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Effects of Biofloc on Water Quality and Growth Performance of the Nile Tilapia (*Oreochromis niloticus*) in a Zero-Water Exchange System

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ABSTRACT
The use of biofloc technology (BFT) can enhance aquaculture productivity, which promotes the attainment of sustainable development goals. With the help of this technology, production might increase while reducing environmental effects. The current study was administered to determine the impact of efficient microorganisms, carbohydrate sources such as molasses and starch, and their mixture in a biofloc (BFt) system on water quality and the growth performance of the Nile tilapia (*Oreochromis niloticus*). A 45-day experiment employing two carbon sources, molas and starch, was carried out to examine the effects of various biofloc systems. A total of 180 healthy Nile tilapia (*Oreochromis niloticus*), with an average body weight of 5.3 ± 0.6g were stocked in each [tank (15 fish/ replicate). Four experimental treatments were designed as follows: T1: control; fish were fed a basal diet without any addition of carbohydrate source. T2: supplementary molas; T3 supplementary starch. T4: with supplementary the mixture from molas and starch. Fish were distributed randomly into twelve circular tanks. Measures of the biofloc conditions in the current investigation were temperature, DO, pH, conductivity and ammonia. Dissolved oxygen concentrations, pH, and ammonia were considerably greater in the biofloc groups compared to the control group; whereas, water temperature and conductivity were not statistically different between groups. Statistically significant ($P \leq 0.05$) differences in the final weight, weight gain, survival rate, SGR, condition factor and FCR were recorded in all treatments compared to the control. Finally, the findings suggested using a combination of useful microbes, molas, and starch as a carbohydrate source in fish water tanks for the optimum effects on tilapia culture raised under a zero water exchange system.

INTRODUCTION

One of the world's fastest-growing food-producing sectors is aquaculture, which is growing at a rapid pace (**Habib *et al.*, 2020**). It is essential for eradicating hunger, boosting health and reducing poverty in underdeveloped countries (**FAO, 2014; Hwihy *et al.*, 2021**). Additionally, aquaculture is a crucial industry for supplying the world's demand for animal protein and will become even more significant as the population of the planet rises (**Jackson, 2007; Magouz *et al.*, 2021**). The aquaculture industry's current global growth rate (8.9-9.1% per year since the 1970s) is essential to adapt to the problem of protein food shortages, which is predominantly present in the developing countries (**Subasinghe, 2005; Gutierrez-Wing & Malone, 2006; Matos *et al.*, 2006; Magouz *et al.*, 2021**). The aquaculture industry in Egypt produces the most farmed fish in Africa (**FAO, 2016; Fitzsimmons, 2016**). Aquaculture must, however, continually contend with a number of issues, including those related to the environment, the economy, the scarcity of materials and the high cost of those ingredients **(De Schryver *et al.*, 2008)**. Therefore, methods to address some of the afore-mentioned problems are needed. Some of the comparatively basic options include biofloc technology (BFT), a novel alternative that eliminates the prior issues and restrictions and revolutionizes aquaculture (**Avnimelech, 2006**). BFT is regarded as a new "blue revolution", based on little to no water exchange and minimising environmental discharges of nutrient-rich effluents (**Walker *et al.*, 2019; Awad *et al.*, 2021**). For zero-water exchange culture systems, biofloc technology (BFT) has lately grown in favor as a more sustainable and long-term alternative (**Avnimelech, 2007; Azim & Little, 2008; De Schryver *et al.*, 2008; Crab *et al.*, 2009**). In this regard, aquaculture water quality parameters have been altered to encourage the growth of some biotic communities in order to increase nutrient recirculation and to use the biomass of such biotic communities as a direct food source for the commercial organisms being cultivated (**Martinez-Córdova *et al.*, 2015**). This lowers maintenance costs and adds value by increasing the rate at which food is consumed (**Azim & Little, 2008; Castro-Nieto *et al.*, 2012**). Biofloc reduces pond water exchange by removing fertilizer from the water and fostering microbial biomass (heterotrophic bacteria) that need ammonia for growth (**Sharma & colleagues, 2015**). BFT has garnered a lot of attention recently for its ability to increase production yield, enhance water quality and recycle feed and protein production in the same culture unit (**Avnimelech, 2006; Crab *et al.*, 2007: Little *et al.*, 2008; Zaki *et al.*, 2020**). To maintain the highest level of heterotrophic bacterial activity, simple carbohydrates like molas must be supplied to the tank on a regular basis for they are easily broken down by bacteria (**Yassien *et al.*, 2021**). However, since bacteria take their time to break down complex carbs including cellulose and starch grains, the level of carbohydrates in the tank remains stable, and the reaction time is prolonged (**Avnimelech, 2009; Serra *et al.*, 2015; Yassien *et al.*, 2021**). Due to its affordability, molasses is frequently and effectively used in shrimp culture ponds to encourage bacterial growth (**Martinez-Cordova *et al.*, 2014; Yassien *et al.*, 2021**). An essential instrument for fishery management is growth performance. It is a crucial factor in determining fish culture success. One of the most crucial elements affecting any farmed fish's capacity to express its genetic potential for growth is nutrition. Additionally, they are significantly impacted by elements, viz. fish behavior, feed quality, daily ratio size, feed intake and water temperature (**Bascnar *et al.*, 2007; Ali *et al.*, 2015**). In addition, the criteria of water quality are crucial for preserving the wellbeing of aquatic animals and serve as a limiting factor (**Khanjani *et al.*, 2020, 2021**). Tilapia is a farmed species that is being produced intensively everywhere in the universe. In 2012, the world tilapia production reached 4.5 million MT (**FAO, 2014**), with eminent anticipations to extend more exponentially in the future. Tilapia intensification is a prime objective to deal with the increased demands and consumption of aquatic animal protein. Clarification of the impact of carbon sources with varying degrees of complexity on tilapia performance, and zooplankton community is still needed. Therefore, the current study sought to examine the impact of carbon sources (molas and starch) on the evaluation of growth performance and the analysis of water quality.

MATERIALS AND METHODS

The current experiment was carried out at the Aquaculture Technology Laboratory, Faculty of Fish and Fisheries Technology, Aswan University, Aswan, Egypt. We investigated the effects of dietary carbon sources on water quality and growth performance of the Nile tilapia, *Oreochromis niloticus*, grown in a biofloc system.

Experimental design

A total of 180 healthy live Nile tilapia (*Oreochromis niloticus*) with an average body weight of 5.3 ± 0.6g were obtained from Sahari *hatchery*, Aswan, Egypt. Fish individuals were kept in a recirculation system to ensure optimum water quality, and the system was thermostatically controlled at 21 ± 3°C. The study was conducted in twelve circular tanks (300 L each) filled with 250 L dechlorinated water. Before the experiment, fish specimens were acclimatized in a laboratory for four weeks before being divided into four equal groups including a control group, with three replicates each (15 fish/replicate). The first group, which served as the control, received a diet with a base level of 30% crude protein (CP). The second, third, and fourth groups consumed the 30% CP basal diet, which contained molas, starch, and mix (molas + starch) as the sources of carbohydrates, respectively. All fish were fed twice a day, at 8:00 am and 4:00 pm, with 7% of their body weight. They were raised for 45 days from the 1st of December 2021 to the 14th of January 2022.

Water quality monitoring

Throughout the study period, daily and weekly measurements of the water's temperature (°C), dissolved oxygen (DO, mg L^{-1}), pH, conductivity, and ammonia were achived using thermometers; portable digital pH metres (Martini Instruments Model 201/digital), and waterproof portable BOD and DO metres (model Hanna waterproof IP67), adding to the total ammonia-nitrogen (TAN, mg L^{-1}) according to **El Sayed (2006)**.

Growth performance parameters

All growth parameters were determined at 15 days intervals. After the feeding trial, the growth parameters, such as initial weight, initial length, final weight gain (FG), total weight gain (LWG), specific growth rate (SGR), condition factor (K), feed conversion rate (FCR), and survival rate (SR) were individually determined using the following equations:

Final weight (FW) = final fish weight (g) – initial fish weight (g).

Specific growth rate (SGR %) = log FW – log IW/ t * 100, Where FW is the final weight of fish (G). Where IW is the initial weight of fish (G), t = total number of experimental days.

Feed conversation ratio (FCR) = feed intake (g) / weight gain (g).

Survival rate (%) = Number of live fishes * 100/Total initial number of fish

Statistical analysis

A database was created using Excel 2013 software to make first analysis using descriptive statistics. Data were presented as mean±SD (Standard Deviation). The results were subjected to a one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance (parameters presented in Tables 1, 2 and 3). Data were analyzed using SPSS (1997) program, Version 16. Differences between means were compared using Duncan's (1955) multiple range tests at $P< 0.01$ level.

RESULTS

Water quality

The average values of the parameters measuring the water quality are shown in Table (1) (temperature, dissolved oxygen, pH, conductivity and total ammonia nitrogen). Temperature (20.2±3.4-21.23±3.58) and conductivity (266.88±37.8-279.8±36.2) of the water quality parameters assessed throughout the experimental period did not reveal any appreciable variations. However, a substantial difference was detected between the control group and other treatments in terms of dissolved oxygen (6.8±1.1-7.6±0.96), pH (7.2±0.18-7.9±0.55) and ammonia (0.81±0.28-1.03±0.17). All parameters of water quality showed tolerable levels for the rearing of *O. niloticus* in Biofloc (Table 1).

Growth performance

The growth performance parameters were investigated every 15 days during the experiment. The average of initial weight of fish was 5.3 ± 0.6g. Statistically significant ($P\leq 0.05$) differences in final weight, weight gain, survival rate, SGR, condition factor and FCR were recorded in the all treatments, compared to the control (Table 2). Fish reared in molas showed the lowest final weight (17.82g), weight gain (12.52g) and SGR (2.29%/day). The highest FCR (1.60) was obtained in the molas group and showed a significant difference with other treatments and the condition factor in molas + starch (12.46). No mortality was observed during the whole time of the experiments, neither in the control group nor in other treatments (SR = 100%) (Table 2).

Table 1. Water quality parameters in rearing tanks of the Nile tilapia *Oreochromis niloticus* under different carbon sources during 45 days of experiment

Parameter	Control	Molas	Starch	Molas+Starch	Range
Temperature	21.23±3.58	21.02±3.25	21.1±3.6	20.2±3.4	(16 – 32)
Do	6.8±1.1^{a}	6.83±1.03 ^{a}	7.3±0.91^{b}	7.6±0.96^{b}	(5.8 – 8.5)
PH	7.2±0.18^{a}	7.6±0.11^{b}	7.5.1±0.18^{b}	7.9±0.55^{ab}	(6.5 – 7.8)
Conductivity	279.8±36.2^{a}	266.88±37.8^{a}	276.4±35.9^{a}	275.9±29.8^{a}	(210 – 230)
Ammonia	1.03±0.17^{b}	0.93±0.19^{b}	0.88±0.19^{a}	0.81±0.28^{ab}	(0.5 – 1.3)

Different letters in rows indicate significant differences (P < 0.05).

Table 2: Growth performance of the Nile tilapia, *Oreochromis niloticus*, as affected by carbon sources of the biofloc system at the end of the experiment

Parameter	Control	Molas	Starch	Molas+Starch
Initial weight (g/fish)	4.7±0.47	5.3±0.41	5.9±0.71	5.3±0.41
Final weight (g fish)	17.2±2.51^{a}	17.82±1.95^{a}	20.25±3.77^{b}	23.42±2.22^{b}
Total weight gain (g fish)	12.5±2.28^{a}	12.52±1.58^{a}	14.35.25±3.79^{b}	18.12±2.21^{b}
Specific growth rate SGR (% day-)	2.13±0.12^{a}	2.29±0.06^{a}	2.94±0.29^{b}	2.70±0.11^{b}
Condition Factor (K value)	1.03±0.14^{a}	6.07±1.8^{b}	1.13±0.16^{a}	12.46±6.40^{ab}
Feed conversation ratio FCR	1.67±0.08^{b}	1.60±0.02^{b}	1.45±0.04^{a}	1.49±0.02^{a}
Survival rate (%)	100%	100%	100%	100%

Different letters in rows indicate significant differences (P< 0.05).

DISCUSSION

Many studies have shown that different microorganisms assembled in biofloc systems play an important role in removing nitrogenous waste, providing food, nutrition, and overall health (**Ju *et al.*, 2008; Ray *et al.*, 2010b; Emerenciano *et al.*, 2013a, b, c; Cardona *et al.*, 2016; Ahmad *et al.*, 2017; Becerril-Cortés *et al.*, 2017; Daniel & Nageswari, 2017**). Additionally, **Khatoon *et al.* (2016)** and **Kamilya *et al*. (2017)** reported that, the existence of microbial floc and the system's ability to maintain water quality may be responsible for the improvement of growth performance in a biofloc system. According to **Ekasari (2014)**, compared to non-biofloc systems such as traditional and recirculating aquaculture systems, the biofloc systems can increase net productivity by 8 to 43%. More importantly, the biofloc nutrient-rich waste can be used as a feed in BFT to reduce environmental difficulties in the aquaculture business. The present study supported the positive impacts of enhanced bioflocs on the Nile tilapia (*Oreochromis niloticus*) water quality and growth performance in a zero-water exchange system. According to these results, replacing the commercial feed in the BFT system with a carbohydrate such as molas or starch promotes the population of heterotrophic bacteria, which enhances water quality and growth performance. The criteria of water quality are crucial for preserving the wellbeing of aquatic animals and serve as a limiting factor (**Khanjani *et al.*, 2020, 2021; awad *et al.*, 2021**). The Biofloc method raises the quality of water by adding additional carbon, which encourages bacteria to take up nitrogen and proliferate, reducing the ammonium content to simpler chemicals more quickly than nitrification (**Kumar *et al.*, 2019; awad *et al.*, 2021**). Exchanging the water in the culture system is essential to maintain the properties of water quality from deterioration. Frequently, using techniques with minimum or zero-water exchange increases nitrogen levels in water (**Iwama *et al.*, 2000; Randall and Tsui, 2002; Zaki *et al.*, 2020**). In the present study, the water quality variables were within the normal range tilapi, as recommended by **Popma and Lovshin. (1995).** The best temperature for biofloc was between 18 ºC to 20 ºC (**De Schryver and Verstraete, 2009**). The present study indicates that the water temperature values in all treatments were higher than the optimum level for biofloc. However, it was still tolerated by the cultured Nile tilapia. Temperature and conductivity were found to not differ substantially between the treated and control groups. Both parameters fell within the permitted limits for floc formation for Nile tilapia farming. DO values remained unchanged throughout the period above 5 mg L^{-1}. According to **Fang *et al*. (2018),** low concentrations of DO in BFT can occur due to the respiration of microorganisms, fish, and prawns. The metabolic activity of heterotrophic bacteria can be adversely affected by DO concentrations below 4 mg L-1, which did not occur in the current investigation and had no impact on the performance of the animals or the formation of the bioflocs. **Thilakan *et al.* (2019) and Hisano *et al.* (2019),** who measured DO between 6 and 7.8 mg L^{-1} during the study period, supported this finding. In contrast, according to **Hwihy *et al.* (2021**), there was less DO in the biofloc than there was in the control. Due to the inhibition of nitrification at low pH, the pH has an impact on the amount of nutrients in the water (**TANG and CHEN, 2015; ZOU *et al.*, 2016).** The pH in this investigation was within the acceptable limit. The pH levels in the treatment groups were found to be significantly different from the control group; this result was verified by **Awad *et al.*, 2021**, who assessed pH levels between 7.7 and 7.9. The Biofloc system is comprised of heterotrophic, autotrophic, and photoautotrophic microorganisms (**BURFORD *et al.*, 2003),** though the increase and prevalence of heterotrophic bacteria are desired to promote the decomposition of organic matter, to reduce the total ammonia nitrogen levels, and to provide an additional food source for animals (**AVNIMELECH, 1999**). In the current study, all treated groups had ammonia levels that were noticeably lower than the control group. The starch-only group displayed the lowest level, followed by molas+starch. The greater absorption and breakdown of carbon as a substrate for heterotrophic bacteria that metabolise ammonia and hence enhance water quality is likely the cause of the faster decrease of ammonia that was observed in the molas and molas+starch groups as simple carbon sources (**Khanjani *et al.*, 2017; El-Shafiey *et al.*, 2018; Khanjani *et al.*, 2021; Awad *et al.*, 2021**). In accordance with this study, the organic carbon source in the biofloc system lowers the total ammonia level and boosts the diversity of microbial communities and ammonia-oxidizing bacteria. Although earlier research has shown that the BFT system has a good impact on fish output, it is still unknown how this improves growth performance. According to one concept, biofloc is a constant in-situ supplemental food source that can give fish extra protein (essential amino acids), polyunsaturated fatty acids, vitamins, and minerals (**Avnimelech, 1999, 2007; Wasielesky *et al.*, 2006; Azim and Little, 2008; De Schryver *et al.*, 2008; Luo *et al.*, 2014**).

The final weight, weight gain, SGR and FCR were shown to have increased significantly ($P > 0.05$) in the current study compared to the control group. The findings of the current research are consistent with those of previous studies on *Oreochromis mossambicus* (**Avnimelech, 1999 and 2007**), *Macrobrachium rosenbergii* (**Asaduzzaman *et al.*, 2008**), *Litopenaeus vannamei* (**Burford *et al.*, 2004; Xu *et al.*, 2013**), *Marsupenaeus japonicas* (**Zhao *et al.*, 2012; Wang *et al.*, 2015**). Regarding BFT's beneficial impacts on growth performance, these findings showed that Nile tilapia can adapt well to novel dietary conditions and that microbial flocs promoted the development and/or activity of digestive enzymes, improving nutrient digestion in the gut and possibly explaining the fish's enhanced feed utilisation and growth performance (**Moss *et al.*, 2001; Xu *et al.*, 2012a, b; Xu and Pan, 2012).** According to numerous authors, the growth performance of shrimp or tilapia was unaffected by the addition of various carbon sources to the biofloc system. The addition of molas, sugar, or cassava starch had no impact on the growth performance of tilapia cultivated in a biofloc system, while both molasses and sugar had a favourable impact on the production of microbial floc (**Silva *et al.*, 2017). Serra *et al.* (2015)** made the same claim for shrimp raised in a biofloc system while looking at various carbon sources (molasses, sugar, dextrose, and rice bran). According to **Khanjani *et al.* (2017),** the addition of molasses, starch, wheat flour, or a combination of them to the biofloc system did not significantly affect the growth of the shrimp. In general, the Nile tilapia fish reared in BFT did not grow differently depending on the source of carbon. Growth in the current study was constant across all carbon sources and the control. The results, however, imply that the usage of molas+starch produced the best growth of Nile tilapia in BFT system. No mortality was observed during the whole time of the experiments, either in the biofloc treatments or control group. (SR= 100%). Under the influence of different carbon sources, the survival rate was reported to be 100% for *O. niloticus* (**Mirzakhani *et al.*, 2019**). It was shown that due to the presence of immune stimulant compounds such as lipopolysaccharide in the wall of biofloc bacteria as well as the presence of antioxidants in biofloc, the immunity and survival in the cultured species increased (**Kim *et al.*, 2014; Walker *et al.*, 2020).** Bioactive compounds in the biofloc improve aquatic survival, which is possibly because of the amount of essential amino acids, fatty acids, and other nutritional compounds (**Xu and pan, 2013**).

CONCLUSION

The success of the biofloc system is significantly influenced by the source of organic carbon that is selected. The results of the current study confirmed the positive effects of biofloc on water quality and growth performance in Nile tilapia with zero-water exchange. However, it is vital to conduct further studies on enhancing the efficiency of nutrient utilisation, whether through cultivating animals directly or indirectly by trapping nutrients and making them available for other uses.

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