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Original Research Article

EVALUATION OF GROWTH AND SURVIVAL OF *OREOCROMIS NILOTICUS IN* A BIOFLOC SYSTEM

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Abstract: *The global demand for Nile tilapia (Oreochromis niloticus) is rapidly increasing. To meet this demand, a green technology known as fast biofloc was introduced, although its intensive application on an industrial scale remains limited.* ***Objective:*** *This study aimed to evaluate the effects of advanced biofloc technology (BFT) on water quality parameters, growth, and survival of Nile tilapia to optimize water quality and maximize overall tilapia growth.* ***Methods:*** *A 90-day experimental study was conducted from May to July 2021 at the Fish Biodiversity Hatchery Chashma, district Mianwali, Punjab, Pakistan. The experiment utilized a controlled and a bio floc treatment, each managed in 10,000 L outdoor tanks. A total of 1,670 Nile tilapia, with an average weight of 3.2 g and length of 2.2 cm, were stocked in each tank. The BFT and control tanks were fed a diet containing 30% crude protein (CP) at 20% of the total fish biomass daily. Additionally, the BFT tank received probiotics, molasses, and salt. Critical parameters such as water quality, floc volume (FV), growth rate, and feed conversion ratio (FCR) were measured. Statistical analyses were conducted at a significance level of $p \leq 0.05$.* ***Results:*** *The nutritional quality of biofloc was suitable for tilapia, and no significant differences were observed in water quality parameters between the BFT and control tanks. Floc volume was only present in the BFT tank, measuring 28 g. The BFT treatment resulted in significantly higher growth rates and performance than the control ($p \leq 0.05$), with net fish production being 72% higher in the BFT tank. The average FCR was 2.2 in the BFT tank and 3.1 in the control tank, indicating more efficient feed utilization in the BFT system.* ***Conclusion:*** *Compared to the control, the bio floc system enhanced the Nile tilapia's water quality, growth performance, and survival rates. These findings suggest that biofloc technology is a viable and effective method for sustainable aquaculture, offering substantial benefits regarding fish growth and resource efficiency.*

Keywords: Aquaculture, Biofloc Technology, Stocking Density, Bacterial Aggregates, C/N Ratio

Introduction

According to FAO (2020), the world's human population is growing daily and should be increased to 9.6 billion on Earth by 2050 (1). Aquaculture is essential for developing countries like Pakistan. It is expected that its production, especially fish products, will increase to 82 million tons by 2050. The usage of fish is much higher than that of other types of meat worldwide. However, global aquaculture still faces a few severe challenges. Many effluents are discharged from the industries. All of these problems produce adverse effects on water parameters (2). Feed cost and protein requirements for the better growth of fish and other aquatic organisms are critical aquaculture challenges. To overcome these problems, aquaculture producers used biofloc technology, an excellent nutritious food source (3). Biofloc is inexpensive compared to the extremely expensive fishmeal. In shrimp culture, 15-30% conventional protein sources are required, which can be gained through bio floc meal without destructive effect on fish species (4). Several studies have indicated that biofloc is much better than fishmeal and other dietary sources such as soy protein, protein hydro-lysate, and lysine. It also improves cultured aquatic organisms' growth performance, survival, and immunity (5). The biofloc system was developed in the 1990s to utilize fish and supplementary feed wastes. It has produced a desirable fish yield. During the past eight years, it has flourished in India, Malaysia, Thailand, and Japan (6). Technically, it is an alternative to optimize the use of aquaculture feeds (7). The application of biofloc in aquaculture controls pathogens in the aquatic environment. A tremendous amount of water change is necessary in conventional fish farming, but in biofloc technology, limited or no water exchange is required. The absorption of nitrogen compounds is often responsible for preserving water quality. Compared to the standard approach, it creates in situ microbial biomass and reduces water pollution. It also enhances larval development, reduces mortality rate, and increases culture feasibility by lowering feed conversion ratio (FCR). It decreases the price of feed and also lowers competition for diseases (8). Compared to conventional aquaculture, biofloc is a good source of natural food, vitamins, and phosphorus for fish growth (9). In biofloc technology, the role of bacteria has fundamental importance. Heterotrophic and nitrifying bacteria coexist in complex biofilms (10). These groups of microorganisms are firstly used to change residues into microbial proteins that can be used as food for other organisms and secondly serve as a natural food source for animals in culture, so the quality of water and cultivation of fish species are improved (11). In this technology, ammonia is the primary waste product secreted by the cultivated organism and decomposes dissolved nonliving matter. The carbon-to-nitrogen ratio (C:

N) is essential in each aquatic environment to convert hazardous inorganic nitrogen molecules into helpful microbial biomass. Any change in the biofloc technology's carbon-to-nitrogen ratio might impact water quality. The bio floc's water quality parameters and composition alter dramatically when the C: N ratio is elevated, favoring heterotrophic bacteria (12). The carbohydrate content of feed and external sources in fish-rearing water may be altered to affect the C: N ratio (13). Sources with high carbon content are more effective in developing flocs because of their ability to form well-organized clusters. For example, molasses breaks down faster than complex carbohydrates like cassava (14). It is commonly utilized as a carbon source in biofloc technology systems to enhance water quality (15). Temperature (^{0}C), pH, alkalinity, dissolved oxygen (DO), salinity, and total dissolved solids are the main parameters of water that should be continually checked in biofloc systems. The comprehension, understanding, and interactions of these water parameters in the biofloc system are necessary to precede fish development and production cycle because the safety ranges of these parameters lead to health, growth, and the avoidance of mortalities (16). The current study aimed to investigate the production of fish (Nile tilapia) and growth performance by the use of 10,000 L water in an 810 cm^3 water area to assess the conversion of feces and residues of supplement feed into feed by microbiota in a biofloc system and to monitor the effect of probiotic bacteria on fish growth and water quality in biofloc system.

Methodology

The experiment was conducted at the Fish Biodiversity Hatchery Chashma, District Mianwali, Punjab, Pakistan.
Two cemented circular water tanks with a diameter of 540 cm containing a capacity of 10,000 L were used to run the experiment. One tank was used as a control tank, and the other was used as a bio floc tank. These tanks were in an open area with shade at the fish hatchery Chashma. A central pipe with a diameter of 5 cm was present in both tanks for the drainage of water and sludge when necessary. A complete aeration system was used to control the oxygen deficiency in the water tank. Probiotics were used in the bio floc tank to check their effect on the growth and survival of Nile tilapia (*Oreochromis niloticus*). A 100w Channel Blower (LP-100, AIR PUMP, China) was used for the aeration of fish throughout the duration of the experiment (24 hours). Five circular plastic pipes with a diameter of 2.5 cm were attached to an aerator and used in both control and biofloc tanks. At the end of each pipe, air stones were attached for proper aeration in water tanks. There was no water exchange in either tank; instead, it evaporated water.
One thousand six hundred and seventy (1670) Nile tilapia (*Oreochromis niloticus*) fishes at an average weight of 3.2 g and length of 2.2 cm were stocked in each control and bio floc circular water tanks in May 2021 as experimental fish. All these fishes were sex-reversal male tilapia, which were collected from a commercial fish farm of Fish Biodiversity Hatchery, Chashma district Mianwali. Floc was prepared for the bio floc tank by the addition of probiotics (200g) of Compro Company, Molasses (400g), and Salt (5kg) dissolved in 4-liter water for a 10,000 L water tank. All fishes were fed a commercial diet with 30% crude protein till the end of the 90-day trial. The feed was given to all fish three times a day. The feeding rates were 20 % body weight per day till the end of the experiment.
The system was checked at least two times a day. Special efforts were made to observe the tank and check the signs of the fish's unconventional behavior and any other problems. Mortalities were removed from the tank without being replaced. The dead fish were measured during sampling and recorded on the date.
The water quality parameters consisting of temperature (^{0}C), pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), Nitrite (NO_2), and Nitrate (NO3) were monitored on a daily basis in both tanks by using scientific instruments. YSI DO200 and YSI pH100 checked dissolved oxygen (DO) and pH. The HACH Fish Farming Test Kit checked TAN, NO2, and NO3 (Model FF-1A, Cat No. 2430-02, Lot A2146). Floc volume (FV) was measured by electronic balance. At the end of the experiment, the length gain (cm), weight gain (g), survival rate, mortality rate, and feed conversion ratio (FCR) were calculated.
Collected water quality and growth performance data were analyzed through an independent t-test using SAS software (version 9.1.). The level of significance was considered as $p \leq 0.05$.

Results

The mean values of water quality parameters under the influence of biofloc technology and control tank are presented in (Table 1). The average mean values of temperature in the control and bio floc tank were 27.71 ± 0.33 and 27.84 ± 0.28, respectively. There was no significant difference in control and biofloc tanks ($p \leq 0.05$). The average pH measurement in the control and biofloc tank was 8.15 ± 0.09 and 8.19 ± 0.07, respectively. There was no significant difference in control and biofloc tanks ($p \leq 0.05$). The average TDS level in the control and biofloc tank was 1.79 ± 0.05 and 1.83 ± 0.06, respectively. There was no significant difference in biofloc and control tanks. The average value of DO in the control and bio floc tanks was 5.36 ± 0.09 and 5.16 ± 0.08. There was no significant difference in both tanks ($p \leq 0.05$). The monitoring results of this study indicated that the total ammonia nitrogen (TAN) mean value was 0.65 ± 0.03 in the control tank and 0.64 ± 0.03 in the bio floc tank. The results of TAN in the biofloc tank were more significant than in the control tank ($p \leq 0.05$). More significant results have been recorded for the biofloc tank after five weeks compared to the control tank. The average measurement of dissolved inorganic nitrogen (NO_2) has been obtained (0.82 ± 0.03^a from the control tank and 0.54 ± 0.02b from the biofloc tank. The control tank has shown more significant results than the biofloc tank ($p \leq 0.05$). Mean values after four weeks were $1.07^a \pm 0.12$ in the control and 0.43b ± 0.03 in the bio floc tank, and in week 7, mean values were 1.03a ± 0.09 in the control tank and 0.43b ± 0.03 in the control tank, which showed more significant results in control as compared to bio floc tank. The flocs volume (FV) was not present in the control tank. The average value of FV has been recorded as 28.91 ± 1.53^a in the biofloc tank ($p \leq 0.05$).
The growth performance and feed utilization of Nile tilapia in biofloc and control groups are given in (Table 2). The maximum increased final average length was 15.41 ± 1.42b in the control tank and 22.72 ± 2.15a in the biofloc treatment tank. Biofloc treatment tank has shown more significant

results than the control ($p \leq 0.05$). The Average body weight increase was higher in the biofloc treatment tank than the control tank. The maximum gain in body weight of *O. niloticus* has been noted at 11.44 ± 1.49a in the biofloc tank and 6.95 ± 0.97b in the control tank. More significant results were observed in the biofloc tank compared to the control tank ($p \leq 0.05$). From the current study, the average feed conversion ratio (FCR) in the control and bio floc tanks was 2.18 ± 0.19 and 1.83 ± 0.13, respectively. The higher fluctuation in FCR was due to the use of more feed in both control and biofloc tanks.

Fish mortality was higher in the control tank than in the biofloc system (Table 3). The average values of survival of fishes were 1311.42 ± 21.87 and 1300.88 ± 26.49 in bio floc and control tanks, respectively. No significant difference has been found in the tanks ($p \leq 0.05$). So, the survival rate in the control tank was recorded as 60%, lower than that of the biofloc tank (72%) over the entire experiment. The average mean mortality of fish has been recorded as 14.58 ± 4.8 and 20.3 ± 4.82 in the bio floc and control tanks, respectively. No significant difference has been found in the tanks ($p \leq 0.05$). The average fish mortality (%) results in biofloc and control tanks were 28.43 ± 1.99 and 30.15 ± 2.76 respectively. There was no significant difference between the tanks ($p \leq 0.05$).

Table 1: Comparison of water quality parameters of Nile tilapia between biofloc and control tank

Parameters	Biofloc	Control	p-value
Temp°C	27.84 ± 0.28	27.71 ± 0.33	0.7682
pH	8.19 ± 0.07	8.15 ± 0.09	0.6886
TDS (mg/l)	1.83 ± 0.06	1.79 ± 0.05	0.6087
DO (mg/l)	5.16 ± 0.08	5.36 ± 0.09	0.1066
TAN (mg/l)	0.64 ± 0.03	0.65 ± 0.03	0.6711
NO_2 (mg/l)	0.54 ± 0.02^b	0.82 ± 0.03^a	<0.0001
Floc (g)	28.91 ± 1.53^a	0.00 ± 0.00^b	<0.0001

a-superscripts on different means within the row differ significantly at $p \leq 0.05$

Table 2: Comparison of growth performance traits of Nile tilapia between biofloc and control tank

Parameters	Biofloc	Control	p-value
Average Body Length (cm)	22.72 ± 2.15^a	15.41 ± 1.42^b	0.0061
Increased Body Length (cm)	1.32 ± 0.09^a	0.84 ± 0.04^b	<0.0001
Increased body Length (%)	10.03 ± 2.3	8.42 ± 1.29	0.5432
Average Body Weight (g)	120.54 ± 20.04	77.6 ± 11.94	0.0703
Increased Body Weight (%)	11.44 ± 1.49^a	6.95 ± 0.97^b	0.0142
Average Body Weight (%)	16.16 ± 2.19	14.34 ± 2.24	0.5631
Number of Fishes	1310.18 ± 21.93	1300.88 ± 26.49	0.7876
Fish Average Body weight (g)	120.54 ± 20.04	77.6 ± 11.94	0.0703
Total Body Weight (g)	146865.94 ± 23467.56^a	91410.24 ± 12428.44^b	0.0408
Increased in Weight (g)	13357.42 ± 1673.86^a	7147.18 ± 640.29^b	0.0010
Feed (20%)	29346.3 ± 4700.74^a	18064.76 ± 2473.85^b	0.0376
FCR	1.83 ± 0.13	2.18 ± 0.19	0.1205

a-superscripts on different means within the row differ significantly at $p \leq 0.05$

Table 3: Comparison of survival and mortality rate of Nile tilapia between biofloc and control tank

Parameters	Biofloc	Control	p-value
Survival of Fishes	1311.42 ± 21.87	1300.88 ± 26.49	0.7599
Mortality of Fishes	14.58 ± 4.8	20.3 ± 4.82	0.4031
Mortality %	28.43 ± 1.99	30.15 ± 2.76	0.6139

a-superscripts on different means within the row differ significantly at $p \leq 0.05$

Discussion

The current study demonstrated the valuable effects of microbial flocs on the growth performance of *O. niloticus* in a minimum water exchange system and improved water quality. Water quality parameters for the culture of any aquatic organisms are most important, especially in biofloc systems, for maintaining the health of such species, recycling of nutrients, and acting as a limiting factor (17). The present study revealed that the temperature was slightly larger in the biofloc tank than in the control tank, but it was within the suitable range of fish culture. No significant difference ($p \leq 0.05$) was found in biofloc and control tanks. The more substantial water temperature in the biofloc tank compared to the control tank was due to the activity of microbes in the biofloc tank. It was observed that the microorganisms within the biofloc system helped maintain water quality parameters by decreasing the concentration of nitrogen compounds (18). Similar findings were also reported in other studies (19, 20). This study is correlated with the work of El-Shafiey *et al.* (2018).

The result of this study indicated that the pH value was significantly lower but found in the normal range in the biofloc tank as compared to the control tank every week, according to the work of El-Shafiey et al. (2018). The decreased pH value might be due to the addition of carbohydrates in the medium, activation of heterotrophic bacteria to break down organic matter, and continuous respiration processes, degradation, nitrification, and assimilation carried out by activated microbes in the biofloc tank (21). In this study, the average concentration of TDS level slightly increased in the biofloc tank compared to that of the control tank. However, it has no significant difference ($p \leq 0.05$) in both the tanks. Similar findings were also reported in other studies, in which the TDS level was also increased in the biofloc tank compared to the control. The higher growth rates of heterotrophic bacteria and the higher microbial biomass were all due to decreased water exchange (22).

The present work represented the lower dissolved oxygen (DO) level in the biofloc tank compared to the control tank. But no significant difference ($p \leq 0.05$) was observed. In this study, the concentrations of nitrites (NO2) were significantly lower ($p \leq 0.05$) in the bio floc tank till the end of the experiment as compared to the control. Generally, this study showed that at the initial stage, the concentration of Nitrite (NO3) was at a lower level. Still, its value increased in the biofloc treatment tank over the control tank with time. Our findings are by Avnimelech (2015). The fluctuations in NO_3 concentrations might be due to the addition of carbohydrates in the bio floc tank (23).

The monitoring results of our study indicated that total ammonia nitrogen (TAN) decreased in the bio floc tank as compared to the control tank, with similar findings reported by Mirzakhani et al. (2019). The concentrations of total ammonia nitrogen (TAN) fluctuated from lower to higher levels due to the addition of carbon to nitrogen (C: N) ratio and rapidly produced heterotrophic bacteria (17). In this study, the growth performance (length and weight) in the biofloc tank was much better than in the control tank due to the probiotics present in the biofloc. Mirzakhani *et al.* (2019) also reported in their study that the survival rate of *O. niloticus* was 100%. In contrast to this study, Aghabarari et al. (2021) gave the highest yield in control treatment and a higher survival rate in biofloc technology. At the beginning of the experiment, the feed conversion ratio (FCR) was observed to be very low. Still, with the proceeding of the experiment, its range fluctuated in control and biofloc tanks. It was noted that the bio floc tank has a lower FCR than the control tank, according to results from Panigrahi et al. (2019). Khanjani *et al*., 2017 also stated that in the biofloc system, the growth rate of fish increased and FCR decreased compared to the control tank due to the use of different carbon sources (18). The floc volume (FV) in a biofloc system is a loose matrix of mucus that is secreted by bacteria and numerous microorganisms and bounded by an electrostatic force of attraction (22). In this study, the level of FV was significantly ($p \leq 0.05$) higher in the biofloc system as compared to the control tank. Moreover, it was observed that biofloc is a good source of probiotics and also contains advantageous probiotic effects in the aquaculture production system (Nguyen *et al.,* 2020). The present study revealed that feeding rates were 20% per body weight, which showed higher significant results ($p \leq 0.05$) in bio floc tank as compared to control that lowered FCR rate in bio floc tank. Our work was according to Aghabarari *et al.* (2021). In the current study, increased survival rates and lower mortality rates were observed in biofloc tanks. Biofloc maintains the water quality parameters as well as supplies more nutrition for the cultured species, which can increase the survival rate and decrease the mortality rate (24).

Conclusion

The increasing food demand of a rapidly increasing worldwide population, water scarcity, and a lack of area for aquaculture expansion are the main impediments to further development. Intensive aquaculture, such as biofloc technology (BFT), is one promising method for meeting the population's growing need for animal protein. The outcomes of the present study showed that the use of BFT in aquaculture improves water quality parameters like temperature, pH, DO, NO_2, NO_3, and TAN and produces beneficial effects on fish growth performance, survival rate, and FCR with the same fish quality at high stocking density (556 fish/m^3). Therefore, it could be argued that BFT would be the most excellent alternative for growth performance and production rate. It also acts as an effective and beneficial tool to control the nitrogenous waste products for sustainable aquaculture production of Nile tilapia (*Oreochromis niloticus*).

Declarations

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All data generated or analyzed during the study are included in the manuscript.
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Conflict of interest

The authors declared the absence of a conflict of interest.

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