Research Article

Evaluation of Growth Rate of Different Concentration Floc in a Biofloc Setup for Nile Tilapia *(Oreochromis Niloticus)* Culture

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Abstract | Nile Tilapia (*Oreochromis niloticus*) is the most common marketable fish species in biofloc throughout the world. It has higher growth rate and is reared in limited resources when compared to other aquaculture species. This research work identified the requirements needed for installation of biofloc setup in winter session; different physico-chemical parameters and optimized the floc density for growth of Nile Tilapia (*O. niloticus*) in the study area. A total of three tanks were taken and the floc concentration was 121.071g in tank first, 131.16g in second and 135.19g in tank third. Total fingerlings (750) stocked were divided in three tanks (dimensions of length was 15feet, height=4.5feet, water level=3.2 feet) with water volume of 17000 liters in each tank. Formulated feed was used with 30% and 40% crude protein (CP) and buffalo meat. Water temperature was maintained at 31°C using heater (500Watts) with thermostat. The results were recorded with significant value in the growth of fish during 4^{th} to 9^{th} weeks. The values of fish weight were recorded higher ($P<0.05$) in group 3 than the group 1 and group 2. The floc concentration, feed and physico-chemical parameters have positive effects on biofloc system at winter session for quality production of Nile Tilapia (*O. niloticus*) production.

Received | January 11, 2024; **Accepted** | April 19, 2024; **Published** | June 05, 2024
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Citation | Alam, A., A. Ullah, I. Khan, Yaseen, R. Ullah, T. Tayyeb, M. Hanif, M. Rahman, M.O. Khan, F. Syed, R. Taj, S. Gul, M. Sadeeq and M. Islam. 2024. *Sarhad Journal of Agriculture,* 40(2): 588-594.
DOI | https://dx.doi.org/10.17582/journal.sja/2024/40.2.588.594
Keywords | Fish, Biofloc system, Physico-chemical parameters, Nile tilapia, Growth, Feeds

Introduction

Aquaculture is the practice of promoting and harvesting aquatic organisms, in confined spaces like tanks, ponds, and cages (FAO, 2010). The world bank reported in 2019 that the global fishing trade employs 200 million people and generating USD 80 million per year (Noman, 2019). In Pakistan the fishery sector offer service to about 400000 fisher men and 600000 people in minor industries (Laghari *et al.*,

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2022). Fish make up the major group of vertebrates, with above 34,000 species known worldwide (Nelson *et al*., 2016). It play crucial ecological roles, serving as both predator and prey in food webs and acting as indicators of ecosystem health (Nelson *et al*., 2016).

Aquaculture is one of the industries that is consistently growing and producing more food than ever before. Due to rapid growth, greater resistance against aillness, quality of protein, tilapia is one of the aquaculture species referred to as aquatic chicken (Abdel-Aziz *et al*., 2021). Water quality is important for high yield production, mostly the physico-chemical parameters of water are affected by industrial sewage, dumping waste at the margin of streams (Islam *et al*., 2021). Aquaculture water quality can be improved with biofloc technology by balancing nitrogen and carbon. This technology has recently gained popularity as a long-term way to control water quality that also adds value by producing protein feed (Deswati *et al*., 2022).

Biofloc technology recycle organic waste using microbial colonies into protein-rich feed for aquatic organisms, thus improving water quality and reducing environmental pollution (Emerenciano *et al*., 2017). The biofloc system has been successfully applied to various fish species, such as Nile tilapia (Emerenciano *et al*., 2017), and catfish (Liu *et al*., 2016) and shrimp (Crab *et al*., 2012), 20% reduction of feed costs (Emerenciano *et al*., 2012), promoting growth and improving fish health (Faizullah *et al*., 2019).

The biofloc system represents a promising approach to sustainable aquaculture that can enhance fish production, reduce environmental pollution, and improve resource efficiency. The success of this system depends on the optimization of key management factors and the development of complementary technologies that can enhance its effectiveness and sustainability. This research study was designed for providing comparative baseline information regarding the growth of Nile Tilapia (*O. niloticus*) in winter season with provision of same environmental conditions and to optimize the density of floc for growth of Nile Tilapia (*O. niloticus*) and to investigate the growth response of Nile Tilapia (*O. niloticus*) in biofloc setup in winter season.

Materials and Methods

Study area

This research work was conducted in district Charsadda and Fish Pathology Research Laboratory (FPRL), College of Veterinary Science and Animal Husbandry (CVS and AH), Abdul Wali Khan University Mardan, Pakistan.

Experimental design and requirements

This experiment was conducted during January-March 2023. An outdoor cemented and circular shape three tanks with dimensions of 14 feet from radius (7 × 7 feet from radius and 4.5 feet height) were designed with water capacity of 9174 liters. Internally covered with tarpaulin (660 GSM-gram per square meter) sheet. The water level in each tank was up to 3 feet and 2 inches, water volume was 17000 liters. The physico-chemical parameters were investigated then used non iodized 1/2kg salt per thousand liters. Again, physico-chemical parameters and focused on Total Dissolved Solid (TDS), range 1580ppm and waited up to 48 hrs. The aeration was started and calculated the carbon (C) and nitrogen (N) ratio for 72hrs before stocking and then 50g Calcium carbonate ($CaCO_3$) per thousand liters was added. The optimum value of water was analyzed and the final probiotic solution was added. A 5000 watts thermostat was placed in water for sustaining of favorable temperature.

The requirements used during this research work were tarpaulin sheet (660 GSM), Moter Hailee H-1 Blow Diaphragm air pump, air controller, silicon pipe 1inch, oxytone, uninterruptible power supply (UPS), electric supply (24-hour), green mesh, plastic pipe (2 inch) for outlet with 2 elbow, total dissolved solid (TDS), ph meter, digital temperature, hygrothermograph meter (temperature range: -50 to 70 °C), small digital scale, Imhoff sedimentation cone (SAN BRANDand#174), fresh water Master ® Ammonia test kit, molasses, Napro® probiotics (*Compro*® Beijing Limited, Spring mansion, Bio-medi, China), potassium permanganate ($KMNO_4$), $CaCO_3$, non-iodized salt, tamarind juice, lemon salt used to decrease the value of ph and formulated feed (Ekasari and Maryam, 2012; Azim and Little, 2008).

Preparation of probiotic solution

The floc concentration was prepared and the 50 liter final solution was set for 25 days (Khanjani *et al*., 2021).

Molasses preparation

249 g molasses were used in 1000 liters water and the total volume of water tank was 9700 liter which

required 2415.3 g molasses. Initially, 249 g molasses were added in 20 liters water in a small plastic bucket and shaked well for 5 minutes at evening time.

Probiotic preparation

4.9 g Napro® probiotics were used in 1000 liters water and the volume of water tank was 9700 liter which required 47.53 g probiotic. Initially, 47.53 g probiotic were added in 5 liters tap water in a small plastic bucket and shaked well for 5 minute to form a homogenized solution. The prepared solution was then mixed with molasses solution to form a final soultion and then shaked well for 5 minutes. Then 15 liter tap water was added to make it 50 liters total volume and then stored in 60 liters plastic drum under shaded environment and the resultant final solution was used 2 liter per day at evening time (Figure 1).

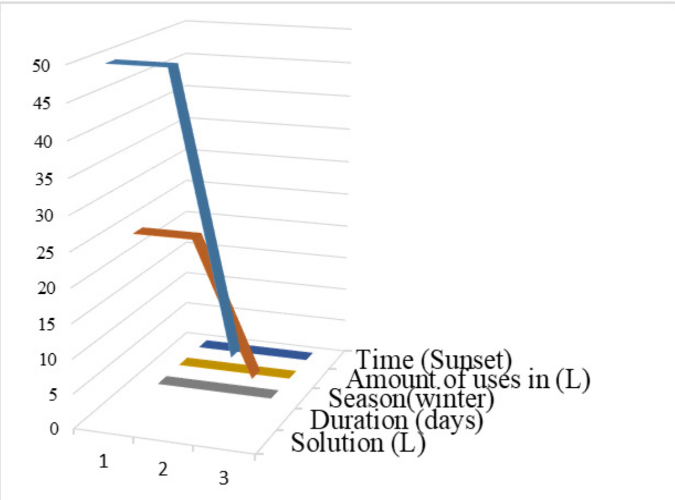

Figure 1: *Schedule of usage of Probiotic solution.*

Physico-chemical analysis of water

The pH and dissolved oxygen (DO) were monitored on daily basis while the ammonia, Nitrite (NO_2), Nitrate (NO_3) and TDS were checked twice a week to monitor the quality of water and to ensure the health and stability of biofloc ecosystem.

Disinfection of fish

All the fingerling was disinfected with $KMNO_4$ before stocking. The solution was prepared by adding 1 spoon $KMNO_4$ in 10 liter tap water and shacked well for 5 minutes. The fingerlings were dipped 10 time for 10 second and were transferred to biofloc system.

Experimental procedure

A total of 750 fingerlings were purchased from National Agricultural Research Centre (NARC), Islamabad and brought to FPRL, CVS and AH, Abdul Wali Khan University Mardan for initial care and then shifted to biofloc area at district Charsadda.

Stocking of nile tilapia

A total of 250 fingerlings of Nile tilapia (*O. niloticus*) were stocked in each tank and used 2 liters floc concentration in 1st tank, 2.5 liter in 2nd tank and similarly used 3 liters floc concentration in 3rd tank daily at evening time (Table 1). The feed with 30% CP was added in 1st tank (Figure 2), the feed with 40% CP was added in 2nd tank (Figure 3) while buffalo meat was added in 3rd tank and then the growth was calculated (Yaseen *et al*., 2016; Sarker *et al*., 2018).

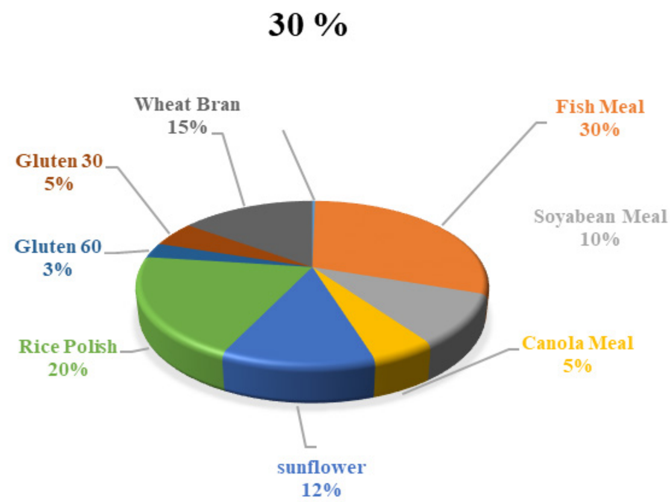

Figure 2: *Formulated feed with 30% crude protein (CP).*

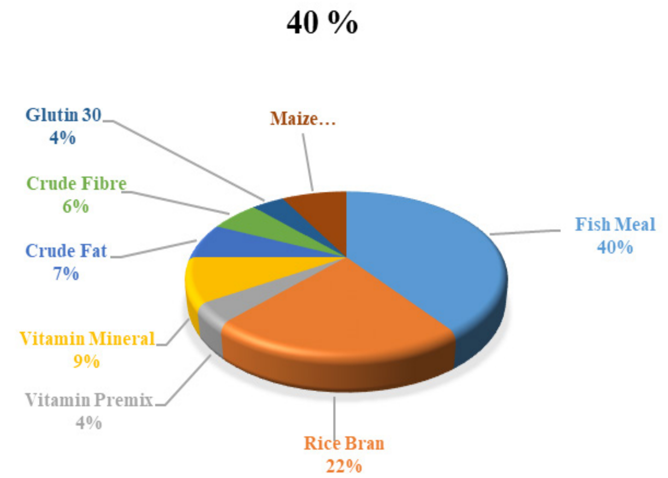

Figure 3: *Formulated feed with 40% crude protein (CP).*

Statistical analysis

The data were presented as mean ± standard error mean (SEM) and were statistically analyzed using SPSS (version 20.0). The group means were analyzed using 1 way ANOVA and group differences were analyzed with a significant level observed at $P<0.05$.

Table 1: *Quantity of bio-feed/probiotics (grams) used, mortality rate and survival rate in winter season.*

Tank No.	Compro® product	Probiotics (g)	Molasses (g)	Total H_20 volume (liters)	MR* (%)	SR** (%)
1st	Napro® Probiotics	121.071	554.91	112	10	90
2nd	Napro® Probiotics	131.16	565	122	2	98
3rd	Napro® Probiotics	135.19	585.17	112	0	100

MR = Mortality rate; SR**= Survival rate*

Table 2: *Analysis of different physico-chemical parameters of H_20 and standard error mean (SEM) with a significant level at P<0.05.*

S. No	Physico-chemical Parameters	Groups	G-1	G-2	G-3	P value
		Weeks	1st, 4th and 7th	2nd, 5th and 8th	3rd, 6th and 9th	
1	pH	SEM	6.30 ± 0.30	6.20 ± 0.40	6.80 ± 0.30	0.06
			6.70 ± 0.40^{ab}	6.90 ± 0.50^{a}	6.30 ± 0.60^{b}	0.04
			6.30 ± 0.40^{b}	6.30 ± 0.70^{b}	7.40 ± 0.70^{a}	0.03
2	NH_3 *	SEM	0.25 ± 0.01^{b}	0.50 ± 0.03^{a}	0.25 ± 0.01^{b}	0.03
			0.50 ± 0.03^{a}	0.25 ± 0.01^{b}	0.50 ± 0.01^{a}	0.03
			0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.01	0.10
3	NO_3 **	SEM	3.0 ± 0.6^{b}	5.0 ± 0.3^{b}	10.0 ± 0.5^{a}	0.02
			5.0 ± 0.3	5.0 ± 0.3	5.0 ± 0.5	0.23
			5.0 ± 0.3	5.0 ± 0.4	5.0 ± 0.4	0.23
4	NO_2 ***	SEM	0.20 ± 0.02^{c}	0.25 ± 0.02^{b}	0.35 ± 0.02^{a}	0.05
			0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.11
			0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	0.112
5	TDS ****	SEM	1300 ± 27	1300 ± 32	1300 ± 35	0.55
			1300 ± 27	1330 ± 35	1340 ± 35	0.180
			1320 ± 20	1340 ± 25	1360 ± 28	0.16
6	Fish weight	SEM	14.20 ± 0.80^{c}	21.75 ± 1.20^{b}	30.44 ± 2.50^{a}	0.01
			36.50 ± 2.50^{c}	45.62 ± 2.20^{b}	53.89 ± 2.80^{a}	0.01
			63.45 ± 3.80^{c}	71.71 ± 3.90^{b}	85.80 ± 2.70^{a}	0.06
7	Fish length	SEM	2.85 ± 0.30	4.35 ± 0.45	5.93 ± 0.20	1.21
			7.60 ± 0.60	10.26 ± 0.45	12.55 ± 0.70	1.79
			14.45 ± 0.90	16.62 ± 0.85	18.21 ± 1.02	1.58

Ammonia; **Nitrat; * Nitrite/Nitrogen dioxide; ****Total dissolved solid.*

Results and Discussion

Probiotic solution

The 50 liters floc were used during the research trial for 25 days in the evening time. The formula used for floc preparation were the same but the amount of floc used for each tank was different. Different floc level were used and the effect on growth and survival rate of Nile tilapia (*O. niloticus*) were optimized (Table 1).

Physico-chemical parameter effects on Nile tilapia

pH: During the first three weeks, the pH did not showed any variation among different groups. During 4th, 5th, and 6th weeks, the pH in group 2 was recorded higher ($p<0.05$) than group 3. During 7th, 8th and 9th week, the pH of group 3 was found higher ($p<0.05$) than group 2 and group 1 (Table 2).

Ammonia (NH_3) concentration: The concentration of ammonia during 7th, 8th and 9th weeks were found constant in all groups. During first three weeks, the NH_3 was found higher ($p<0.05$) in group 2 as compared to group 1 and group 3. The NH_3 concentration was recorded higher ($p<0.05$) during 4th, 5th, and 6th weeks in group 3 and group 1 as compared to group 2 (Table 2).

Nitrat (NO_3) level: During 1st, 2nd and 3rd weeks, the NO_3 in group 3 was recorded higher ($p<0.05$) as compared to group 1 and 2. The NO_3 level did not showed noticeable variation in all groups during 4th to 9th weeks of the trial (Table 2).

Nitrogen dioxide (NO_2) level: The water NO_2 contents were remained unaffected during 4th to 9th weeks of the research trial. The results showed that in first three weeks, the NO_2 in group 3 was recorded higher (P<0.05) as compared to group 1 and 2. However, group 2 also showed higher (p<0.05) content of NO_2 than group 1 and the value of total dissolved solids (TDS) was remained unaffected (Table 2).

Growth parameters

During 4th to 9th weeks, body weight of fish was found higher (P<0.05) in group 3 than in the groups 2 and group 1. However, in group 2, it was recorded greater (P<0.05) compared to the group 1 and length of fish was found constant (Table 2).

Recycling in biofloc system

Biofloc system is a modern aquaculture technique which recycles organic waste by using microbial populations into protein-rich feed for aquatic organisms thus improving water quality and reducing environmental pollution and is suitable for Nile Tilapia culture in winter session and should be maintained water temperature for higher yield (Figure 4).

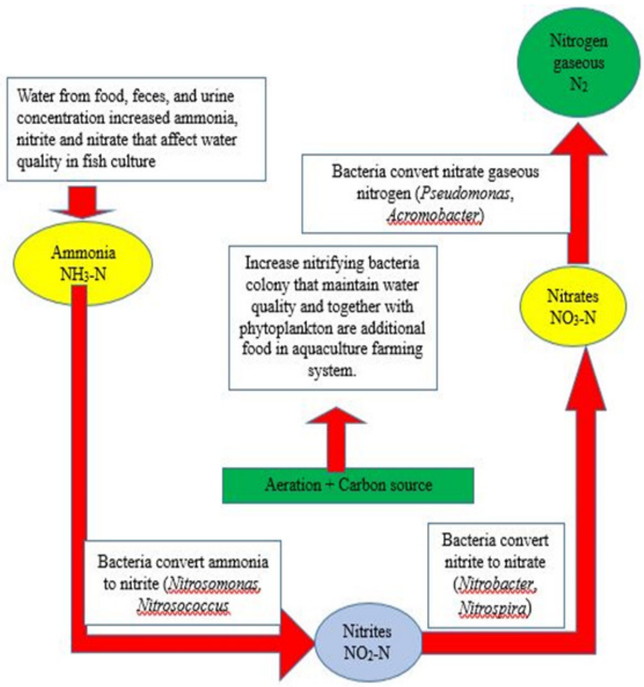

Figure 4: *Recycling in Biofloc system.*

The biofloc system represents a promising approach to sustainable aquaculture and enhanced fish production as good protein source for human consumption, reduce environmental pollution and improve resource efficiency. Nile tilapia is amongst the freshwater fish species commonly used in aquaculture production. In recent years, the use of biofloc technology (BFT) in tilapia farming has gained attention due to its potential benefits in refining water quality and reduce feed cost (Emerenciano *et al*., 2012). The present study evaluated the growth response of Nile tilapia (*O. niloticus*) fish in biofloc setup in winter season and different physico-chemical factors of water for the growth of tilapia were optimized. The three replicates of biofloc tanks were formed containing same number of same species. Each group were raised differently on the basis of feed and floc volume to show a significance. The pH was not found significant on different treatments of *O. niloticus* (Azim and Little, 2008; El-Sherif and El-Feky, 2009; Ekasari and Maryam, 2012). The pH parameter was recorded higher in group 2 (p<0.05) than group three while the pH of group three was found higher (p<0.05) than group 2 and group 1 during 7th, 8th and 9th week of the research trial. The tolerable range of pH between 4-11 as documented by Balarin and Hatton (1979). Our results suggested that the optimum range of pH between 6-9 and temperature between 18°-30°C were found balancing pH and temperature for freshwater fish farming operations (Azim and Little, 2008).

The high concentration of total ammonia nitrogen (TAN) cause mortality in the 600 fishes per m^3 in biofloc technology (Vicente *et al*., 2020). In first three weeks, the NH_3 content was found higher (p<0.05) in group 2 than group 1 and group 3. In 4th, 5th and 6th weeks, the NH_3 content was recorded higher (p<0.05) in group 3 and group 1 than group 2. The NO_2 and NO_3 concentration was found vary throughout the research trial as were documented by Avnimelech *et al*. (2012). The Nitrite (NO_2) concentration recorded above 5.0mg and best range below 1.0mg (Avnimelech *et al*., 2012). Our result recorded that the NO_2 in group 3 was found higher (P<0.05) than group 1 and 2 while group 2 also have higher (p<0.05) content of NO_2 than the group 1.

The feed and floc volume has direct effect on the growth of Nile talapia (*O. niloticus*) (Somerville *et al*., 2014) and similar results were recorded in indoor BFT tanks. Formulated feed with composition of 24% and 35% crude protein were used by Vicente *et al*. (2020) while we used 30% CP, 40% CP and pure buffalo meat in this experiment. The TDS ranges were recorded between 1300 ± 32 to 1360 ± 28 which is similar to the findings of Vicente *et al*. (2020) wherein the total suspended solid was kept in BFT at about 1300 ppm. The ponds were covered with polyethylene sheets to maintain proper water temperatures and their physico-chemical parameters in BFT. Starch was added to the ponds to encourage the growth of bio-flocs and to prevent the water quality from deterioration (Siddik *et al*., 2014). Different concentration of floc in each tank were used and 10% mortality was recorded.

In general, the data obtained in the present study revealed higher fish yield production results with minimum mortality.

Conclusions and Recommendations

It is concluded that biofloc contributed remarkably to the development and high production of fish in light-restricted indoor environment. Biofloc's nutritional value was suitable for Nile tilapia *(O. niloticus*) and the growth of Nile tilapia *(O. niloticus*) was found enhanced during the research trial. In order to maintain system integrity and meet the principles for compromised parameters, water quality must occasionally be actively modified. The biofloc technology (BFT) plays a significant role as an effective alternative system in which nutrients are continuously regenerated, cost-efficient and environment friendly. This study demonstrated a significant difference of Nile tilapia (*O. niloticus*) growth response in winter season. It is recommended that biofloc setup is very good alternative system instead of earthens pond. Further research works no molecular and genetic identification of Nile tilapia throughout the province Khyber Pakhtunkhwa are immensely needed. Also, financial support to the local fish farmers by Government, awareness seminars about the fish farming, trainings workshops on the high growth rate of (*O. niloticus*) and economic importance of biofloc system are strongly recommended for the uplift of those with limited resources.

Acknowledgments

We are thankful to the technical and supporting staff of Fish Pathology Research Laboratory (FPRL), College of Veterinary Sciences and Animal Husbandry (CVS and AH), Abdul Wali Khan University Mardan, Pakistan for their full support during the research trial.

Novelty Statement

The research and experimental work on the subject title is original and new in the field of aquaculture in Khyber Pakhtunkhwa, Pakistan.

Author's Contribution

Asad Alam: Investigation, Writing-original draft preparation.
Asad Ullah: Supervision, Methodology.
Imad Khan: Conceptualization.
Yaseen: Project administration.
Rafiq Ullah and Tahira Tayyeb: Validation.
Muhammad Hanif and Maiz ur Rahman: Software.
Muhammad Owais Khan and Fatima Syed: Data curation.
Raheela Taj: Resources.
Shumaila Gul: Writing-review and editing.
Muhammad Sadeeq: Visualization.
Muneeb Islam: Formal analysis.

Conflict of interest
The authors have declared no conflict of interest.

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