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Research Article

Effect of *Nannochloropsis oculata* and Isochrysis *galbana* on growth, water quality, biofloc, carcass fatty acids profile, and intestinal bacteria in Nile tilapia (*Oreochromis niloticus*) raised in zero-water exchange system

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Abstract

In this study, the effects of various algal resources on water quality, growth performance, body fatty acid composition, and intestinal bacteria of Nile tilapia were studied under a zero-water exchange biofloc system. *Oreochromis niloticus* with an initial mean weight of 2.73±0.27 g, was cultured in five experimental tanks (150 L) including the control group (T1), biofloc group (T2), biofloc+ *N. oculata* group (T3), biofloc+ *I. galbana* group (T4), and biofloc+algal (T5) for 60 days. A total number of 225 *O. niloticus* was used for this study (15 individuals in each replication). The results showed the highest total ammonia nitrogen (4.54±0.05 mg L^{-1}) in the biofloc treatment (p<0.05). There were significant differences in the growth performance among the control group with other treatments (p<0.05). In terms of fillet quality, the highest amount of saturated fatty acids (39.79±0.25%) was found in the biofloc group. The highest monounsaturated fatty acids (41.87±0.99) and polyunsaturated fatty acids (33.25±0.93%) were obtained in the biofloc+algal treatment (p<0.05). All intestinal bacteria in the biofloc group were higher than the control group (P<0.05). The current study demonstrated that biofloc could promote the growth performance of unsaturated fatty acids in the fish fillet.

Introduction

Fish is one of the most important protein sources for the global population, hence the need to increase fish production to meet the increasing demand for protein. Due to the rapid expansion of aquaculture, fish feed is considered an essential component, constituting over 50–70% of the total operating cost in aquaculture (FAO, 2018). Since only about 26 percent of the nitrogen and 30 percent of the phosphorus in the feed are used by fish, the accumulation of fish waste and uneaten feed causes bond nitrogen and ammonia load to rise in semi-intensive and intensive fish culture systems (Baluyut and Balnyme, 1995). Continuous and partial water exchange is required, especially in intensive fish culture, to maintain good water quality, which may be expensive (Boyd, 1990; Datta, 2012). Through the uptake of water nitrogen and conversion to microbial protein, biofloc technology (BFT) is a recent technique created to maintain water quality (Xu and Pan, 2014; Abakari *et al.*, 2020; Li *et al.*, 2023; González-Camejo *et al.*, 2020). According to Emerenciano *et al.* (2017), bioflocs comprise a variety of heterogeneous conformer microorganisms, including bacteria, microalgae, protozoa, phytoplankton, rotifers, annelids, nematodes, copepods, cations, colloids, organic polymers, undigested feed, and dead cells. All local formers aggregate to form a mass that fish can eat, promoting nutrient recycling and enhancing fish growth performance (Avnimelech, 2009; Zhao *et al.*, 2012). The culture system's carbon-to-nitrogen ratio must be kept constant as one of the main requirements for biofloc formation. The carbon/nitrogen ratio should be between 10:1 and 20:1 to promote the growth of biofloc, and the carbon sources can vary (Ballester *et al.*, 2010). Fish immune system and disease resistance improve in BFT (Long *et al.*, 2015; Panigrahi *et al.*, 2018).

The Nile tilapia due to its traits and benefits, such as its capacity to consume a variety of nutrients (phytoplankton, suspended particles in the water column, and microbial masses) (Bosisio *et al.*, 2017), quick development, simple adaptation to a diet of artificial foods and intensive culture (Fitzsimmons *et al.*, 2011) has been acknowledged as an essential species for cultivation worldwide (Wang and Lu, 2016).

Species with characteristics like tolerance to moderate oxygen levels, extreme success are linked to feeding on debris and adapting to dense populations that can be cultured in biofloc system (Khanjani and Sharifinia, 2020). Avnimelech (2009) and Samocha *et al.*, (2017) reported tilapia can be cultured in biofloc system under intensive and super-intensive conditions. According to Durigon *et al.* (2019), this species can also eat microorganisms that depend on biofloc. How the biofloc system is managed depends critically on the kind of carbon source used (Panigrahi *et al.*, 2019).

Microorganisms are essential in many ecosystem materials cycles because of their functions as producers and decomposers (Mickalide and Kuehn, 2019; López-Mondéjar *et al.*, 2020). The nitrogen-related material cycle is one example of this (Cirri and Pohnert, 2019; Urakawa *et al.*, 2019), in which certain microorganisms use nitrogen for anabolic or catabolic processes

(Takai 2019; Dai *et al.,* 2020). These procedures eliminate ammonia, other pollutants, and nitrogen-based compounds, e.g., protein synthesis occurs (Pilgrim *et al.*, 1970). According to Zhang *et al.* (2020) and Huo *et al.* (2020), the function of microorganisms in this situation has led to their industrial use. For instance, microbial processes treat nitrogen compounds in city wastewater. In addition, microbes are employed to remove nitrogen-based substances produced by aquatic organisms in water, including small- and large-scale home aquariums and fish farms (Miranda-Baeza *et al.,* 2017; Putra *et al.,* 2020). Improved techniques are being developed because the water exchange produces wastewater and requires high water levels (Timmons *et al.,* 1998).

Previous studies on the components of microbial communities in biofloc systems have been carried out to enhance biofloc technology's potential (Wei *et al.,* 2020). Ray *et al.* (2009) and Wang *et al.* (2019) mentioned that more research on microalgae is required to further our understanding of biofloc technology, even though previous studies on pollutant removal and floc formation have advanced this understanding (Da'Silva and Kyndt, 2020; Dauda, 2020). It is well known that microalgae can produce organic nitrogen-related compounds like proteins using ammonia and nitrate (Cui *et al.,* 2020). Photosynthesis can produce antioxidants like astaxanthin, lutein, hydrocarbons, and lipids (including unsaturated fatty acids) (Kawale and Kishore 2019; Cui *et al.,* 2020; Li *et al.,* 2020). The productivity and quality of agricultural products can be improved by feeding these substances made by microalgae to aquatic creatures (Crab *et al.,* 2010; Dauda, 2020; Khanjani and Sharifnia, 2020). However, more investigation is still required into the functions and characteristics of microalgae in biofloc technology.

The consumption of algae is a source of enriched plant protein and can change the target species' lipid profile and body chemistry (Chisti, 2007; Richmond, 2004). This lipid profile is not present in the environment's native food organisms, nor are the fatty acids omega-3 produced by freshwater fish (Martins *et al.,* 2013). About 50% of marine and freshwater algae lipids are polyunsaturated fatty acids (PUFAs) (Martin *et al.,* 2006; Chisti, 2007). Freshwater algae have a higher concentration of omega-6 fatty acids, while marine algae contain more omega-3 fatty acids than land-based algae. As a result, freshwater fish have lower levels of PUFAs from the omega-3 family than marine fish (Wan Rosli *et al.,* 2012). Algae may be added to freshwater fish feed to promote growth and increase the amount of DHA in fish fillets, thereby enhancing the meat's nutritional value.

Nannochloropsis Oculata contains 31.2% crude protein and 36% fat. *N. oculata* thrives in a wide range of salinities (0-36 g/L), without flagella, green in color, and has a 4-6 micron-diameter round (Ayerza and Coates, 2001). *Isochrysis galbana* has a high growth rate of 41 to 46% protein and 22% fat in dry chemical composition. However, it can grow in salinities between 10 and 30 ppt. This alga swims quickly in the water and rotates its cells as it does so. It has two flagella with a

golden-coloured spherical shape and a diameter of 4–8 microns (Hoffmann, 1999).

This study aims to evaluate the effects of including microalgae as a source floc in a biofloc system with zero exchange water on growth performance, water quality, biofloc characteristics, fatty acid profiles of fish fillets, and intestinal bacteria in Nile tilapia (*Oreochromis niloticus*) for 60 days in five groups including control, biofloc, biofloc+ *N. oculata*, biofloc+ *I. galbana*, and biofloc+algal.

Materials and methods

Sari Agricultural Sciences and Natural Resources University in Iran's Mazandaran province served as the site of the current study. Fingerlings of the Nile tilapia were purchased from Yazd, Iran, with a mean weight and length of 2.73±0.27 g and 2.78±0.26 cm, respectively. For this experiment, fifteen 300-litre circular polyethylene tanks were also created. Fifteen fingerlings were placed in each tank (225 fingerlings in total) after it had been filled with 150 liters of 12 ppt salinity water. For 60 days, the experiment was carried out. A control group (with water exchange, 90% every week) and four different biofloc treatments (no water exchange) considered (Table 1).

Table 1: Characteristics of treatments based on the different biofloc for cultivating Nile tilapia fingerlings.

T1	T2	T3	T4	T5
Control	Biofloc	Biofloc + *Nannochloropsis oculata*	Biofloc + *Isochrysis galbana*	Biofloc + algal (*Nannochloropsis*+ *Isochrysis*)

Algal were cultured with Gaillard medium in photoperiod of 12:12 with 3500 lux light densities and at a temperature of 24°C, and then they reached the high density (at the end of logarithmic phase after 9 days for *N. oculata* and 11 days for *I. galbana*) by centrifugation (80 million cells per mL for *I. galbana* and 120 million cells in mL for *N. oculata*). 80 and 150 million cells/ml made up the stock of microalgae used in this experiment. Each tank received a daily 120 ml addition of each microalgae *N. oculata* and *I. galbana*. Eight days before moving the fish to tanks, algae in equal amounts was transferred. The amount of microalgae added to the biofloc treatments throughout the experiment was every week. The diet contained 40% crude protein and was produced by the Faradaneh Company in Iran. Feed was administered twice daily at 08:00 and 20:00. The feeding ratio was estimated according to the percentage of body weight of 3%, then calculated every 15 days throughout the experiment. There was a 25% decrease in feeding in the biofloc treatments compared to the control group.

To create biofloc, the nitrogen and carbon source was added to the treatment tanks. The biofloc was initially made by adding 104 g of molasses containing 41.6 g of carbon (molasses containing 40g per 100g carbon) and 100 g of a formulated diet containing 26 percent protein, totalling 4.16 g of nitrogen (based on equation N×6.25=amount of protein so N×6.25=26 and then N was obtained 4.16 g), to the biofloc tanks. The 10:1 C: N ratio was

thought to exist. According to Avnimelech (2009), it was assumed that microbial communities absorbed 40% of the carbon in the carbonaceous material.

Water quality parameters

Daily temperature, pH, and dissolved oxygen (DO) were measured by ATC-686 (made in China). To determine settled solids (mL), the mixture was given 20 minutes to resolve after adding one liter of water to a graduated conical funnel, and it was conducted weekly (Avnimelech, 2009). To measure total suspended solids (TSS)(g/L), 100 ml of water was filtered with S&X 42 filter paper and put in an oven at 105°C for three hours to dry. A spectrophotometer was used to measure the levels of total nitrogen (TAN), nitrite, nitrate, and total carbon (mg/L) (APHA, 1998). To determine TDS, the sample was first passed through a sieve with a pore diameter of less than 0.2 μm. Then the filtered water was evaporated. The solids remaining after drying were measured. TDS concentration was obtained from the following equation:

$TDS = (W_{d+TDS} - W_d) / V$

where W_{d+TDS} is total weight of the container and the remaining mass of the dried solution (mg), W_d is total weight of the container (mg) and V is sample volume (L).

Biological and chemical oxygen demand (mg/L) was measured every two weeks using a spectrophotometer (APHA, 1998). Briefly, total organic carbon (TOC) is calculated as the difference between the TC and inorganic carbon channels. Heated-persulfate instruments utilize a digestion vessel heated to 105°C. Samples are added by direct injection. After inorganic carbon is removed by acidification (by acidifying with H_2SO_4 to pH=2; it takes about 15-30 mL for 10 min), a measured amount of persulfate solution (Sodium peroxydisulfate 10%, Ammonium peroxydisulfate 15%, and Potassium peroxydisulfate 2%) is added to the sample. After an oxidation period, the resulting CO_2 is sparged from the solution and carried to an infrared analyzer specifically tuned to the absorptive wavelength of CO_2. The instrument's microprocessor converts the detector signal to organic carbon concentrations in mg/L based on stored calibration data (ASTM, 1994).

Growth Performance

Measuring fish length and weight was performed on the first day and every 15 days of the trial. Growth performance indices: including weight gain (WG), length gain (LG), body weight index (BWI), Daily weight gain (DWG), specific growth rate (SGR) and survival rate (SR), and nutrition indices, including protein efficiency ratio (PER) and feed conversion ratio (FCR) were calculated based on the Khanjani *et al*. (2020).

Sample collection

For biofloc, the experiment was completed by passing water through 20-micron nets. The biofloc from each treatment was separately gathered and placed in a container. For fish, feeding was stopped 24 hours before sampling. Three fish were randomly selected from each tank. Clove (*Eugnia carioplyllum*) is used for anaesthetic fish at 3 ml plant extract per litre (Javahery *et al*., 2012). Then, sampling from the beginning of the midgut in entirely

sterile conditions was done for microbial assessment (Askarian *et al.,* 2012) and from fillets for fatty acid profiles.

Fatty acid profiles

The fatty acid composition of fish fillet and biofloc was determined using Miquel and Browse (1992) method. In brief, 200 mg of fish fillet and biofloc were heated to 80°C in 1 ml of a mixed H_2SO_4 2.5 percent and methanol 98 percent (1:40, v/v) for one hour in a Teflon lined screw cap glass tube. The mixture was cooled to room temperature, and then 500 µL of hexane and 1.5 ml of NaCl 0.9 percent (w/v) were mixed and added to the samples. The samples were centrifuged for 10 min at 4000 rpm, and the supernatant (1 µL) was injected to Gas chromatograph (GC) to determine the fatty acid profiles (Miquel and Browse, 1992).

Microbial assessment

After weighing, the samples were divided into small pieces in a sterile container and homogenized with 0.9% physiological serum (9 times the weight of the samples) for 2 minutes. To count the bacterial flora of the intestinal tissue, dilutions (1:10) were prepared from the homogenous solution (Askarian *et al*., 2012). For the preparation of the culture medium, incubation, and counting total bacteria was used TSA culture medium was used (Vahdat *et al.,* 2018), and PCA culture medium was used to count the mesophilic bacteria in the intestine (Audenaert *et al*., 2010). MRS culture medium was used to count lactic acid bacteria (Bansal *et al.,* 2013).

Statistical analysis

The Kolmogorov-Smirnov test checked the normal distribution of data. The results were analyzed using One-Way ANOVA, and the Tukey test was used to compare treatment means at 5 percent (p<0.05). The statistical analysis was done by SPSS (version 22).

Result

Table 2 displays the mean (±SD) of water's physicochemical parameters throughout the experiment. Temperature, pH, and dissolved oxygen measurements revealed no difference between treatments (p>0.05). TAN values of 1.99 and 4.54 mg/L were found in the control and biofloc treatments, significantly different from other groups (p<0.05). According to the results, there were significant differences between the control and biofloc treatments for nitrite (NO_2), nitrate (NO_3), total dissolved solids (TDS), settled solids (SS), and total suspended solids (TSS), with the biofloc algal treatment having the highest SS throughout the experiment (p<0.05). The differences in total carbon between the biofloc groups were significant, with biofloc+algae having the highest value (704.99 mg/L) (p<0.05). Significantly (p<0.05) higher values were seen for BOD (3.58±0.50 mg/L) and COD (24.21±0.52 mg/L) in biofloc treatment (Table 2).

The mean (±SD) of growth parameters for different treatments is revealed in Table 3, which showed significant differences between the control group and biofloc treatments (p<0.05). The lowest PER (1.32±0.00) was obtained in the control group, while the highest PER was observed in biofloc with algal treatment (1.73±0.06)

and showed a noticeable distinction from other treatments (p<0.05). The survival percent of Nile tilapia and FCR in different treatments showed no differences (p>0.05).

Table 2: Physicochemical parameters of water during fish rearing.

Parameters	Control	Biofloc	Biofloc + *N. oculata*	Biofloc + *I. galbana*	Biofloc + Algal
pH	8.38±0.10[a]	8.55±0.09[a]	8.35±0.07[a]	8.55±0.10[a]	8.30±0.08[a]
Temperature (ºC)	27.50±0.22[a]	27.39±0.22[a]	27.30±0.32[a]	27.60±0.33[a]	27.71±0.33[a]
O_2 (mg/L)	6.89±0.15[a]	6.47±0.15[a]	6.44±0.15[a]	6.71±0.14[a]	6.41±0.16[a]
TAN (mg/L)	1.99±0.03[a]	4.54±0.05[c]	3.83±0.06[b]	3.84±0.07[b]	3.48±0.63[b]
NO_2 (mg/L)	3.11±0.14[a]	20.69±0.31[b]	19.84±0.14[b]	21.28±0.26[b]	22.08±0.51[b]
NO_3 (mg/L)	3.55±0.17[a]	23.83±1.00[b]	22.86±0.18[b]	24.50±0.29[b]	25.43±0.46[b]
TDS (mg/L)	3.05±0.01[a]	3.52±0.21[b]	3.54±0.00[b]	3.55±0.01[b]	3.57±0.14[b]
Total Carbon (mg/L)	0	678.02±0.45[b]	643.07±0.49[a]	685.77±0.61[b]	704.99±0.35[c]
COD (mg/L)	20.47±0.47[b]	24.21±0.52[c]	8.96±0.35[a]	9.43±0.43[a]	8.55±0.56[a]
BOD (mg/L)	1.89±0.03[a]	3.58±0.50[b]	1.84±0.05[a]	1.80±0.09[a]	1.97±0.09[a]
TSS (g/l)	0	0.25±0.01[a]	0.31±0.01[b]	0.32±0.01[b]	0.36±0.02[b]
SS (ml/L) or biofloc volume index	0	32.50±0.65[a]	47.00±0.23[b]	52.00±0.65[c]	57.50±0.50[d]

Data are expressed as mean±SD. Values in the same row with different letters are significantly different (p<0.05).

Table 3: Growth performance of Nile tilapia *O. niloticus* fingerlings cultivated under different bioflocs at the end of 60 days of the experiment period (mean ± SD).

Growth Factor	Initial weight (g)	Final weight (g)	Initial length (cm)	Final length (cm)	WG (g)	LG (cm)
Control	2.73±0.27	57.69±0.20[a]	2.78±0.26	13.65±0.52[a]	54.98±0.09[a]	10.87±0.67[a]
Biofloc	2.73±0.27	68.58±2.31[b]	2.78±0.26	16.11±0.44[b]	65.85±2.18[b]	13.33±0.24[b]
Biofloc + *N. oculata*	2.73±0.27	70.05±0.98[b]	2.78±0.26	16.50±0.08[b]	67.31±1.39[b]	13.72±0.13[b]
Biofloc + *I. galbana*	2.73±0.27	67.10±2.96[b]	2.78±0.26	16.73±0.10[b]	64.37±3.17[b]	13.95±0.09[b]
Biofloc + Algal	2.73±0.27	72.09±2.98[bc]	2.78±0.26	16.59±0.16[b]	69.36±2.61[b]	13.81±0.13[b]

Table3 (continued):

Growth Factor	BWI (%)	DWG (g/day)	SGR	SR (%)	FCR	PER
Control	1099.20±41.96a	0.88±0.00a	2.55±0.06a	94.11±5.26a	1.02±0.01a	1.37±00.0a
Biofloc	840.16±33.62b	1.02±0.03c	3.24±0.06b	98.03±3.03a	1.02±0.04a	1.64±0.05b
Biofloc + N. oculata	814.53±64.07b	1.04±0.02c	3.25±0.09b	98.03±3.03a	1.02±0.02a	1.68±0.03b
Biofloc + I. galbana	780.26±63.91b	0.99±0.05b	3.31±0.05b	100a	1.06±0.05a	1.60±0.07b
Biofloc + Algal	775.99±12.62c	1.06±0.04c	3.44±0.05b	100a	1.04±0.04a	1.73±0.06c

Data are shown as mean±SD. Values in the same column with different letters differ significantly (p<0.05).
Body weight index (BWI), daily weight gain (DWG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR)

On different sampling days, the weight of the reared Nile tilapia showed significant differences (p<0.05) among control and biofloc treatments. However, there were no significant differences between biofloc groups (p>0.05). Total length revealed marked differences (p<0.05) among the control and biofloc treatments in all sampling times (p<0.05). Also, there were significant differences in control and biofloc treatments during the growing time (p>0.05) (Fig. 1).

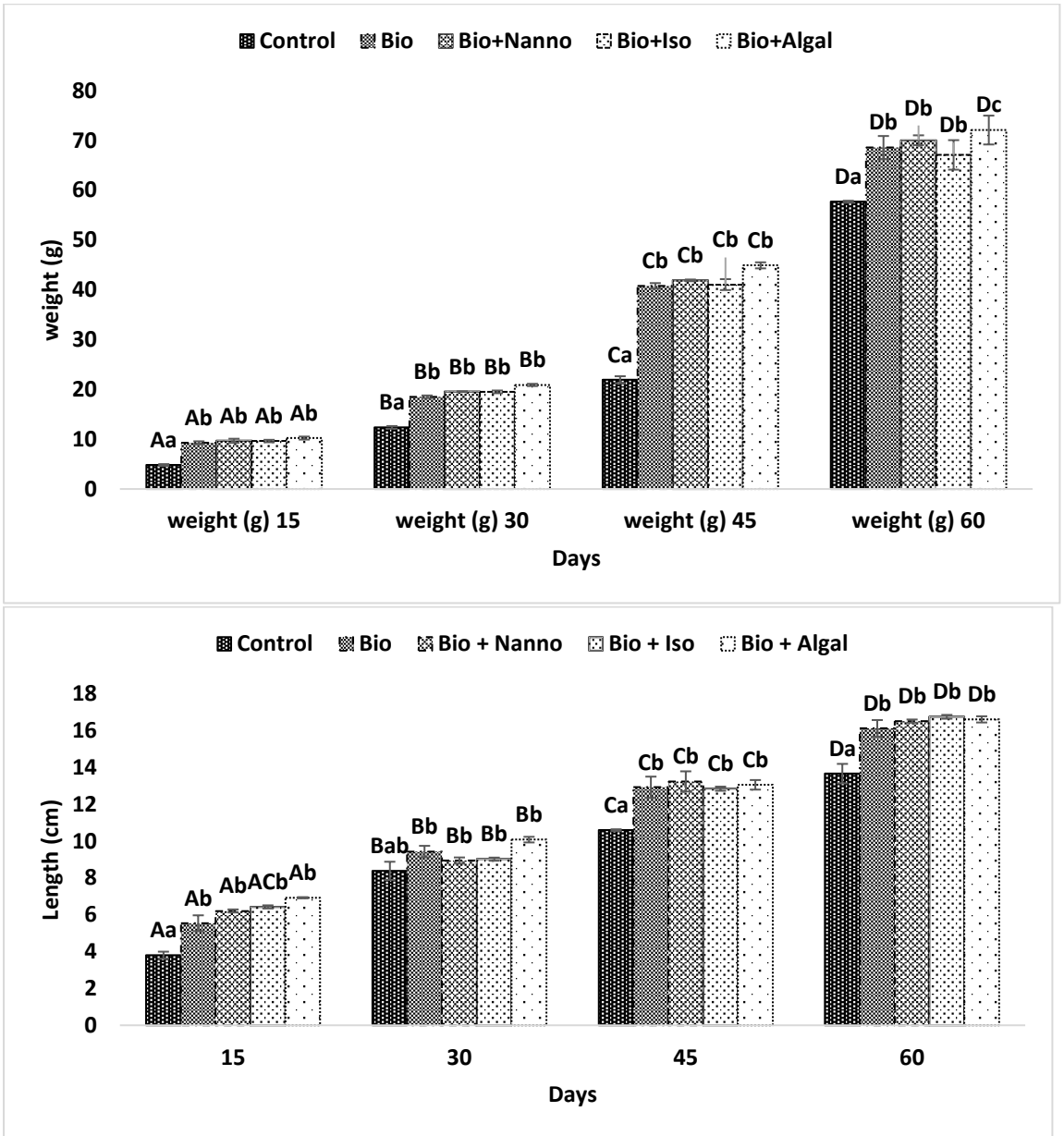

Figure 1: Total weight (g) and total length (cm) of Nile tilapia cultured at different experimental treatments. a,b,c,… indicate significant differences between groups on the same day. A, B, C,… indicate significant differences between the same treatments in growing time (p<0.05).

Fatty acid profiles of Nile tilapia are presented in Table 4. Myristic acid (C14: 0), palmitic acid (C16:0), Arachidic acid (C20:0), and lignoceric acid (C24:0) showed lower values in biofloc+*N. oculata* and biofloc+algal groups significantly

(p<0.05). Stearic acid (C18:0) and Behenic acid (C22:0) showed the highest values significantly in biofloc+*N. oculata* and biofloc+algal treatments respectively (p <0.05). Myristoleic acid (C14:1n5), Palmitoleic acid (C16:1n7), cis-Vaccenic acid (C18:1n7), Erucic acid (C22:1n9) and Nervonic acid (C24:1n9) showed highest values in biofloc+algal treatment significantly (p<0.05). oleic acid (C18:1n9) was the highest fatty acid in biofloc+*N. oculata* significantly (p<0.05). Eicosenoic acid (C20:1n9) showed the most value in biofloc+*N. oculata*, biofloc+*I. galbana* and biofloc+algal groups significantly (p<0.05). Linoleic acid (C18:2n6) and Arachidonic acid (C20:4n6) demonstrated the highest values in biofloc+algal treatments significantly (p<0.05). also, Eicosadienoic acid (C20:2n6) and Eicosapentaenoic acid (C20:5n3) showed highest values in biofloc+*N. oculata* group significantly (p<0.05). Docosatrienoic acid (C20:3n3) and Docosahexaenoic (C22:6n3) showed no different meaning between the treatments (p>0.05). alpha-linolenic acid (C20:3n3) was the highest value in biofloc+*N. oculata* and biofloc+algal groups significantly (p<0.05). The amount of SFA (39.79±0.25) in biofloc treatment and; values in MUFA (41.87±0.99) and PUFA (33.25±0.93) were highly marked significantly in the biofloc+algal group (p <0.05) (Table 4).

Table 4: Fatty acid profiles of *O. niloticus* cultured in different biofloc treatments at the end of the experimental period (Mean±SD).

Fatty acid profiles (%)	Control	Biofloc	Biofloc + *N. oculata*	Biofloc + *I. galbana*	Biofloc + Algal
C14	5.91±0.06^{c}	7.09±0.07^{d}	4.13±0.04^{a}	5.02±0.05^{b}	3.60±0.46^{a}
C16	10.20±0.35^{b}	12.24±0.42^{c}	7.14±0.25^{a}	11.73±0.41^{c}	6.59±0.08^{a}
C18	1.22±0.12^{ab}	1.46±0.14^{bc}	0.85±0.08^{a}	1.39±0.13^{b}	1.74±0.11^{c}
C20	10.66±0.48^{b}	12.79±0.58^{c}	7.46±0.33^{a}	12.26±0.55^{c}	6.34±0.52^{a}
C22	3.43±0.46^{c}	4.12±0.05^{e}	2.40±0.03^{b}	3.94±0.05^{d}	2.18±0.05^{a}
C24	1.75±0.14^{b}	2.09±0.17^{b}	1.22±0.10^{a}	2.00±0.16^{b}	1.21±0.21^{a}
Total Saturated	**33.16±0.21^{c}**	**39.79±0.25^{e}**	**23.20±0.14^{b}**	**36.35±0.22^{d}**	**21.65±0.22^{a}**
C14: 1n5	0.74±0.06^{a}	0.66±0.05^{a}	0.92±0.07^{a}	0.88±0.07^{a}	1.65±0.28^{b}
C16: 1n7	1.39±0.08^{ab}	1.25±0.07^{a}	1.73±0.10^{b}	1.66±0.09^{b}	3.44±0.16^{c}
C18: 1n7	1.33±0.03^{a}	1.20±0.30^{a}	1.66±0.41^{a}	1.59±0.40^{a}	3.95±0.08^{b}
C18: 1n9	20.68±0.07^{b}	18.61±0.06^{a}	25.85±0.09^{c}	24.81±0.08^{b}	24.87±0.25^{b}
C20: 1n9	2.50±0.16^{ab}	2.25±0.15^{a}	3.12±0.20^{b}	2.99±0.21^{b}	3.21±0.33^{b}
C22: 1n9	2.81±0.03^{b}	2.53±0.03^{a}	3.51±0.04^{d}	3.37±0.04^{c}	3.70±0.05^{e}
C24: 1n9	0.38±0.04^{a}	0.34±0.03^{a}	0.47±0.05^{a}	0.45±0.04^{a}	1.02±0.31^{b}
Total monounsaturated	**29.82±0.21^{b}**	**26.83±0.19^{a}**	**37.26±0.27^{c}**	**35.78±0.25^{c}**	**41.87±0.99^{d}**
C18: 2n6Cis	2.57±0.06^{a}	2.31±0.06^{a}	3.22±0.08^{b}	7.71±0.21^{c}	10.75±0.37^{d}
C18: 3n3	3.56±0.11^{a}	3.20±0.09^{a}	4.45±0.12^{b}	3.20±0.09^{a}	4.68±0.43^{b}
C20: 2n6	10.77±0.33^{ab}	9.70±0.30^{a}	13.46±0.41^{c}	9.69±0.30^{a}	10.81±0.31^{b}
C20: 4n6	1.43±0.12^{ab}	1.29±0.11^{a}	1.79±0.15^{bc}	1.28±0.11^{a}	2.15±0.07^{c}
C20: 3n3	1.23±0.32^{a}	1.11±0.29^{a}	1.54±0.41^{a}	1.11±0.29^{a}	1.78±0.20^{a}
C20: 5n3	2.51±0.04^{ab}	2.60±0.04^{ab}	3.14±0.11^{b}	2.26±0.04^{ab}	2.08±0.64^{a}
C22: 6n3	0.40±0.09^{a}	0.36±0.08^{a}	0.50±0.12^{a}	0.36±0.08^{a}	0.97±0.55^{a}
Total polyunsaturated	**22.48±0.46^{b}**	**20.23±0.41^{a}**	**28.09±0.57^{d}**	**25.62±0.55^{c}**	**33.25±0.93^{e}**

Values in the same raw with different letters differ significantly (p<0.05).

Fatty acid profiles of biofloc are presented in Table 5. Myristic acid (C14:0) showed the highest value in Biofloc+*I. galbana* treatment significantly (p<0.05). Palmitic acid (C16:0) and lignoceric acid (C24:0) showed the highest and lowest values significantly in the control group, respectively (p<0.05). Stearic acid (C18:0) and Arachidic acid (C20:0) demonstrated the highest values, and Behnic acid (C22:0) was the lowest value in biofloc+*N. oculata* treatment significantly (p<0.05). Cis-Vaccenic acid (C18: 1n7), oleic acid (C18:1n9), erucic acid (C22:1n9) and nervonic acid (C24:1n9) showed highest values in biofloc+*I. galbana* treatment significantly (p<0.05). Myristoleic acid (C14:1n5) was the highest fatty acid in biofloc+*N. oculata* significantly (p<0.05). Eicosenoic acid (C20: 1n9) was the highest fatty acid in the biofloc group significantly (p<0.05). Palmitoleic acid (C16:1n7) was the highest fatty acid in biofloc and biofloc+*N. oculata* significantly (p<0.05). Linoleic acid (C18: 2n6) showed the highest amount in the control treatment significantly (p<0.05). Alpha-linolenic acid (C18:3n3) and Eicosadienoic acid (C20:2n6) showed the highest amounts in biofloc+*N. oculata* groups significantly (p<0.05). Docosatrienoic acid (C20:3n3) and Arachidonic acid (C20:4n6) and Eicosapentaenoic acid (C20:5n3) demonstrated highest values in biofloc+*I. galbana* treatments significantly (p<0.05). Although, Docosahexaenoic (C22:6n3) showed no difference in significance between the treatments (p>0.05). The amount of SFA (35.25±0.23) in biofloc+ *I. galbana* treatment and; values in MUFAs there were no significant differences between treatments, and PUFA (35.89±0.62) were marked significantly in biofloc+*N. oculata* group (p<0.05) (Table 5).

Table 5: Fatty acid profiles of biofloc obtained at the end of the experimental period (Mean±SD).

Fatty acid profiles (%)	Biofloc	Biofloc + *N. oculata*	Biofloc + *I. galbana*	Biofloc + Algal
C14	2.18±0.06^{a}	1.91±0.04^{a}	8.46±0.25^{c}	5.44±0.15^{b}
C16	18.56±0.09^{c}	15.54±0.08^{a}	16.73±0.24^{b}	15.44±0.40^{a}
C18	7.00±0.05^{b}	8.56±0.10^{c}	5.15±0.19^{a}	7.19±0.15^{b}
C20	1.09±0.05^{a}	3.72±0.07^{d}	2.51±0.11^{b}	3.26±0.10^{c}
C22	1.15±0.02^{b}	0.56±0.04^{a}	1.65±0.24^{b}	1.17±0.15^{b}
C24	0.31±0.06^{a}	0.58±0.04^{b}	0.75±0.08^{b}	0.69±0.02^{b}
Total Saturated	**30.30±0.06^{a}**	**30.88±0.16^{a}**	**35.25±0.23^{c}**	**33.21±0.37^{b}**
C14: 1n5	0.56±0.02^{a}	2.86±0.09^{c}	0.67±0.04^{a}	1.85±0.02^{b}
C16: 1n7	6.96±0.13^{c}	7.02±0.05^{c}	4.64±0.09^{a}	6.11±0.06^{b}
C18: 1n7	11.18±0.27^{ab}	10.54±0.35^{a}	11.56±0.23^{b}	10.59±0.06^{a}
C18: 1n9	9.36±0.20^{bc}	7.20±0.36^{a}	9.90±0.04^{c}	8.98±0.16^{b}
C20: 1n9	0.85±0.07^{b}	0.47±0.08^{a}	0.40±0.01^{a}	0.45±0.03^{a}
C22: 1n9	0.23±0.13^{a}	0.26±0.02^{a}	1.57±0.06^{c}	0.96±0.04^{b}
C24: 1n9	0	0.40±0.08^{a}	1.36±0.13^{c}	0.92±0.11^{b}
Total monounsaturated	**29.13±0.02^{a}**	**28.73±0.74^{a}**	**30.10±0.64^{a}**	**29.88±0.05^{a}**
C18: 2n6Cis	15.65±0.26^{d}	13.66±0.22^{c}	10.56±0.14^{a}	12.71±0.04^{b}
C18: 3n3	0.31±0.04^{a}	9.71±0.08^{d}	4.94±0.05^{b}	7.69±0.07^{c}
C20: 2n6	0.88±0.05^{a}	8.29±0.30^{d}	3.87±0.09^{b}	4.88±0.04^{c}
C20: 4n6	3.26±0.30^{b}	2.43±0.08^{a}	4.58±0.03^{c}	3.68±0.06^{b}
C20: 3n3	0.02±0.00^{a}	0.39±0.04^{b}	1.53±0.01^{d}	1.00±0.03^{c}
C20: 5n3	0.48±0.01^{a}	0.57±0.03^{a}	1.90±0.06^{c}	1.29±0.08^{b}
C22: 6n3	0.75±0.02^{a}	0.86±0.10^{a}	0.79±0.02^{a}	0.86±0.04^{a}
Total polyunsaturated	**21.34±0.26^{a}**	**35.89±0.62^{d}**	**28.16±0.23^{b}**	**32.13±0.37^{c}**

Values in the same raw with different letters differ significantly (p<0.05).

The means (±SD) of the total logarithm of bacteria (CFU) are shown in Table 6. For the total number of bacteria, the control group showed the lowest values, the total number of mesophilic bacteria, and the total number of lactic acid bacteria significantly (p<0.05). The total height number of bacteria and the number of lactic acid bacteria were observed in biofloc groups (15.66±0.51), while the number of mesophilic bacteria was revealed highest in biofloc (8.33±0.52), biofloc+*N. oculata* (7.67±1.36) and biofloc+*I. galbana* (7.33±1.86) significantly (p<0.05) (Table 6).

Table 6: Logarithmic mean (±SD) of the total intestinal number of bacteria, mesophilic bacteria and lactic acid bacteria (log cfu/ml) at the end of the experiment in different treatments

Bacteria (CFU/mL) ×10	The total number of bacteria	Total number of mesophilic bacteria	Total number of lactic acid bacteria
Control	4.66±1.03^{a}	3.00±0.89^{a}	2.67±0.52^{a}
Biofloc	15.66±0.51^{d}	8.33±0.52^{c}	8.33±1.03^{c}
Biofloc + *N. oculata*	10.67±1.36^{c}	7.67±1.36^{c}	4.00±0.00^{b}
Biofloc + *I. galbana*	10.33±1.86^{c}	7.33±1.86^{c}	4.00±0.89^{b}
Biofloc + Algal	8.66±0.51^{b}	5.00±0.89^{b}	4.67±1.04^{b}

Values in the same column with different letters differ significantly (p<0.05).

Discussion

Water quality parameters

Water quality is influenced by some factors crucial for maintaining aquatic species' well-being and may even be a limiting factor (Sharifinia *et al.*, 2020). This study used the physicochemical properties of water, like pH and temperature-salinity, nitrite, nitrate, TAN, TSS, and salinity, to measure the appropriate kind of fish farming (Emerenciano *et al.*, 2017). There are three principal pathways to remove hazardous N species in aquaculture: (1) photoautotrophic removal by algae, (2) immobilization by heterotrophic bacteria as proteinacious microbial biomass and (3) chemo-autotrophic oxidation to nitrate by nitrifying bacteria (Ebeling *et al.*, 2006). The relative importance of each varies with system type and production intensity. In the present experiment, although removal by algae and immobilization of heterotrophic bacteria was encouraged, the system was dominated by nitrifying bacteria. Further research on how to minimize nitrification in BFT systems is needed. TAN and NO_2–N concentrations were elevated, sometimes reaching critical levels in BFT tanks during the experiment. The carbon requirement to minimize TAN needs further evaluation. The calculation might include NO_2–N concentration along with TAN concentration as also suggested by Azim and Little (2008).

One of the factors is temperature factors that affect biofloc formation (Hostins *et al.*, 2015); Some effects of temperature on the oxygen dynamic have been previously described, such as a decrease in the solubility of O_2 in the water column with increasing temperature (Boyd, 1990). Likewise, the temperature may affect the respiratory rate of aquatic organisms and, consequently, the zootechnical performance. For example, in *L. vannamei*, the respiratory rate increases when temperature is increased from 25˚C to 30˚C, negatively affecting food consumption,

growth and survival and it causes the formation of larger particles in the formation of flocs (Boyd, 1990; Stimpson *et al.,* 2005). In the current study, the temperature was maintained within a floc formation range suitable for growing fish like by El-Shafiey *et al.* (2018).

One of the main ones is the salinity parameter that may be influenced by heterotrophic bacteria that process nitrification (Khanjani *et al.,* 2020). Hence, this factor is influenced by the formation of the biofloc and the efficiency of aquacultured organisms possible in BFT. In the present study, the pH and DO were held to be sufficient, specifically in the control and biofloc treatments. Adding molasses to the biofloc tanks decreases oxygen compared to the control group, but increasing aeration eliminates the lack of oxygen. Consumption of feed with flocs (Kim *et al.,* 2014; Khanjani. *et al.,* 2017) made higher respiration rates as well as the generation of carbon dioxide, causing pH reduction in the environment (Khanjani *et al.,* 2017; Khanjani *et al.* 2020).

The biofloc with algae is a part of the BFT groups that exhibited the lowest levels of TAN, BOD, and COD and the highest NO_2 and NO_3 levels between BFT groups, which showed a larger population as opposed to biofloc treatment like Correia *et al.* (2014). This study looked at the nitrogen content. Ingredients used in biofloc treatments were less than what was stated by Mirzakhani *et al.* (2019). Managing heterotrophic bacteria's nitrogen-based substances should be adequately developed, and bacteria that can take up carbon more quickly derived from simple sugars like molasses and starch could lead to population growth (Khanjani *et al.,* 2017). It was discovered that adding organic carbon sources into the water-poor system exchange stops TAN from rising content, which was in line with other researchers' findings (García-Ríos *et al.,* 2019; Ahmad *et al.,* 2016, 2019). Total nitrogen from ammonia, when using molasses, total carbon decreased in biofloc with algae, but there was a difference not important the quicker decrease of ammonia using straightforward carbon sources. Metabolically active bacteria in heterotrophs water reduce ammonia quality (Khanjani *et al.,* 2017; El-Shafiey *et al.,* 2018).

Ratios of TAN and NO_3 concentrations were lower in BFT treatments versus the control treatment because of the accumulation due to a lack of organic matter exchange of water during biofloc treatments, the addition of carbon source effective in was water column (Li *et al.,* 2023). According to these findings, heterotrophic bacteria straight a forward carbon source that breaks down molasses more effectively than complex carbon sources like malted barley. These results are supported by the results of Silva *et al.* (2017), Khanjani *et al.* (2021), and Panigrahi *et al.* (2019) studies.

Growth efficiency

The current study exhibited that algal in biofloc, there was no discernible impact on the growth efficiency of consistently tilapia fingerlings. The biofloc treatments performed better than the control group. Bioflocs have probiotics characteristics (Ferreira *et al.,* 2015), which are essential nutrients (like fatty acids, minerals, and

amino acids) (Ju *et al.,* 2008). Also, bioflocs have many organic substances, including carotenoids, chlorophylls, phytosterols, bromophenols, and substances like antibacterial compounds (Crab *et al.*, 2010; Najdegerami *et al.* 2016) that provide along with artificial diet complete feed for aquaculture aquatic animals (Khanjani and Sharifinia, 2020). Various sources claim that the biofloc in the cultivation system leads to better growth performance in aquaculture aquatics (Ahmad *et al.,* 2016; Najdegerami *et al.*, 2016; Panigrahi *et al.,* 2019; Adineh *et al.*, 2019), which is possibly a result of the scarcity of water exchange.

In the current study, the lowest and highest PER and FCR were identified in the control and biofloc with algal groups, respectively, which showed a notable distinction with biofloc treatments. Various research has suggested that biofloc in the farming system increases protein and feed effectiveness productivity ratio (Ahmad *et al.,* 2016; Mirzakhani *et al.,* 2019; Panigrahi *et al.,* 2019). Panigrahi *et al*. (2019) reported 0.87 to 1.6 for shrimp FCR under various carbon sources in the biofloc system for tilapia. PER amounts for tilapia ranged in size from 1.8 to 2.6 based on Mirzakhani *et al.* (2019) and 1.79 to 2.33 by Durigon *et al.* (2019) reports.

In the current study, the survival rate in bioflocs groups showed no significant differences when contrasted with the control group. In light of the high costs associated with water exchange, biofloc treatments were, without that, observed to comparable survival and growth to the control group while also lowering water costs. Reports on survival rates were made C. gairpinus scored from 22 to 90% (Dauda *et al.,* 2017), 100% in favour of *O. niloticus* based on Mirzakhani *et al.* (2019), and 81 to 100% for *L. vannamei* by Panigrahi *et al.* (2019) while under the influence of various suppliers of carbon, the research being done. About 98 to 100% of tilapia survived in the biofloc treatments, which was not superior to the control group with 94% survival. The presence of demonstrated substances that stimulate the immune system beta-glucan and peptidoglycan in the wall is lipopolysaccharide also, the presence of biofloc bacteria immunity and bio floc's antioxidant the species' survival in culture growth (Kim *et al.,* 2014; Walker *et al.,* 2020). bioactive substances in biofloc increase aquatic survival, for example, could result from how much essential fatty acids, amino acids, and other dietary components (Xu and Pan, 2014).

Schizochytrium sp. has been added more frequently, and when 40 g per kg of *Schizochytrium* sp. was added to the feed, tilapia growth showed a linear increase. Compared to the control, there was a 47.67% difference in final weight and weight gain (Santos *et al.,* 2019). Saiyasaeng *et al.* (2014) was added Schizochytrium sp. at a rate of 70.5% (75 g/kg). Greater ultimate weight and weight gain (42% and 82%, respectively) were observed in Nile tilapia (*Oreochromis niloticus*) feed. The presence of Schizochytrium sp. improved the growth of the channel catfish (*Ictalurus punctatus*) by a weight gain of 12.35% (Li *et al.,* 2009). Based on our result, the presence of algal in biofloc treatments increased weight gain amounting to 1.15-fold significantly compared to the control group.

Even though there was ample evidence that the biofloc significantly impacted fish growth and production, the low FCR and production levels were well below what would be considered commercially viable. Little *et al.* (2008) evaluated the 10 to 28 kg of fish per cubic meter of ultimate biomass in indoor and outdoor BFT systems. These outputs are significantly less than those of traditional recirculating aquaculture systems, which have a standing biomass of more than 100 kg fish per cubic meter with oxygenation and 70–80 kg fish per cubic meter with aeration (Timmons *et al.,* 2002). The poor fish growth and production, though, could have many causes. Biofloc causes increased turbidity, which lowers visibility and, consequently, artificial feed intake. Even though a floc separator was employed, it was challenging to keep the TSS level at 500 mg/L; frequently, it exceeded 1,000 mg/L, in particular in the experiment's second half. A crucial problem in managing BFT systems was also identified by Little *et al.* (2008) as maintaining ideal floc levels. Second, there were varying water quality parameters, including high pH and alkalinity swings, high levels of inorganic nitrogen species, and other factors that could have a long-term impact on FCR.

Fatty acids profiles

More research was done to determine if the biofloc contained vital fatty acids for the fish. There were 30-35% SAFA, 21-35% PUFA, and 28–30% MUFA. Tacon *et al.* (2002) calculated that the biofloc harvested from a shrimp-cultured zero-exchange outdoor system fed different commercial diets contained 35–38% crude protein, 5–9% crude lipid, and 7–10% ash. A lower percentage of polyunsaturated fatty acids, about 22% for Tacon *et al.* (2002) and 7% for Azim and Little (2008) than in the current study was also reported. Jauncey (2000) showed that Nile tilapia needs about 0.5-1% of its lipid diet's n-6 fatty acids. However, a better quality biofloc was reported in the similar system without fish (Azim *et al.,* 2008) indicating that *in situ* floc utilization by fish could have an effect on the biochemical composition of floc. *Schizochytrium* sp.'s presence in the feed changed the lipid profile of tilapia. The fillet increased omega-3 fatty acids and DHA levels during the experimental period. When 40 g per kg of *Schizochytrium* sp. (Santos *et al*. 2019) In the current study, compared to the control group, the amount of omega-3 fatty acids and DHA of tilapia increased by 2.92 and 5.9 times, respectively. The components comprise the feed composition and the different inclusion levels of *Schizochytrium* sp.. provided varying amounts of the same fatty acid profile, primarily from the omega-3 family. The Fresh *Schizochytrium* sp. was discovered by Ludevese-Pascual *et al.* (2016) and had 19.4% of the total lipid average in weight. In the current study, the inclusion of algae in biofloc treatments increased the levels of MUFA and PUFA in the fatty acid profiles of tilapia fillets, demonstrating the direct impact of biofloc with algae on tilapia. In general, freshwater fish require n-6 fatty acids for maximal growth (Rodriguez *et al.,* 1997). In addition, freshwater fish have an innate ability to convert C-18 PUFA to HUFA and hence can presumably satisfy their EFA requirement with diets containing C-18

PUFA, which occurs in vegetable oils (Sargent *et al.*, 2002).

Since palmitate is the primary product of fatty acid synthase, a regulatory enzyme in the *de novo* synthesis of fatty acids. Elongation and desaturation of palmitate either in the mitochondria or on the surface of endoplasmic reticulum generate longer saturated and unsaturated fatty acids in organisms. Both stearic acid and palmitic acids were dominant in all the tissues in the current study, a finding that agrees with studies of Suloma *et al.* (2008) and Akpinar *et al.* (2009). Excess carbohydrates are sources of acetyl CoA, precursor of palmitic acid which is the first fatty acid to be produced during fatty acid synthesis. Stearic acid, C18:0, is elongation product of palmitate, thus its composition is likely to be determined by levels of palmitic acid. Palmitic acid is also an anabolic precursor to biosythesis of longer fatty acids. MUFAs constituted the largest proportion of total unsaturated fatty acids in all the tissuesWith Oleic acid, C18:1-n9 being the most dominant MUFA, a finding which collaborates with Satue and Lopez (1996), Alemu (2017), Mwanja *et al.* (2010), Olsen *et al*. (1990), Jabeen and Chaudry (2011), and Luo *et al.* (2010).

Tilapia is a freshwater fish that can tolerate higher salinity ranges for rearing. One disadvantage, however, is that high levels of vegetable oils in fish diets will decrease the concentrations of beneficial n-3 HUFA in fish fillets. Hence, fillet nutritional quality declines (Kaushik, 2004). Given this, using a "wash-out" feeding strategy just before harvesting is the best solution to enrich the flesh levels of beneficial n-3 HUFA (Ng and Chang, 2004). The fatty acid compositions of neutral lipids in fish muscle follow the feed quite closely than those of polar lipids (Jobling, 2001; Sargent *et al.*, 2002). A previous study reported that the dietary source and composition of fatty acids determine the fatty acid composition of the fish body (Sargent *et al.*, 2002; Lim and Webster, 2006; Yildirim *et al.*, 2007; Yeganeh *et al.*, 2012; Patterson and Gatlin, 2013). *Nannochloropsis* contains significant amounts of n-3 fatty acids, especially EPA. The inclusion of *N. oculata* in biofloc tanks produced significant changes in the fatty acid content of the fish at the end of the present study. The changes include higher levels of n-3 fatty acids; in the fish, the medium received biofloc+*N. oculata*. The composition of HUFA, like 20:5n-3 and 22: 6n-3 in Nile tilapia, is reported to depend on the fatty acid composition of the diets (Kanazawa *et al.*, 1980; Lim and Webster, 2006). Moreover, in the present study, the rate of production of HUFA in fish fillet at the end of the rearing was reflected by the composition of HUFA in the biofloc composition, which represented high HUFA in biofloc with algae, especially in Biofloc + *I. galbana* and Biofloc + Algal groups. The amount of SFA, MUFA, and PUFA in I.galbana were 32.3%, 22.6%, and 43.5%, respectively (Tibaldi *et al.*, 2015). A stepwise increase in the incidence of total SFA and MUFA in muscle was expected due to the relative abundance of the same fatty acids in the corresponding biofloc. Similar changes in the same fatty acid profile of the total lipid fraction in fish muscle tissue have been observed in other studies when increasing levels of palm oils replaced marine lipids in the diet of a vast range of fish species,

including salmonids and marine carnivorous teleosts (Ng and Gibon, 2011). Barclay and Zeller (1996) noted that *Schizochytrium* sp. has 24% DHA. Also, Arterburn *et al.* (2007) showed that 40% of the fat extracted from *Schizochytrium* sp. consists of DHA. In tilapia research, *Schizochytrium* sp. was added to feed and increased the body's amount of PUFAs, especially DHA content (Sarker *et al.*, 2015). Compared to the other sources, this source increases the amount of DHA in these fish's bodies by 100 g of Schizochytrium sp. Enriching live food (such as rotifers and *Artemia* nauplii) with *Schizochytrium* sp., DHA levels in tilapia increased by 10.5 percent due to replacing high DHA supplementation in fish feed (Barclay and Zeller, 1996).

Bacterial

It is well known that the intestinal microbiota acts as the host's "second genome" or "extra organ" and is essential to maintaining host health (O'Hara and Shanahan, 2006). The relative abundance profile of the intestinal microbiota in different hosts has been the subject of prior research (Li *et al.*, 2015; Zhernakova *et al.*, 2016). No particular patterns when comparing the eukaryotic or prokaryotic microbial communities in any samples; differences were found in the number of reads, species richness, or species diversity (Yun *et al.*, 2022). These findings confirm earlier research (Sanjit and Bhatt, 2005) that found no relationship between species diversity, species richness, or community scale.

Furthermore, no overt connections between the prokaryotic and eukaryotic microbial communities were discovered in the samples (Santi *et al.*, 2019). However, the distribution of intestinal microbiota in aquatic vertebrates is unknown regarding absolute abundance. A prior 10^8–10^{10} CFU/mL study demonstrated the intestinal bacterial densities of seven freshwater fish species by direct microscopic clump counts (DMCC) (Sakata *et al.*, 1980). The current study found the highest total concentration of intestinal microbial in the tank biofloc, whereas the lowest concentration was found in the control group. Another research reported that the bacterial density of rainbow trout intestine varied from 1.0×10^7 CFU/mL to 1.8×10^8 CFU/mL at different sampling dates by DAPI direct counts (Huber *et al.*, 2004). In this study, the intestinal bacterial density of farmed Nile tilapia (*Oreochromis niloticus*) was more than 15×10^{10} CFU/mL. It was inconsistent with the previous study, which showed that the bacterial density of 5.0×10^9 bacterial CFU/mL was detected in the intestines of tilapia by direct microscopic clump counts (DMCC) (Sakata *et al.*, 1980). In ponds that use biofloc systems, microbial organisms can break down ammonia, and other nitrogen compounds can be converted to safer compounds for aquatic life (Abakari *et al.*, 2020). This function is carried out by bacteria participating in certain nitrification and either through the denitrification procedure or an algal group using photosynthesis to create organic compounds with nitrogen (Ray *et al.*, 2009; Abakari *et al.*, 2020; Luo *et al.*, 2020). In terms of mesophilic and lactic acid bacteria in the tilapia gut, the results of the present study demonstrated that biofloc tanks have relatively large-

scale microbial communities without algae. As a result, Ray *et al.* (2009) and Abakari *et al.* (2020) predicted that the biological mechanisms involved with nitrogen compounds would differ depending on the microbial community's composition. The bacterial group would be crucial to the mechanisms relating to nitrogen compounds when zooplankton or other closely related organisms predominate in microbial communities, as in all biofloc groups (Ray *et al.*, 2009; Abakari *et al.*, 2020). In contrast, the algal group would control the metabolic process in situations where it makes up the majority of the microbial community, such as in biofloc groups with algal, where algae also make up more than half of the entire microbial community (Ray *et al.*, 2009).

The media fish/shrimp (water) accumulated more types of bacteria than samples from the aquatic animals' intestines. Reports of higher bacterial diversity in aquaculture systems for clear water and biofloc than observed in the shrimp's intestine *Litopenaeus stylirostris* (Cardona *et al.*, 2016). The digestive tract is less aerobic, and resident flora, acids, bile salts, enzymes, and mucus create an unfavourable environment for many bacteria and discourage colonization (Cain and Swan, 2011). The colonization of bacteria in the digestive tract relies on the ability of bacteria to survive in different regions of the tract (Harris, 1993). At this point, the posterior region of the Nile tilapia intestine represents approximately 6.5% of the total intestinal length (Smith *et al.*, 2000).

Among the microbial species that mainly constituted the microbial communities of the studied tilapia tanks, the two algal species were *N. oculata* and *I. galbana*, which are photosynthetic floating microalgae (Li *et al.*, 2023; Resende *et al.*, 2015). They absorb nitrogen sources, such as ammonia, and synthesize protein through metabolism, including photosynthesis (Slegers *et al.*, 2013; Mostafaei *et al.*, 2023). Therefore, the algal species in these tilapia tanks are expected to play significant roles in the nitrogen cycle and as producers in the ecosystems of the microbial community and tilapia tanks (Cheng *et al.*, 2023; Mostafaei *et al.*, 2023). As the results showed, in the tilapia tanks using biofloc technology, the microbial communities differ in terms of the major species in each experimental group due to the presence of algae. Still, they seem to have common features in terms of their function and ecological role. However, as the bacterial group consists of various major species, relatively clear differences exist among microbial communities with the function of bacterial species (Abakari *et al.*, 2020). Generally, the studied microbial communities had similarities in nitrogen-related metabolism and ecological roles, despite observed differences in environmental factors and the composition of microbial communities. Thus, this study proposes that various metabolic processes are conducted by various species from the bacterial group, which plays many roles in tilapia intestinal with biofloc systems. In the current study observed, biofloc groups had a more significant number of bacteria in the tilapia gut than the control, because the number of bacteria in biofloc media was more than the control medium.

Conclusions

The current study generally demonstrated that tilapia produced when cultured in vitro using biofloc; could promote growth performance, an unsaturated fatty acid in fish fillet (HUFA and PUFA), and bacteria compared to the control group. Although using algae in a biofloc system could not affect on growth performance during the 60-day experimental period compared to the biofloc system, it seems that improved growth performance could be obtained if the experimental period was extended. Fatty acid profiles are improved and more noticeable in complex algae compared to other biofloc treatments and control groups. The tilapia cultured in biofoc+algae could be recommended for improving human health because of the effect of HUFA and PUFA on health and the prevention of some diseases. This study confirmed a direct influence of the dietary composition of biofloc on fatty acid composition of different tissues of *O. niloticus*. Biofloc inclusion of algal in the system resulted in the elevated levels of body n-3 and n-6 fatty acids, suggesting a possible utilization of algal in biofloc system of fish. Moreover, the influence of supplemented algae in the biofloc system on the immune and antioxidant condition of Nile tilapia remains open to question, and it should be surveyed in future studies.

Competing interests

There is no conflict of interest to declare by the authors.

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