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BIO-FLOC TECHNOLOGY AND ITS APPLICATIONS TOWARDS IMPROVEMENT AQUACULTURE PRODUCTIVITY OF TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURE: SOLUTIONS TO DOMESTIC AQUA CULTIVATION**

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ABSTRACT

Over the course of ten weeks, this study was investigated the effect of Bio-floc technology (BFT) on several parameters such as water quality, production performance, feed retention, and the health profile of tilapia (*Oreochromis niloticus*) in a zero-water exchange tank system. The findings show encouraging trends that validate the efficiency of BFT in maintaining optimal parameters for tilapia aquaculture. The BFT system maintained critical water quality parameters within recommended ranges for Tilapia Culture. The Specific Growth Rate (SGR) 6.64 % per day reflects significant growth rates, highlighting the effectiveness of the system. Furthermore, the average daily increase in fish length was 22.8 %. Keeping water quality parameters like total ammonium nitrogen (TAN), nitrite, nitrate, and chlorine within safe limits throughout the experiment ensured a tilapia-friendly environment. TAN levels remained consistently below the critical threshold of 0.5 mg/L, nitrite concentrations remained consistently below 0.5 mg/L, and nitrate levels remained significantly below the harmful threshold of 50 mg/L, promoting optimal nutrient utilization. Furthermore, there were no detectable levels of hazardous chlorine compounds. Adequate dissolved oxygen concentrations above 5 mg/L were maintained, ensuring sufficient oxygen. Overall, these findings support the potential of BFT as an environmentally sustainable approach providing optimal conditions for tilapia aquaculture environments.

Keywords: Bio-floc Technology, water quality, aqua culture, Tilapia.

INTRODUCTION

One of the most challenging difficulties ahead is meeting the nutritional requirements of a predicted world population of 8 billion individuals by 2030. This has intended to improve productivity and sustainable food production, thereby effectively satisfying global food demand. Over the course of over sixty years, it has been observable that the world's consumption of fish and fish products has increased significantly, surpassing the rate of growth in the worldwide population. Aquaculture has attracted a lot of attention recently because of its significant effects on the world's food supply. The technique known as aquaculture involves the breeding and nurturing of a variety of aquatic creatures. According to projections by FAO (2018), aquaculture will continue to be the primary catalyst of global fish production growth. By 2030, the estimated development in aquaculture output is predicted to reach 109 million tons, a significant rise of 32% (or 26 million tons) above the levels of production observed in 2018 (Ogello *et al*., 2021).

Aquaculture typically involves the discharge of substantial quantities of untreated water, which may result in water scarcity and contamination. Bio-floc technology (BFT) is an environmentally sustainable alternative to conventional aquaculture. Bio-floc technology is an approach utilized in aquaculture to improve water quality through the regulation of carbon and nitrogen within the system. In addition to its other benefits, the technology has recently garnered interest as a sustainable approach to water quality management that generates proteinaceous feed on-site (De Schryver *et al*., 2008). Bio-flocs technology (BFT) is an environmentally sustainable technology that operates by facilitating the large-scale cultivation of microorganisms in their natural habitat. The microbial components play a significant role in the natural establishment and stability of a heterotrophic microbial community. These microorganisms have significant functions in various aspects: (i) they contribute to the preservation of water quality by absorbing nitrogen compounds and producing microbial protein on-site; (ii) they enhance the feasibility of culture by improving feed conversion ratio (FCR) and reducing feed costs; (iii) they compete with pathogens; (iv) they help maintain favorable water quality; (v) they contribute to biosecurity measures; and (vi) they aid in the sequestration of greenhouse gases BFT is seen as the emerging "blue revolution" due to its ability to facilitate the continuous recycling and reuse of nutrients in the culture medium, hence minimizing or eliminating the need for water exchange (Emerenciano *et al*., 2017).

The carbon-nitrogen ratio, the microbial community, the nutritional content, the dietary stimulant, and the immuno-physiological all have important relationships with Bio-flocs. the Microbial Community and the CN ratio

*Dr. Kashif Ahmed; Department of Chemistry, N.E.D. University of Engineering & Technology, Karachi 75270, Pakistan. Phone No: 03012275358; ** Paper is based on MS thesis are the most significant (De Schryver *et al*., 2008). In minimum or zero water exchange systems—also referred to as intensive systems—two main functional categories of bacterial populations are essential for preserving water quality. These include chemoautotrophic nitrifying bacteria and heterotrophic ammonia-assimilating bacteria. Phytoplankton, bacteria, and aggregation of living and dead particulate organic matter make up microbial ensembles. Furthermore, the components that make up the Bio-flocs are diverse. Diatoms such *Thalassiosira*, *Chaetoceros*, and *Navicula* made up the majority of phytoplankton, which made up 24.6% of the total composition. Three percent of the Bio-flocs were made up of bacterial biomass, of which roughly two thirds were gram-negative and one third were gram-positive. It was determined that a protozoan population consisting of 0.5% amoeba, 1.5% rotifers, and 98% flagellates was responsible for a minor percentage of the bio-flocs. Furthermore, 33.2% of the bio-flocs were made up of detritus, and 39.25% of the composition was made up of ash in the remaining fraction (De Schryver *et al*., 2008).

Immobilizing toxic inorganic nitrogen compounds is facilitated in part by the carbon-nitrogen ratio (C/N) in the aquatic environment. An autotrophic system can become heterotrophic due to variations in the carbon-to-nitrogen ratio. The contents of nitrite nitrogen (NO_2-N) and total ammonia nitrogen (TAN) can be effectively controlled by either autotrophic nitrification or heterotrophic assimilation after a fully grown Bio-flocs community is established. Even at higher stocking densities, this management helps keep TAN and NO_2-N concentrations within acceptable bounds for the organisms under cultivation. The BFT (Bio-flocs Technology) system's zero-water exchange mechanism causes nitrate to progressively build up over time (De Schryver *et al*., 2008).

Several studies have provided evidence of positive outcomes in the areas of brood stock maintenance, larval production (Ekasari *et al*., 2015), enhancement of the immune system (Long *et al*., 2015), improved absorption of nitrogen and phosphorus (Avnimelech and Kochba, 2009), and subsequently, a reduction in food conversion rate. Various approaches to organic fertilization have been suggested for the purpose of Bio-floc formation in the context of Nile tilapia production. These strategies encompass the utilization of diverse carbon-to-nitrogen ratios (Pérez-Fuentes *et al*., 2016) as well as different carbon sources (Crab and Verstraete, 2012).

Under limited or no water exchange, the focus of this study is on the growth performance, feed efficiency, nitrogen retention, water quality, hematological profile, nutritional composition of Bio-floc, and high production rates of Tilapia (*Oreochromis niloticus*).

MATERIALS AND METHOD

Materials and Chemicals

A total of two 120-liter virgin polypropylene (PP) barrels were utilized, exhibiting both durability and environmental friendliness. The Experiment also employed a fish probiotic containing a diverse range of bacteria and enzymes, including *Bacillus subtilis, Bacillus Licheniformis, Enterococcus faecalis, Bacillus Pumilus* and *Bacillus Magaterium*. The carbon source utilized in the system was sugar cane molasses. The maintenance of water quality, specifically nitrogen compounds, was determined using a 12:1 (C: N) ratio, TAN concentration, and carbon quantity of molasses. The experiment involved no water exchange in the BFT system.

Fish, Experimental Conditions, And Feeding Cycle

Tilapia (*Oreochromis niloticus*), was utilized in our experiment due to its notable characteristics, which include an omnivorous diet, resistance to nutrient cycling diseases, rapid growth rate, and ability to thrive in diverse environmental conditions. The experimental conditions were established based on the ambient temperature of Karachi from May to August, resulting in an average temperature range of 30°C (86°F) to 35°C (95°F).

Calculations And Statistics

Calculations and statistics were used to calculate the survival cycle and growth cycle of Tilapia as reported in literature (Azim and Little, 2008; Anand *et al*., 2014; Emerenciano *et al*., 2017; Martins *et al*., 2019).

Assessment Of Water Quality Parameters

The water parameter measurements were undertaken at regular intervals of three days. The criteria encompassed in this study comprise total ammonia nitrogen (TAN), pH, nitrite, dissolved oxygen, chlorine, and the water hardness test. The tests in this study were performed utilizing testing materials obtained from Wuhu Jinghui Biotechnology Co., Ltd. on a tri-day basis. A comprehensive series of tests was completed during the 10-week investigation. If the parameters do not adhere to the substantial constraints that have been discussed in Emerenciano *et al*. (2017) changes were made in the constitutes and the parameters.

Bio-floc reactor and Cultivation Unit

We utilized approximately one hundred liters of water in the cultivating device for the experiment, resulting in a Bio-floc-to-water ratio of 1%. 120 grams of molasses, 12 grams of aquatic microorganisms, and 1.2 grams of sodium comprised the Bio-flocs (Azim and Little, 2008; Anand *et al*., 2014).

Cultivation Unit

The Unit for Bio-floc reactor effectively utilized Following three days of forming multicultivation flocs, as in the Bio-floc reaction, these flocs were introduced to the cultivating unit (Fig. 1). The unit dedicated to cultivation was Tilapia.

Fig 1. Bio-floc Cultivation Unit, The Figure indicates the Bio-floc introduced in the Cultivating Unit.

RESULTS AND DISCUSSION

Growth & sustainability

Weight and length of the fish were recorded on a weekly basis, and the condition factor (K) was computed. The SGR (specific growth rate) was calculated using the data that was gathered. The calculated SGR was approximately 6.64% per day on average identified which signifies substantial rates of growth. The aforementioned value illustrates the efficacy of the zero-water exchange system in facilitating the growth of tilapia. The tilapia within the system demonstrated significant expansion in length, averaging around 22.8 % weekly growth (Fig 4, 5). This result highlights the efficacy of the system in facilitating the development of fish. The condition factor of the tilapia varied between 1.2 and 2.1, Fig.6 suggesting that the fish maintained a body mass that was comparatively greater than their length. This range is consistent with optimal conditions for aqua cultured tilapia. Moreover, it was demonstrated that the zero-water exchange system functioned exceptionally well in preserving water quality parameters at acceptable levels. The calculated Specific Growth Rate (SGR) is approximately 6.64% per day based on the provided data. This indicates the average daily growth rate of the fish during the 70-day experimental period. Varying growth and sustainability were reported in literature (Azim and Little, 2008; Anand *et al*., 2014; Martins *et al*., 2019).

Survival rate

The results of a ten-week experimental study investigating the viability of tilapia in a zero-water exchange tank cultivation system were documented through analysis. The seeding density utilized throughout the investigation was one fish per liter. The investigation observed weekly survival rates ranging from 90 % to 100 % which suggests that overall survival was favorable. Taking into account mortality rates associated with measurements and handling, the calculated survival rate of 60 % at the conclusion of the 10-week experiment provides valuable insights into the obstacles and potential advantages of zero water exchange tank systems. The observed discrepancy in weekly survival rates implies that the aggregate survival rate might have been impacted by mortality that occurred during measurements. Different survival rates are reported by various researchers (Azim and Little, 2008; Anand *et al*., 2014; Martins *et al*., 2019).

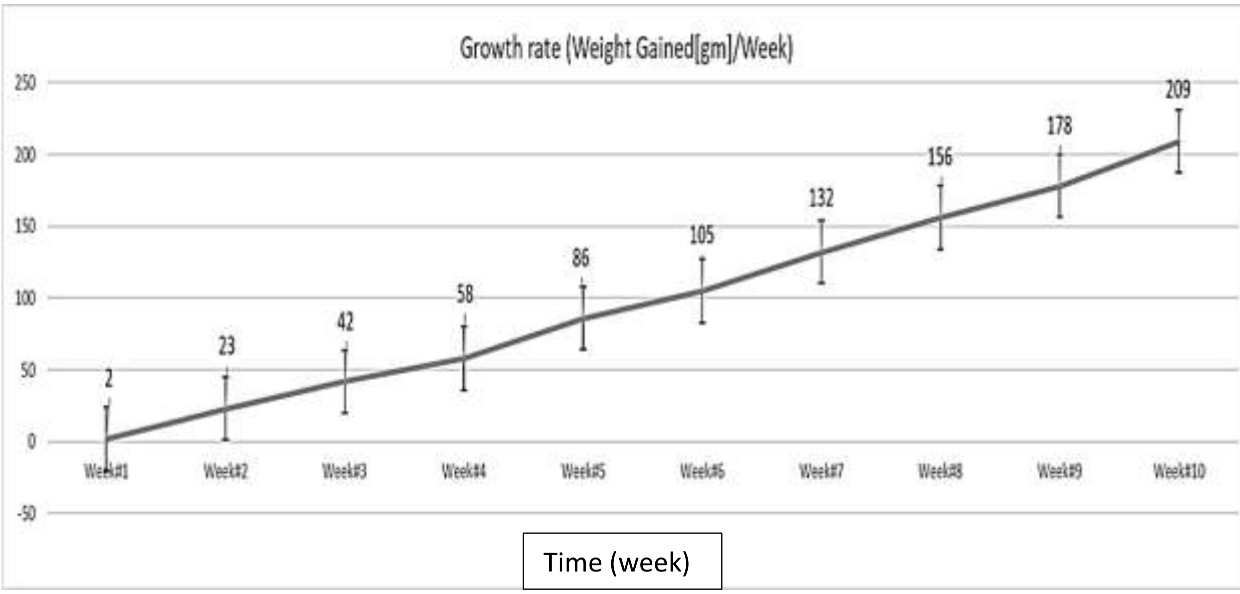

Fig 2. Depicts the graph illustrating increasing trends for weight gained/week.

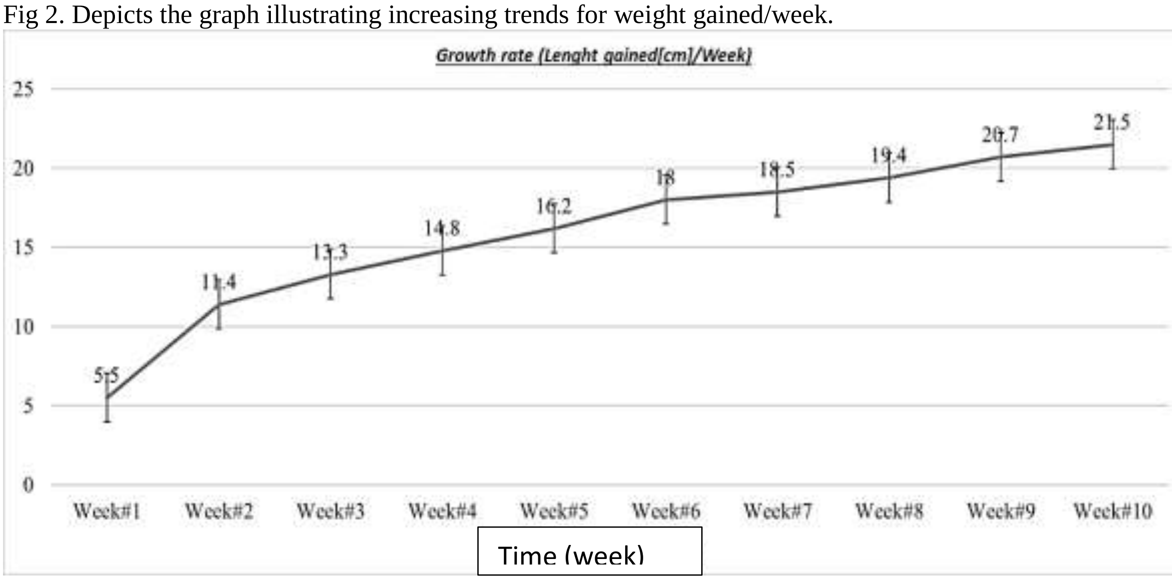

Fig 3. Depicts the graph illustrating increasing trends for length gained/week.

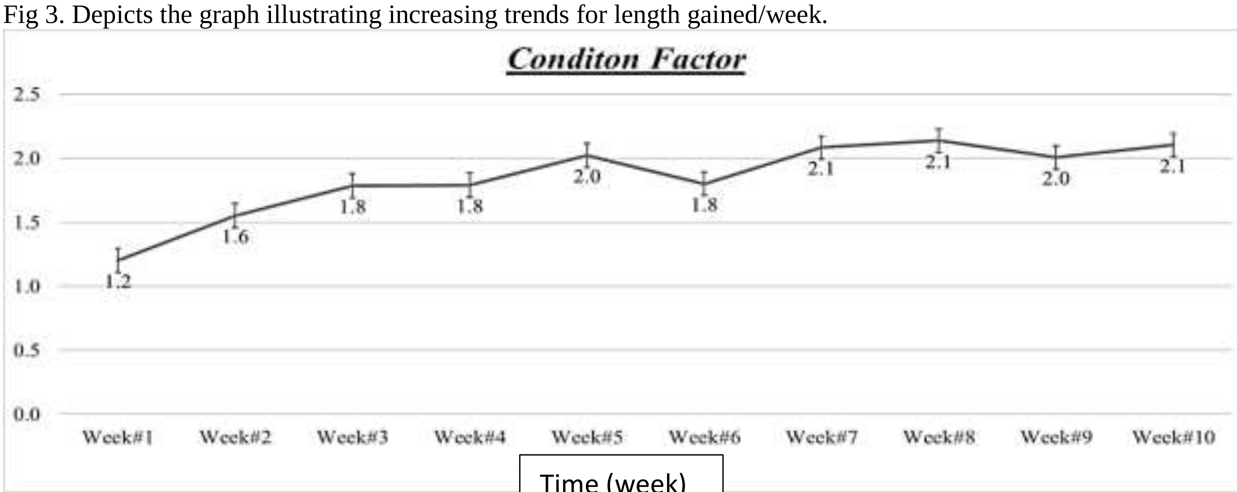

Fig 4. Depicts the graph illustrating increasing trends for Condition factor. As condition factor increase the 1.5 marks it indicates higher body mass for their length

Table 1. illustrates the Calculation of trends of survival rate per week.

Week	Initial number of fish	Number of surviving fish at the end of the week	Weekly Survival Rate %
Week#1	100	90	90.0
Week#2	90	81	90.0
Week#3	81	75	92.6
Week#4	75	70	93.3
Week#5	70	70	100.0
Week#6	70	65	92.9
Week#7	65	64	98.5
Week#8	64	60	93.8
Week#9	60	60	100.0
Week#10	60	60	100.0

Targeted Approach Water Dynamics

Throughout the Experiment, pH levels were constantly kept within the recommended range for tilapia culture (usually 6.5-8.5). TAN levels stayed below the crucial threshold of 0.5 mg/L, showing that ammonia generation within the system was well-managed. Nitrite levels constantly maintained below the crucial level of 0.5 mg/L, ensuring that harmful effects on fish health were reduced. Nitrate levels were kept under the required range of less than 50 mg/L, encouraging both fish health and effective nutrient use. Chlorine levels were low, indicating that no hazardous chlorine compounds were present in the system. Dissolved Oxygen: Dissolved oxygen levels frequently above the acceptable limit for tilapia, generally greater than 5 mg/L, guaranteeing enough oxygen for fish respiration. Similar types of results were also reported in literature (Anand *et al*., 2014; Martins *et al*., 2019).

pH

Throughout the 10-week trial, pH levels (Fig. 5) were constantly maintained within the recommended range for tilapia culture (usually 6.5-8.5); the ph. remained around 7.0-7.6. This steady pH range was critical for tilapia's general health and development, since it enabled proper nutrient absorption while reducing stress on the fish. Furthermore, keeping a steady pH level creates an ideal environment for helpful bacteria that aid in waste breakdown and water quality maintenance (Anand *et al*., 2014; Martin *et al*., 2019).

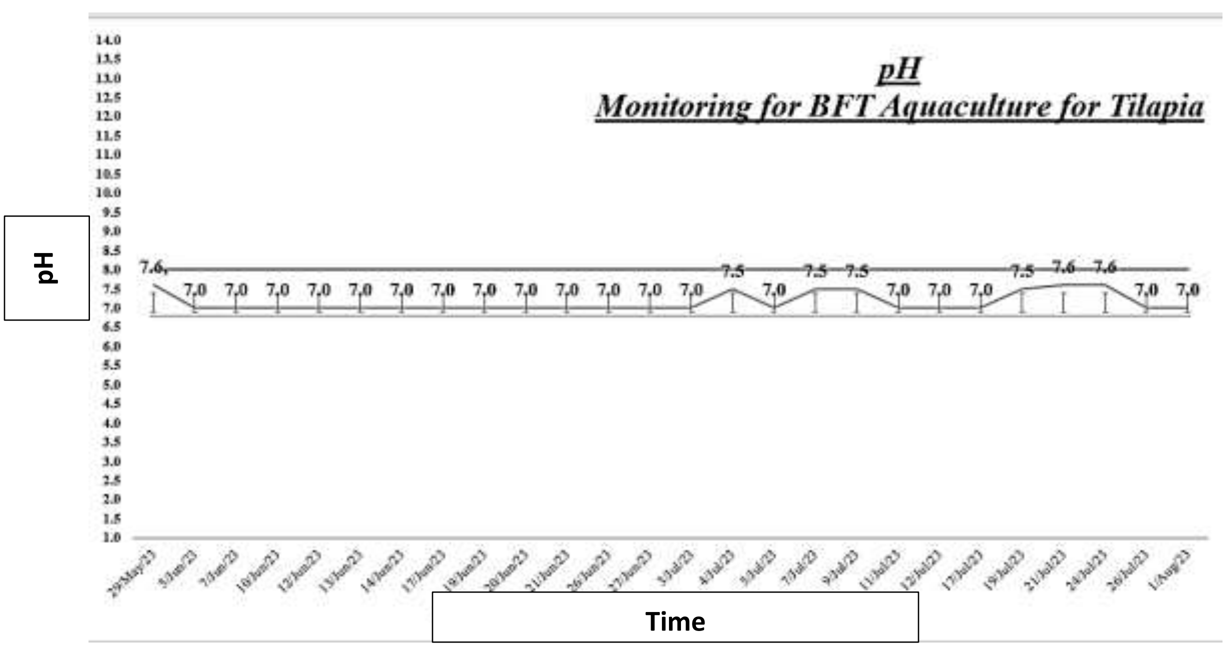

Fig 5. Depicts the graph illustrating the sampling conducted and the result documented from the pH test.

Total Ammonia Nitrogen

TAN, which stands for total ammonia concentration ($NH_3NH_4^+$), is an essential parameter for the surveillance and regulation of TAN levels in order to mitigate the risk of ammonia toxicity (Azim and Little, 2008; Anand *et al*., 2014; Martins *et al*., 2019). In general, the optimal TAN limits for tilapia cultivation are between 0.5 mg/L and 2.0 mg/L (milligrams per liter), with a preference for the lower end of the range. Tilapia exhibits a remarkable capacity for tolerating high concentrations of TAN in contrast to certain other fish species. They are capable of withstanding TAN concentrations as high as 2.0 mg/L for prolonged durations. Throughout the duration of our ten-week experiment, we recorded (Fig. 6) TAN readings ranging from 0.2 mg/L to 0.99 mg/L, with an action limit of 1.0 mg/L. There were two occurrences in which the recorded TAN levels were 1.2 mg/L. Throughout the comprehensive investigation, it was discovered that an increase in temperature increases the concentration of unionized ammonia (NH_3), which is a toxic form of ammonia.

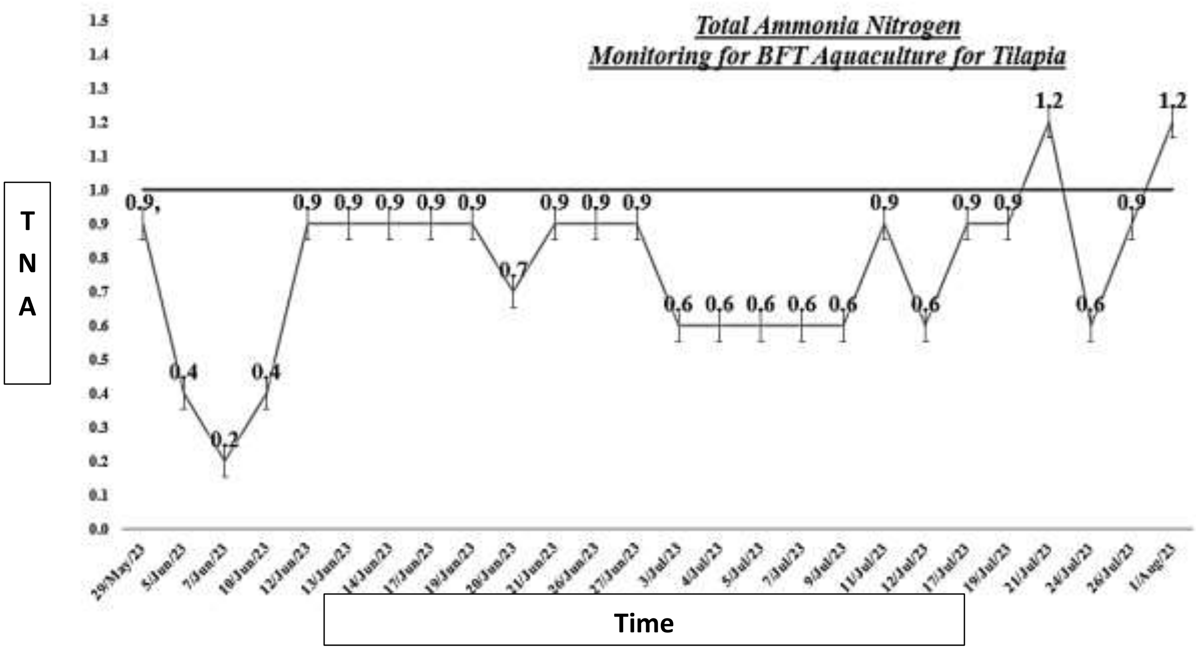

Fig 6. Depicts the graph illustrating the sampling conducted and the result documented from the TAN test.

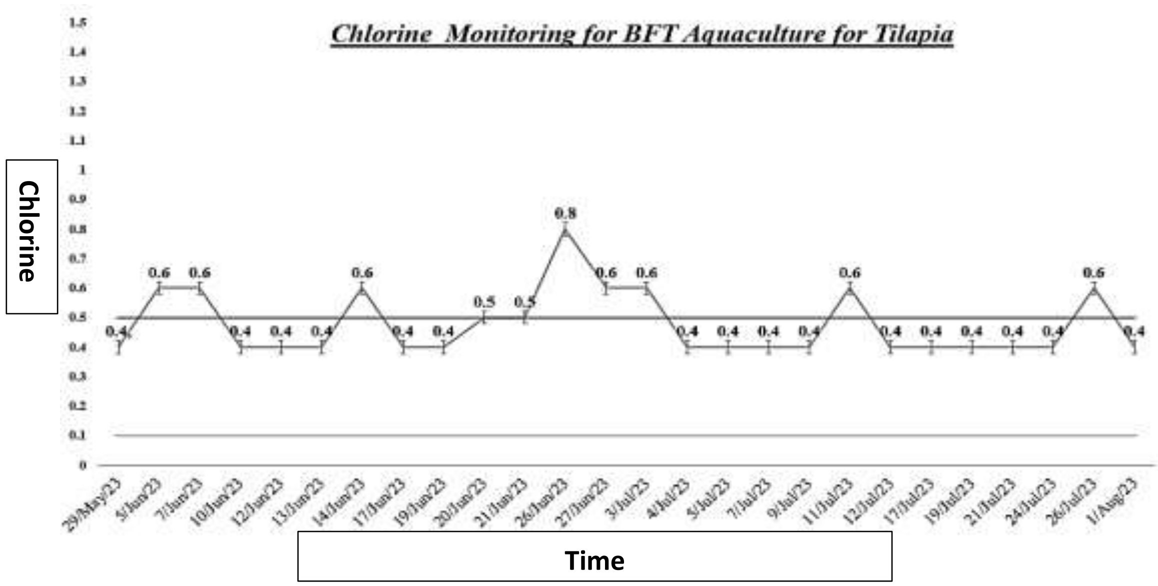

Fig 7. Chlorine observed concentrations typically falling within the range of 0.4 to 0.8.

Chlorine

It is recommended to maintain chlorine levels as near to zero as feasible in Bio-floc Technology (BFT) systems utilized for tilapia production (Azim and Little, 2008; Anand *et al*., 2014; Martins, *et al*., 2019). Chlorine is frequently employed as a disinfectant in water treatment procedures, with observed concentrations typically falling within the range of 0.4 to 0.8. (Fig. 7).

Nitrite

During our 10-week Experiment (Fig. 8) we saw trace amounts of nitrite at around 0.0 mg/l. However, we encountered three instances where the acceptable limits were surpassed. Subsequent Investigation study and the root cause analysis revealed that the primary factor contributing to this issue was excessive feeding and subsequent depletion of oxygen (Azim and Little, 2008).

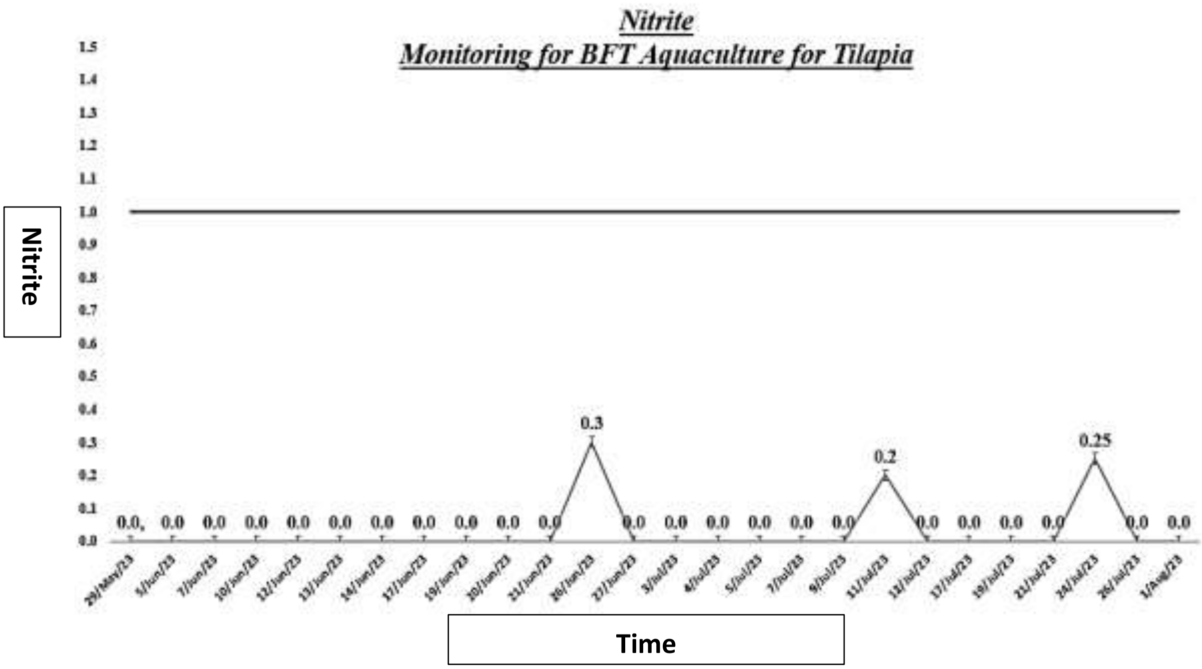

Fig 8. Depicts the graph illustrating the sampling conducted and the result documented from the nitrite test.

Nitrate

During our 10-week study (Fig. 9) on tilapia culture, the nitrate levels were consistently measured at 0. However, there were conditions where the nitrate level exceeded the specified action limit. A root cause analysis was conducted to investigate the underlying factors contributing to this issue. It was determined that excessive feed input resulted in the accumulation of nitrogenous compounds, including nitrate, within the system. Additionally, low oxygen levels were found to impede the denitrification process in the Bio-floc system, thereby leading to the accumulation of nitrate (Azim and Little, 2008; Anand *et al*., 2014; Martins *et al*., 2019).

Dissolved Oxygen

Our 10-week experiment (Fig. 10) revealed that a concentration of at least five and up to eight was obtained using the tri-day sampling basis. We periodically measured our dissolved oxygen concentration at about 4, which was the action line boundary line where we adjusted our stock density. The addition of more aeration and oxygenation systems allowed for a sustained saturation of the depleted oxygen. On the other hand, insufficient concentrations of dissolved oxygen can lead to hypoxia, which is marked by respiratory distress and possibly even death, if the saturation is not reached (Azim and Little, 2008). By maintaining a higher concentration of dissolved oxygen, we ensure a more salubrious ecosystem for the aquatic organisms and microbial communities that depend on them. It was determined that keeping the right level of dissolved oxygen in the water can also help improve the overall quality of the water and stop harmful algal colonies from growing.

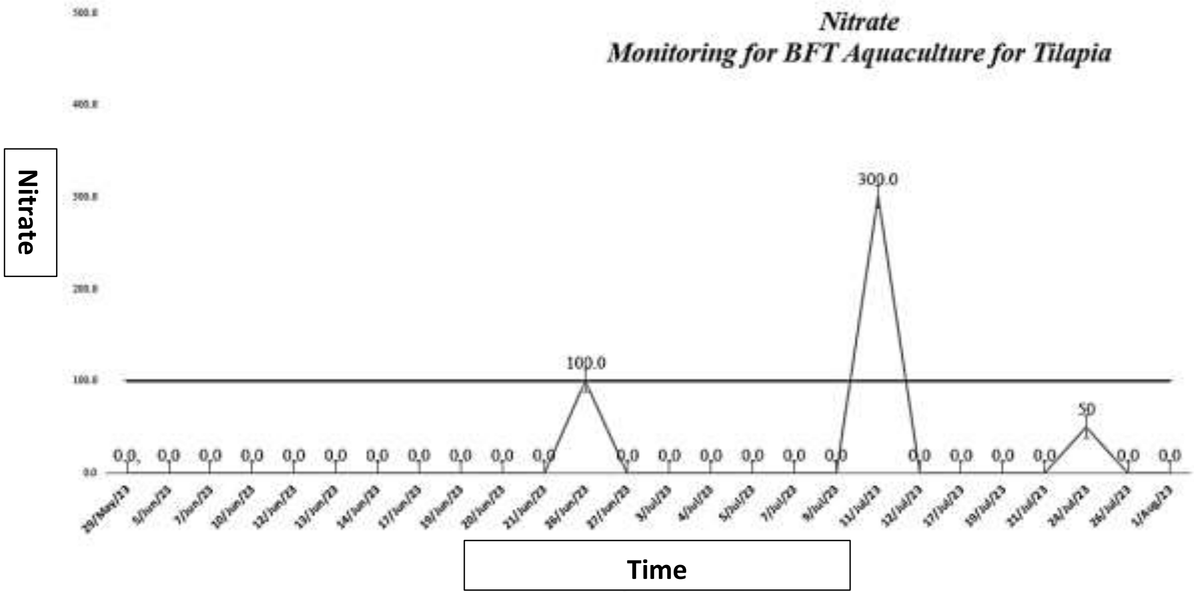

Fig 9. Depicts the graph illustrating the sampling conducted and the result documented from the nitrate test.

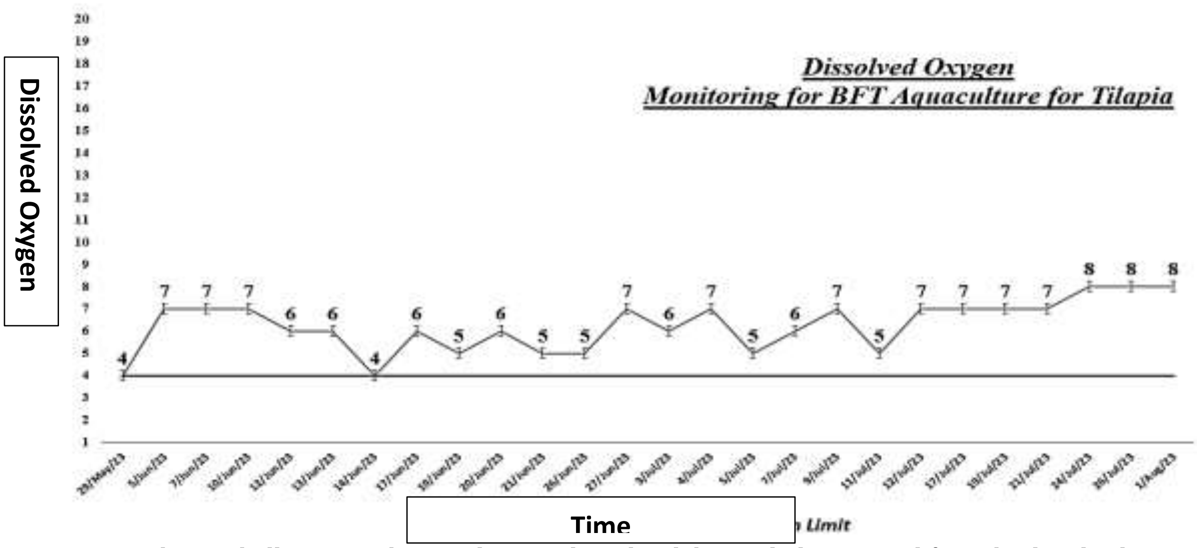

Fig 10. Depicts the graph illustrating the sampling conducted and the result documented from the dissolved oxygen test

CONCLUSION

The results of this ten-week trial show that maintaining water quality indicators within the prescribed ranges and cultivating tilapia in a zero-water exchange tank system is both feasible and effective. This environmentally sustainable strategy provides the opportunity to promote effective nutrient consumption while also creating an ideal environment for tilapia development. The system continuously maintained important water quality measures during the experiment at or below the recommended thresholds for tilapia farming. The pH levels, which are essential to fish health, were consistent and ranged from 6.5 to 8.5 on average.

The computed Specific Growth Rate (SGR) of roughly 6.64% per day on average shows that tilapia grow at significant rates, indicating that the zero-water exchange system efficiently fosters tilapia growth. With an average daily rise of 22.8%, the fish showed significant length growth, demonstrating the system's effectiveness in promoting fish development. According to the condition factor, the tilapia's body mass, which stayed comparatively bigger than their length, was generally steady under ideal aquaculture circumstances. This offered more proof of the fish's general wellbeing and excellent condition.

The maintenance of TAN levels below the critical threshold of 0.5 mg/L signifies the effective management of ammonia. By maintaining nitrite concentrations below the critical threshold of 0.5 mg/L on a consistent basis, potential damage to fish health was minimized. The nitrate concentrations were significantly below the minimum threshold of 50 mg/L, which is detrimental to fish health and hinders optimal nutrient utilization. The lack of hazardous chlorine compounds in the system was confirmed by the low chlorine levels. As dissolved oxygen concentrations surpassed the permissible threshold for tilapia, which is generally above 5 mg/L, sufficient oxygen.

In conclusion, the results of this study offer strong evidence in favor of the viability of tilapia farming applications for zero-water exchange tank systems. These systems support ecologically friendly and efficient aquaculture practices, minimize input costs, meet sustainability goals, and offer a flexible design appropriate for settings with limited acreage. The results underline the potential of this environmentally responsible approach and further the development of responsible and efficient aquaculture technology, adding to the body of evidence supporting the global need for sustainable food production.

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