/ml) as a food additive were added simultaneously with the practical diet during the entire fish culture period. Fish were fed *ad libitum* twice daily at 9:00 am and 3:00 pm, five days a week. The control group received only the practical diet with the same feeding protocol.

Starch was used as a source of organic carbon in the biofloc system and added in a liquid form one time daily for five days a week. The total daily amount of carbon source was calculated according to (Hargreaves, 2013; Pérez-Fuentes et al., 2016).

Water Quality

Water quality variables were measured every week during the experimental period (75 days). Dissolved oxygen and temperature were monitored using a dissolved oxygen meter (Professional Plus, USA). pH was measured in the water column of the tanks by pH meter (HI 8314 model). The settleable solids were measured after 20 minutes by Imhoff cones (after filling the cone) (Avnimelech, 2009). Chemical

variables (NH_4, NO_2, NO_3, PO_4, and Total Alkalinity) were estimated according to the procedures laid down in APHA (2017).

Proximate Composition

Diets, fish carcass, and biofloc samples were analyzed for dry matter (DM), ash content, crude protein (N x 6.25) by the Kjeldahl method using a Kjeltech auto-analyzer according to AOAC (2012). Crude fat was measured according to Bligh & Dyer (1959).

Phytoplankton Identification and Enumeration

Drop method was applied for counting and identifying phytoplankton species (APHA, 2017), triplicate samples (5µl) were taken and examined under inverted microscope ZEISS IM 4738, with magnification power 20 and 40x. The results of phytoplankton density were presented as the number of cells per liter (cell/l). Phytoplankton identification was performed according to Popovsky & Pfiester, 1990; Krammer & Lange-Bertalot, 1991; Edmondson, 1992; Verlencar & Desai,

2004; Lee, 2008; Bellinger & Sigee, 2010 and 2015; Munshi et al., 2010.

Statistical Analysis

At the end of the experiment, data collected were subjected to one-way analysis of variance (ANOVA) using statistical software (SPSS 18) to detect significant differences in all parameters. Duncan's new multiple range tests (Duncan, 1955) was used to detect individual differences between treatment means. All data were represented as means ± standard deviation (SD), and a rejection level of ($P>0.05$) would be used for all statistical analysis.

Pearson's correlation was performed to assess the relationship between the increased growth rate of Nile tilapia with surrounding physical, chemical, and biological factors. These relations were also examined with a normalized principal component analysis (PCA).

Results

Distribution and abundance of phytoplankton in the biofloc system and Nile tilapia gut after *C. vulgaris* addition

Different concentrations of *C. vulgaris* were used and compared in this experiment. The results indicated that increasing *C. vulgaris* concentration enhanced the growth of phytoplankton in the biofloc system, and consequently, in tilapia's gut contents compared to control.

Through the experiment period, the maximum phytoplankton standing crop was observed at T1, that received the highest concentration of *C. vulgaris*, reaching about 46 $\times 10^6$cell/l and 173 $\times 10^6$cell/gut in both biofloc system and tilapia gut, respectively. Their population density was gradually declined with the other treatments T2, T3, and T4. The least density of phytoplankton in both biofloc system and tilapia gut were reported with control treatment, recording 6×10^6cell/l and 40 $\times 10^6$cell/gut, respectively.

Phytoplankton composition, which grown in the biofloc system, was dominated with Chlorophyta, forming about 73.8% of the total phytoplankton density. While Bacillariophyta represented the second group concerning density, representing approximately 14.1%. Charophyta, Euglenophyta, and Cyanobacteria were presented with low densities, reaching about 7.3%, 3.0%, and 1.8%, respectively.

Studying the composition of phytoplankton in fish gut contents and the biofloc system revealed that it was more or less the same. It was predominated by Chlorophyta, forming about 80.6% of the total phytoplankton density found in fish gut, followed by Bacillariophyta with about 12.2% and Charophyta representing about 7.2%.

The average density of *C. vulgaris* in tilapia gut and the biofloc system at the four different treatments during the experimental period is shown in Figure 1.

Phytoplankton abundance in the biofloc treatments and in tilapia gut during the present study revealed that the dominant species was *C. vulgaris* as shown in Tables 1 and 2. The average percentages of *C. vulgaris* present in tilapia gut during the different treatments along the experimental period are shown in Figure 2.

Growth performance, feed efficiency, and survival rate of Nile tilapia

Growth performance increased with the increase of *C. vulgaris* addition in the biofloc system (Table 3). The best productive values for Nile tilapia were recorded in T1 and T2 treatments, which were distinctly superior to the other groups. The control group significantly recorded the lowest final average body weight of 28.47g ($P\leq 0.05$) among all dietary groups.

Fish fed with T3 treatment (Table 3) reported substantially higher feed intake compared to other treatments (44.36), while control and T4 treatment (34.84 and 37.45, respectively) ($P\leq 0.05$) recorded the lowest values compared to other treatments. Use of algae in the biofloc system decreased preferably the feed conversion ratio (FCR) values, whereas the most significant FCR (feed convertion ratio) values were observed for T1 and T2 treatments (1.28 and 1.47, respectively). In contrast, the insignificant values ($P\geq 0.05$) were recorded for T3 (1.76) and T4 (1.65) treatments. Fish fed with T1 and T2 treatments recorded the highest significant PER (protein efficiency ratio) values (2.59 and 2.25, respectively). Otherwise, T3 had the lowest PER values (1.89 %) (Table 3).

Concerning protein results for the present study, T1 and T2 treatments showed higher protein ratios than other treatments, while T2 and T3 treatments showed higher whole-body lipid levels than other treatments (Table 4).

Physico-chemical variables and biological correlations

The correlation matrix (Table 5) cleared that PO_4 ($r=0.73$, $P<0.05$) was a limiting growth factor for phytoplankton growth, especially *C. vulgaris* in a biofloc system.

Statistical analysis showed a significant positive correlation ($r=0.62$, $P<0.05$) between Nile Tilapia growth rate and *C. vulgaris* density in the gut (Table 5).

PCA Figure 3 cleared that Nile Tilapia growth rate is highly coordinate positively with PO_4, NO_3, and alkalinity ($r=0.90$, 0.80, and 0.79, respectively, $P<0.05$), and negatively with pH ($r= -0.85$) and DO ($r= -0.45$).

Discussion

It was observed that using *C. vulgaris* as feed additive in the biofloc system led to the dominance of Chlorophyta (73.8% of the total phytoplankton density). Hence in the gut of tilapia, forming about 80.6% of the total phytoplankton density in the gut. Our result showed that *C. vulgaris* represented a high average of 67.7% from tilapia's gut content along the experimental

period. Ahmed et al. (2019) mentioned that phytoplankton communities in a biofloc system for Nile tilapia cultivation were dominated by Chlorophyceae. The current study showed that using *C. vulgaris* as a feed additive led to the increasing growth performance of Nile tilapia; this result agrees with Mahmoud et al. (2020). The best productive values (growth performance) for Nile tilapia were recorded in T1 that was distinctly superior to the other treatments. This could be due to the addition of the highest amount of *C. vulgaris*. *Chlorella* could be used as a good additive and could promote the growth performance and physiological parameters of gibel carp (*Carassius auratus gibelio*) (Zhang et al., 2014)

Statistical analysis indicated that the most important factor affecting Nile Tilapia growth was *C. vulgaris* addition, and some chemical variables that affect the growth of phytoplankton, especially *C. vulgaris* such as PO_4 as mentioned by Ahmed et al. (2019).

As the results revealed, algae in the biofloc system reduced the feed conversion ratio (FCR) values significantly, whereas the most significant FCR values were observed at T1 and T2 treatments (1.28 and 1.47, respectively). These results were in agreement with the results of Emerenciano et al. (2017), who revealed that algae in the biofloc system play a vital role in reducing feed conversion ratio (FCR); this may be due to the high digestibility of *C. vulgaris*, resulting in stimulation of fish intestinal flora and subsequently increasing the activity of digestive enzymes and efficient diet use (Khani et al., 2017). Furthermore, fish that received the highest *C. vulgaris* concentration in T1 and T2 treatments recorded the most significant PER values (2.59 and 2.25, respectively). Giving that protein in *C. vulgaris* can reach up to 60%, our obtained results indicated that it could be potentially used as a fish feed additive. Xu et al. (2014) showed that *Chlorella* could be a good choice as a fish feed due to the best crude protein level, a significant concentration of polysaccharides, lipid,

Table 1. List of phytoplankton species recorded in a biofloc system and their abundance during the experiment period.

Species	Biofloc system treatments				
	Control	T1	T2	T3	T4
Phylum: Chlorophyta					
Actinastrum hantzschii	-	-	+	+	+
Ankistrodesmus falcatus	+	+++	++	++	+
Chlorella vulgaris	+	++++	++++	+++	++
Coelastrum microporum	+	+	-	-	-
Crucigenia tetrapedia	+	++	+	++	+
Eudorina elegans	-	+	-	-	-
Kirchneriella lunaris	+	++	++	+	+
Monactinus simplex	+	++	+	-	-
Monoraphidium convolutum	+	+++	++	+	+
Nephrocytium limneticum	-	++	+	+	-
Oocystis borgi	+	+	-	-	-
Scenedesmus ecornis	-	+	-	+	+
Schroederia jadayi	-	+	-	+	+
Tetradesmus incrassatulus	-	++	+	-	-
Tetraëdron minimum	+	++	-	+	-
Tetraselmis suecica	+	++	+	-	++
Phylum: Bacillariophyta					
Amphora ovalis	-	+	-	-	+
Aulacoseira granulate	+	-	-	+	+
Cocconeis placentula	-	+	-	-	+
Cyclotella meneghiniana	+	-	+	+	-
Cymbella lanceolata	-	-	-	+	+
Gyrosigma attenuatum	+	-	-	-	+
Lyrella lyra	-	++	+	-	-
Navicula radiosa	-	-	+	-	+
Nitzschia linearis	-	++	+	-	+
Nitzschia sigma	+	+	+	-	-
Pantocsekiella ocellata	+	-	++	+	-
Pinnularia major	+	+	+	-	-
Phylum: Charophyta					
Closterium sp.	+	++	+	+	+
Cosmarium abbreviatum	+	+	-	-	-
Elakatothrix gelatinosa	+	++	-	+	-
Staurastrum anatinum	-	-	+	+	-
Phylum: Euglenophyta					
Phacus acuminatus	+	+	+	-	+
Phylum: Cyanobacteria					
Merismopedia elegans	+	++	+	+	+
Total	21	26	21	18	19

Note: abscent (-), present (+), moderate abundance (++), high abundance (+++), very high abundance (++++)

Table 2. List of phytoplankton species recorded in Nile Tilapia gut and their abundance during the experiment period.

Species	Tilapia gut treatments				
	Control	T1	T2	T3	T4
Phylum: Chlorophyta					
Actinastrum hantzschii	-	-	+	-	+
Ankistrodesmus falcatus	+	+	+	+	+
Chlorella vulgaris	+	++++	+++	++	++
Crucigenia tetrapedia	+	+	+	+	+
Kirchneriella lunaris	+	++	+	-	-
Monactinus simplex	+	+	+	-	-
Monoraphidium convolutum	+	+	-	+	+
Oocystis borgi	+	-	-	-	-
Scenedesmus ecornis	-	+	-	-	+
Tetradesmus incrassatulus	-	+	-	-	-
Tetraëdron minimum	+	+	-	-	-
Tetraselmis suecica	+	-	+	-	-
Phylum: Bacillariophyta					
Amphora ovalis	-	+	-	-	+
Aulacoseira granulate	+	-	-	+	-
Cyclotella meneghiniana	-	-	-	+	-
Cymbella lanceolata	-	-	-	+	+
Lyrella lyra	-	-	+	-	-
Navicula radiosa	-	-	+	-	+
Nitzschia linearis	-	+	+	-	-
Pantocsekiella ocellata	-	-	+	-	-
Pinnularia major	+	+	+	-	-
Phylum: Charophyta					
Closterium sp.	+	+	+	+	-
Elakatothrix gelatinosa	+	+	-	+	-
Staurastrum anatinum	-	-	+	+	-
Total	13	14	14	10	9

Note: abscent (-), present (+), moderate abundance (++), high abundance (+++), very high abundance (++++)

Table 3. Growth performance and feed efficiency of Nile tilapia through 75-days under biofloc condition.

Parameters	Treatments				
	Control	T1	T2	T3	T4
Final body weight (g)	28.47 ± 1.25^{c}	37.01 ± 1.69^{a}	33.82 ± 1.07^{ab}	31.38 ± 1.41^{bc}	29.33 ± 1.33^{bc}
Gain (g)	22.42 ± 1.26^{b}	30.60 ± 1.32^{a}	27.07 ± 1.03^{bc}	25.15 ± 0.74^{b}	22.65 ± 0.82^{b}
SGR (%/day)	2.24 ± 0.15^{ab}	2.59 ± 0.03^{a}	2.38 ± 0.01^{ab}	2.33 ± 0.07^{ab}	2.23 ± 0.11^{b}
Feed intake (g)	34.84 ± 0.48^{c}	39.33 ± 1.83^{a}	39.98 ± 1.23^{ab}	44.35 ± 0.46^{a}	37.45 ± 1.04^{bc}
FCR	1.55 ± 0.94^{abc}	1.28 ± 0.18^{c}	1.47 ± 0.07^{bc}	1.76 ± 0.08^{a}	1.65 ± 0.12^{a}
PER (%)	2.14 ± 0.11^{abc}	2.59 ± 0.13^{a}	2.25 ± 0.14^{ab}	1.89 ± 0.09^{c}	2.01 ± 0.04^{bc}

Note: Each value represents mean ± SD (n = 3). Values within the same row with different superscript letters are significantly different (P<0.05). Specific growth rate (SGR), feed conversion ratio (FCR) Protein efficiency ratio (PER), were calculated as follows: SGR (%/day) = 100 (ln W2 - ln W1). TW2: The final weight of fish in g. W1: is the initial weight of fish in g. ln: is the natural log. T: is the time in days. FCR = Feed intake (g)/ Weight gain (g). PER = Weight gain, g / Protein intake, g.

Table 4. Whole-body composition (% on dry matter basis Dm) of Nile tilapia at the start and end of the experiment.

Treatments	Moisture%	Protein %	Lipid (Ether Extract) %	Ash%
Control	82.84 ± 0.46^{a}	56.78 ± 1.79^{b}	11.22 ± 0.91^{d}	26.08 ± 0.18^{a}
T1	81.35 ± 0.68^{ab}	63.09 ± 1.22^{a}	17.06 ± 1.31^{b}	18.96 ± 2.61^{b}
T2	80.04 ± 0.12^{bc}	62.18 ± 0.83^{a}	18.71 ± 0.50^{ab}	18.06 ± 0.57^{b}
T3	79.04 ± 1.08^{c}	60.70 ± 1.59^{ab}	19.83 ± 1.45^{a}	18.87 ± 0.62^{b}
T4	80.04 ± 1.49^{a}	59.97 ± 2.04^{ab}	12.43 ± 1.96^{c}	24.30 ± 1.42^{a}
Initial	85.95±0.34	61.86±0.9	7.04±0.95	24.93±0.45

Note*:* Each value represents mean ± SD (n = 3). Values in the same columns with different superscript letters are significantly different (P<0.05).

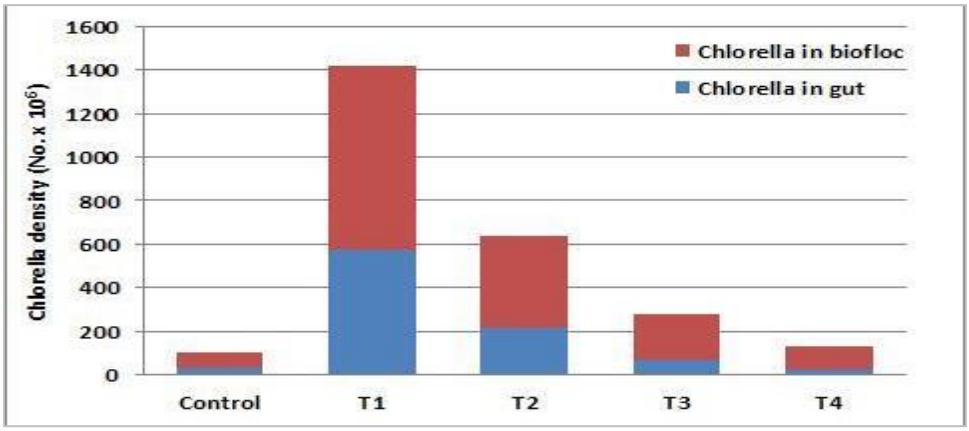

Figure 1. The average density of C. vulgaris in tilapia gut and in the biofloc system at different treatments

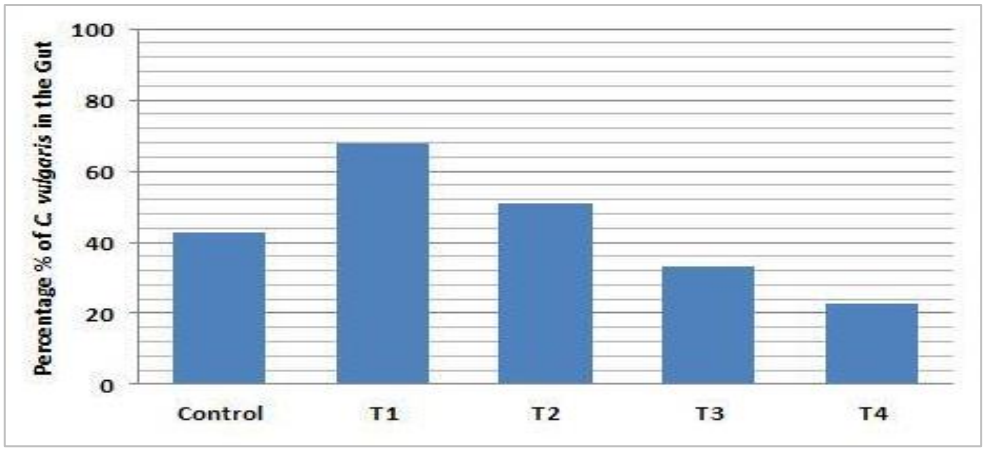

Figure 2. The percentage of *C. vulgaris* presence in tilapia gut at different treatments

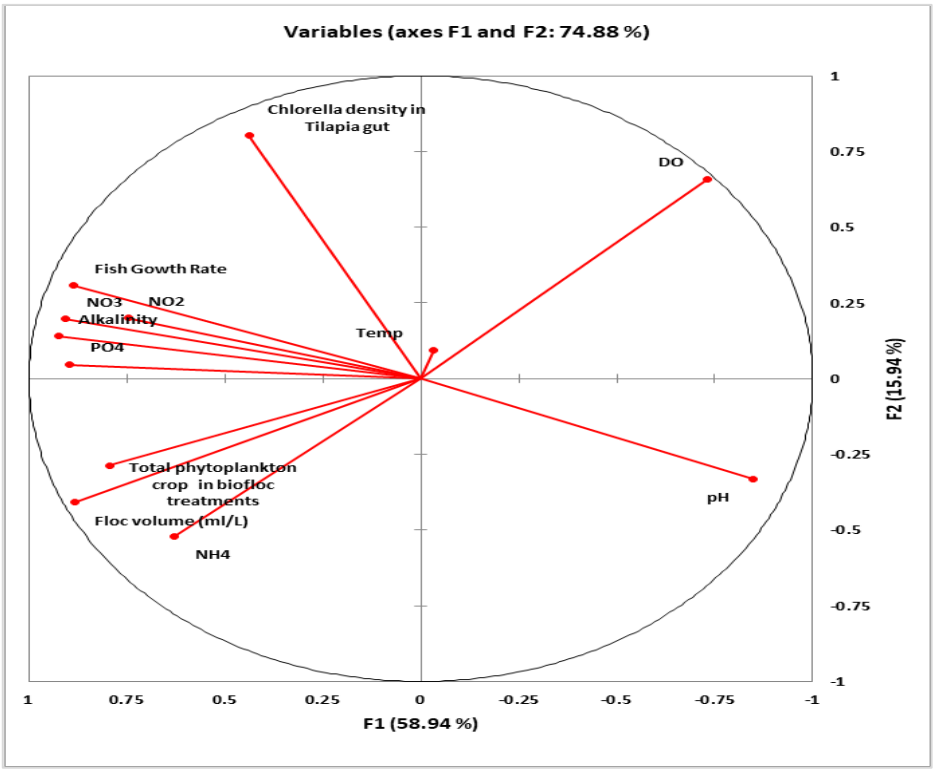

Figure 3. PCA performed on Nile tilapia growth rate, surrounding physico-chemical and biological factors

Table 5. Pearson's correlation between Nile tilapia growth rate, surrounding physico-chemical and biological parameters.

Variables	Fish Growth Rate	pH	Temperature	DO	NH_4	NO_2	NO_3	PO_4	Alkalinity	Floc volume	*Chlorella* density in Tilapia gut	Total phytoplankton crop in biofloc treatments
Fish Growth Rate	1											
pH	-0.85	1										
Temperature	-0.06	0.03	1									
DO	-0.45	0.44	0.13	1								
NH_4	0.26	-0.39	0.10	-0.79	1							
NO_2	0.60	-0.55	-0.05	-0.40	0.36	1						
NO_3	0.80	-0.77	-0.03	-0.54	0.46	0.92	1					
PO_4	0.90	-0.86	-0.05	-0.64	0.50	0.45	0.69	1				
Alkalinity	0.79	-0.75	-0.08	-0.58	0.51	0.90	0.96	0.71	1			
Floc volume (ml/L)	0.72	-0.63	-0.11	-0.92	0.69	0.50	0.69	0.81	0.72	1		
Chlorella density in Tilapia gut	0.62	-0.66	0.15	0.22	0.03	0.34	0.47	0.48	0.46	0.06	1	
Total phytoplankton crop in biofloc treatments	0.68	-0.52	0.17	-0.71	0.58	0.45	0.57	0.73	0.64	0.84	0.15	1

Values in bold are different from 0 with a significance level alpha=0.05

minerals, and other bioactive components involved in many physiological activities.

Concerning lipid contents, the results showed that lipid content varied with varying *C. vulgaris* concentrations, increased levels of *C. vulgaris* resulted in a gradual decrease in fish lipid content, this may be due to that lipid content of *C. vulgaris* is slightly low reaches approximately 12.5% (Blas-Valdivia et al. 2011). Giving fish a higher concentration of *C. vulgaris* resulted in decreasing the whole-body lipid levels. Our concluded results were consistent with that of Badwy et al. (2008), who stated that feed conversion ratio, growth performance, and productive protein values were more proficient in fish fed diets containing 50% of both *Chlorella* and *Scenedesmus* spp., moreover carcass analysis showed higher dry matter and crude protein content, but lower lipid content.

Protein results for the present study confirmed that the use of higher concentrations of phytoplankton (T1 and T2 treatments) resulted in higher protein ratios than other treatments. This is attributed to the use of *C. vulgaris* as a dietary additive that resulted in enhancing tilapia's growth performance as mentioned by Maliwat et al. (2017).

Also, the increased concentration of phytoplankton in the biofloc system led to increased mean floc volume, as shown in T1 and T2 treatments compared to the control. Rajkumar et al. (2016) found that the floc volume in the first 15 days was slow due to the clean surfaces of the reservoir and the lower biological density at the start of the experiment. Then the volume increased gradually throughout the experiment, and the variance was constant over time.

Conclusion

Phytoplankton is the primary source of natural feed for Nile tilapia (*Oreochromis niloticus*) farming in the biofloc system. Introducing microalgae as a protein source might further increase aquaculture's efficacy and subsequently increasing human food production. From the data obtained, it could be concluded that increasing concentration of *C. vulgaris* in the biofloc system improved the growth performance of Nile tilapia under the biofloc condition.

Ethical Statement

All experiments were approved by NIOF Committee for ethical Care and Use of Animals/ Aquatic Animals (NIOF-IACUC) Egypt, with certificate code: NIOF-FW4-F-21-R-003.

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No funding was received to assist with the preparation of this manuscript.

Author Contribution

Nasser Flefil is the author of the idea, cultivating and preparing *chlorella vulgaris* and adding it to fish feed, and he is involved with **Abd-Ellatif Hussian** in developing the design of the algae concentrations, **Ahmed Aboseif** bought and raised fish, set up a biofloc system, calculated feed, analyzed fish growth parameters, and ANOVA statistical analysis. **Abd-Ellatif**

Hussian made Pearson correlation and PCA. All authors are involved in writing and reviewing this manuscript.

Conflict of Interest

The authors declare that they have no potential conflict of interest to report with respect to the research, authorship and publication of this article.

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References

Ahmed, N.M., Flefil, N.S., Tayel, S.I., Mahmoud, S.A., & Soliman, A. (2019). Biological treatment of ammonia using biofloc system for *Oreochromis niloticus* fish. *Egyptian Journal of Aquatic Biology and Fisheries*, 23(4), 639 – 657. http://doi.org/10.21608/ejabf.2019.60912.

AOAC (2012). Official methods of analysis. 19th ed. Gaithersburg: AOAC. 3000p.

APHA (2017). Standard Methods for the examination of water and wastewater. 23rd ed. Washington: 1546 pp.

Arifin, N.B., Fakhri, M., Yuniarti, A., & Hariati, A.M. (2018). Phytoplankton community at intensive cultivation system of whiteleg shrimp, *Litopenaeus vannamei* in Probolinggo, East Java. *International Journal of Fisheries and Aquatic Studies*, 6(2), 42-46. https://doi.org/10.18860/elha.v6i3.4800.

Avnimelech, Y. (2009). Biofloc technology: a practical guidebook. Baton Rouge: The World Aquaculture Society, 182 p.

Avnimelech, Y. (2015). Biofloc Technology, a Practical Guidebook, 3rd Edition, World Aquaculture Society, 258p.

Badwy, T.M., Ibrahim, E.M., & Zeinhom, M.M. (2008). Partial Replacement of Fish Meal with Dried Microalga (*Chlorella spp* and *Scenedesmus spp*) in Nile Tilapia (*Oreochromis niloticus*) Diets. In 8th International Symposium on Tilapia in Aquaculture, Egypt, pp. 801–811.

Ballester, E.L., Abreu, P.C., Cavalli, R.O., Emerenciano, M., Abreu, L., & Wasielesky, W. (2010). Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition*, 16:163-172. https://doi.org/10.1111/j.1365-2095.2009.00648.x.

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25: 207-210. https://doi.org/10.1016/j.biotechadv.2006.11.002.

Bellinger, E.G., & Sigee, D.C. (2010). Freshwater algae: Identification and use as bioindicators. 1st edition by John Wiley and Sons, Ltd, 284 pp.

Bellinger, E.G., & Sigee, D.C. (2015). Freshwater algae: Identification, Enumeration and use as bioindicators. 2nd edition by John Wiley and Sons, Ltd, 290 pp.

Blas-Valdivia, V., Ortiz-Butrn, R., Pineda-Reynoso, M., Hernndez- Garcia, A., & Cano-Europa, E. (2011). *Chlorella vulgaris* administration prevents $HgCl_2$ caused oxidative stress and cellular damage in the kidney. *Journal of Applied Phycology,* 23, 53–58. https://doi.org/10.1007/s10811-010-9534-6.

Bligh, E.G., & Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37: 911– 917. https://doi.org/10.1139/o59-099.

Duncan, D.B. (1955). Multiple range and multiple F test. *Biometrics*,11, 1-42**.** https://doi.org/10.2307/3001478**.**

Edmondson, W.T. (1992). Ward and Whipple's Freshwater biology, 2nd edition. John Wiley and Sons, New York, p 1248.

Emerenciano, M., Ballester, E.L., Cavalli, R.O., & Wasielesky, W. (2012a). Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* Latreille, 1817. *Aquaculture Research*, 43(3), 447-457. https://doi.org/10.1111/j.1365-2109.2011.02848.x.

Emerenciano, M., Cuzon, G., Goguenheim, J., Gaxiola, G., & Aquacop, (2012b). Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. *Aquaculture Research*, 44, 75–85. https://doi.org/10.1111/j.1365-2109.2011.03012.x

Emerenciano, M., Martinez-Córdova, L.R., Martínez-Porchas, M., & Miranda-Baeza, A. (2017). Biofloc technology (BFT): a tool for water quality management in aquaculture. In: Water quality. Tutu H. (ed), *Intech Open*, http://dx.doi.org/10.5772/66416

Enyidi, U.D. (2017). *Chlorella vulgaris* as Protein Source in the Diets of African Catfish *Clarias gariepinus*. *Fishes*, 2, 17. https://doi.org/10.3390/fishes2040017.

FAO (2018). The state of world fisheries and aquaculture 2018: Meeting the sustainable development goals. Rome: FAO. 227p.

Goda, A.M., Ahmed, S.R., Nazmi, H.M., Aboseif, A.M., Taha, M.K., Fadda, S.H., Baromh, M.Z., El-Haroun, E., & Davies, S. (2020). Assessment of a high protein distillers dried grain (HP-DDG) augmented with phytase in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, haematological status, immune

response and related gut and liver histology. *Aquaculture*, 529, In press. 735617. https://doi.org/10.1016/j.aquaculture.2020.735617.

Hargreaves, J.A. (2013). Biofloc Production Systems for Aquaculture. Southern Regional Aquaculture Center. (SRAC) Publication No. 4503, p. 1-12. https://aquaculture.ca.uky.edu/sites/aquaculture.ca.uky.edu/files/srac_4503_biofloc_production_systems_for_aquaculture.pdf.

Ilavarasi, A., Mubarakali, D., Praveenkumar, R., Baldev, E., & Thajuddin, N. (2011). Optimization of various growth media to freshwater microalgae for biomass production. *Biotechnology*, 10, 540–545. 10.3923/biotech.2011.540.545

Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W., Kuo, W.C., & Horgen, F.D. (2008a). Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquaculture Research*, 39, 118–133. https://doi.org/10.1111/j.1365-2109.2007.01856.x.

Khani, M., Soltani, M., Shamsaie Mehrjan, M., Foroudi, F., & Ghaeni, M. (2017). The effects of *Chlorella vulgaris* supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences*, 16(2), 832-843. http://aquaticcommons.org/23121/1/IFRO-v16n2p832-en.pdf.

Krammer, K., & Lange-Bertalot, H. (1991). Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae In Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (Eds.). Süsswasserflora von Mitteleuropa. 2(3): 1-576. Gustav Fisher Verlag, Stuttgart, Germany.

Lee, R.E. (2008). Phycology.4th Edition. Cambridge University Press, New York. 547 pp.

Liñán-Cabello, M.A., Paniagua-Michel, J., & Hopkins, P.M. (2002). Bioactive roles of carotenoids and retinoid in crustaceans. *Aquaculture Nutrition*, 8, 299-309. https://doi.org/10.1046/j.1365-2095.2002.00221.x.

Mahmoud, E.A., El-Sayed, B.M., Mahsoub, Y.H., El-Murr, A.I., & Neamat-Allah, A.N. (2020). Effect of *Chlorella vulgaris* enriched diet on growth performance, hemato-immunological responses, antioxidant and transcriptomics profile disorders caused by deltamethrin toxicity in Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 102, 422-429. https://doi.org/10.1016/j.fsi.2020.04.061.

Maliwat, G.C., Velasquez, S., Robil, J.L., Chan, M., Traifalgar, R.F., Tayamen, M., & Ragaza, J.A. (2017). Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). *Aquaculture Research*, 48, 1666–1676. https://doi.org/10.1111/are.13004.

Mbonde, A.S., Limbu, S.M., Shoko, A.P., & Mgaya, Y.D. (2017). Phytoplankton and food selectivity in Nile tilapia reared in earthen ponds under monoculture and polyculture with African sharptooth catfish. *Journal of Aquaculture in the Tropics*, 32(1), 15-38. https://www.researchgate.net/publication/317240586.

Munshi, J.D., Roy, S.P., & Munshi, D. (2010). Manual of Freshwater biota. Narendra Publising House, Delhi, p. 1–455.

Nimrat, S., Tanutpongpalin, P., Sritunyalucksana, K., Boonthai, T., & Vuthiphandchai, V. (2013). Enhancement of growth performance, digestive enzyme activities and disease resistance in black tiger shrimp (*Penaeus monodon*) post larvae by potential probiotics. *Aquaculture International*, 21, 655-666. https://doi.org/10.1007/s10499-012-9600-y.

Pérez-Fuentes, J. A., Hernández-Vergara, M. P., Pérez-Rostro, C. I., & Fogel, I. (2016). C: N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture*, 452, 247-251. https://doi.org/10.1016/j.aquaculture.2015.11.010.

Popovsky, J. & Pfiester, L. (1990). Dinophyceae (Dinoflagellitida) subwasserflora von Mitteleuropa. Herausgegeben von. H. Ettl J. Gerloff. H. Heynig D. Mollenhauer. Band 6 Gustav Fischer Verlag. Jena, Stuttgart. pp: 272.

Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V., & Purushothaman, C.S. (2016). Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, 47(11), 3432-3444. https://doi.org/10.1111/are.12792.

Ray, A.J., Dillon, K.S., & Lotz, J.M. (2011). Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels ofbiofloc management. *Aquaculture Engineering*, 45(3), 127–136. https://doi.org/10.1016/j.aquaeng.2011.09.001.

Rodriguez-Garcia, I., & Guil-Guerrero, J.L. (2008). Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods. *Food Chemistry*, 108(3), 1023–1026. https://doi.org/10.1016/j.foodchem.2007.11.059.

Roy, S.S., & Pal, R. (2015). Microalgae in aquaculture: a review with special references to nutritional value and fish dietetics. *Proceedings of the Zoological Society*, 68: 1-8. https://doi.org/10.1007/s12595-013-0089-9.

Salin K.R., Arun V.V., Nair C.M., & Tidwell J.H. (2018). Sustainable Aquafeed. In: Hai F., Visvanathan C., Boopathy R. (eds) Sustainable Aquaculture. Applied

Environmental Science and Engineering for a Sustainable Future. Springer, Cham. https://doi.org/10.1007/978-3-319-73257-2_4.

Sarker, P.K., Gamble, M.M., Kelson, S., & Kapuscinski, A.R. (2016). Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium sp.* and of protein and essential amino acids from freshwater *Spirulina sp.* feed ingredients. *Aquaculture Nutrition*, 22, 109-119. https://doi.org/10.1111/anu.12230.

Verlencar, X.N., & Desai, S.R. (2004). Phytoplankton identification Manual. National Institute of Oceanography, Dona Paula. 35pp.

Xu, W., Gao, Z., Qi, Z., Qiu, M., Peng, J., & Shao, R. (2014). Effect of dietary *Chlorella* on the growth performance and physiological parameters of gibel carp, *Carassius auratus gibelio*. *Turkish Journal of Fisheries and Aquatic Sciences*, 14, 53–57.

Zhang, Q., Qiu, M., Xu, W., Gao, Z., Shao, R., & Qi, Z. (2014). Effects of Dietary Administration of *Chlorella* on the Immune Status of Gibel Carp, *Carassius auratus gibelio*, *Italian Journal of Animal Science*, 13(3), 3168, https://doi.org/10.4081/ijas.2014.3168.