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Examination of different C/N ratios, heterotrophic bacteria and plankton abundance on the growth of Nile Tilapia (*Oreochromis niloticus*) in biofloc system

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Abstract

Nile Tilapia is a versatile fish renowned for its rapid growth and occurs in a wide variety of freshwater habitats. The trial was done to evaluate the optimum percentage of C:N ratio in biofloc system. A trial was conducted at IAAS, Paklihawa Campus, Bhairahawa, Nepal from 1st March, 2024 to 28st July, 2024 upto 150 days. Altogether 15 circular polytank (500 liter) each were used with stocking density of 40 fish 0.4 m^{-3} with continuous aeration. Completely Randomized Design (CRD) was set up with 5 treatments, each replicated thrice. The treatments were: (i) T1-without floc (Control) (ii) T2-with floc (C:N-10:1) (iii) T3-with floc (C:N-15:1) (iv) T4-with floc (C:N-20:1) and (v) T5-with floc (C:N-25:1). Molasses was taken as a carbon source. An intitial average weight of mono-sex tilapia fry (4.9±0.8 g) was stocked and 25% CP based pellet feed was given twice daily at 3% of body weight in all the treatments. At initial stage, there was no significant different in Temperature, pH, Ammonia, Nitrite and TSS at $p < 0.05$ in all the treatments. But, there was significance different in Dissolved Oxygen, Floc level and TDS at $p < 0.05$. Similarly, there was no significance different among the treatments in Initial Mean Weight (IMW), Total Initial Weight (TIW) and Initial Number (IN). But there was significantly different among the treatments in final mean weight, final harvest number, final harvest weight, DWG, GFY, NFY, and AFCR among the treatments. The highest final mean weight was found in T5 (58.8±7.6g) and the lowest final mean weight was found in T1 (13.0±0.4g). Result showed final harvest number was found highest in T5 (35.3±1.7) and the lowest in T1 (23.3±0.9). Similarly, the highest final harvest weight was found in T5 (2104.8±68.1g/tank) whereas the lowest final harvest weight was found in T1 (304.4±5.0 g/tank). There was no significantly different among the treatments in the mean value of abundance of bacteria, rotifers and copepda at $p < 0.05$. But there was significantly different among the treatments in the mean value of Cladocera and Protozoa at $p < 0.05$. The study suggests that T5 is found the best option for the growth performance of Nile tilapia in biofloc system.

Keywords: Biofloc technology, Tilapia, C/N Ratio, Probiotics, Bacterial loading, Planktons

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Introduction

Biofloc technology (BFT) can offer a promising solution to environmental and sustainable aquaculture production system. BFT converts waste such as Nitrogen (N), Organic Carbon, other nutritional waste from plankton and fish into bio flocs. In turn these bio flocs become natural food sources for fish and other aquatic organisms. These have implications on reducing the additional need for external inputs, such as feed and chemicals. Moreover, BFT has the potential to improve yields and economic performance by promoting efficient resource utilization, such as water and energy (Khanjani *et al*., 2024). Despite its numerous advantages, BFT have many challenges, such as high energy demand, high initial/running costs, waste (effluent, suspended solids, and sludge) management, opportunistic pathogens (vibrio) spread and a lack of understanding of operational/aquatic/microbial dynamics. BFT offers a cleaner production option that promotes circularity practices by enhancing growth performance and economic benefits. In spite of advantages and challenges of BFT, key technical, biological and economic aspects are needed to optimize its application and further promote its adoption and overcome the current challenges. Biofloc technology (BFT) is a water quality management strategy where, as carbon sources, molasses, jiggery, sugars, wheat flour, glycerol or complex carbohydrates stimulate heterotrophic bacterial growth (Gurung *et al*., 2024). These heterotrophic bacteria converts toxic nitrogenous waste and produce substances suspended solids in the form of debris, support to produce microalgae, zooplankton and others to aggregate into larger particles called as bioflocs. These large size flocs due to their larger size help to increase the chances of their consumption by various aquatic animals.

Bioflocs maintain water quality providing constant nutrition. The consumption of these bioflocs by fish in turn increases the ability to promote animal growth which is largely based on the fish's ability to collect and consume these particles (Romano and Dauda, 2018). Biofloc technology is acknowledged for its ecologically conscious methodology, which diminishes the pollution burden on water resources by completely eliminating water outflow. Aquaculture can expand in an environmentally friendly way by following sustainable development goals and fostering integrated systems that have minimum impact on the environment (Minaz *et al.*, 2023). This technology emphasis on microbial populations, nutrient recycling and water quality preservation highlighting its environmentally benign and economically feasible characteristics (Devi *et al.*, 2015; Emerenciano *et al.*, 2013; Ray *et al.*, 2020).

Additionally, BFT boosts the immune system of aquatic species by diminishing the likelihood of prevalent illnesses and fostering their overall well-being. Nevertheless, the technology needs meticulous control of variables and most probably knowledge to use the combination of carbon-to-nitrogen ratio, water temperature and dissolved oxygen in order to uphold a stable and fruitful environment (Lal *et al.*, 2024). It has been understood that maintaining a higher carbon and nitrogen ratio supports the establishment of microbial community mainly consisting of heterotrophic bacteria, which plays crucial role

in managing water quality and supplying sustenance for the cultivated organisms (Ahmad *et al.*, 2017). The microbial community effectively transforms the nitrogen molecules into microbial protein, consequently resulting in reduced feed expenses, improved feed conversion ratios and enhanced economic feasibility of aquaculture operations (Yu *et al.*, 2023b; Bossier *et al.*, 2017; Emerenciano, 2017).

Generally, carbon-to-nitrogen (C/N) ratios of 10 to 20 or more are recommended in bio floc fish farming system, with higher ratios helping in increasing biofloc production (Silva *et al.*, 2017). Moreover, higher biofloc production is not always favorable due to the necessity for more aeration to support higher microbial respiration rates any disruptions to air flow can result in the loss of an entire crop. Hence, optimum ratio of carbon-to-nitrogen (C/N) is required to operate the system efficiently. There are different studies proposing the higher ratio of C/N values, however, in the present study we also examined the most appropriate C/N ratio favorable for growth of fish.

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Materials and Methods

Location of the experiment

The experiment was conducted at the Aquaculture Research Center of Institute of Agriculture and Animal Science (IAAS), Paklihawa Campus, Bhairahawa from 1st March, 2024 to 28st July, 2024 (150 days).

Experiment details

The experiment was conducted in 15 circular poly tanks with each 500-liter water capacity. For the experiment, a Completely Randomized Design was used having the five treatments and three replications. In this experiment there were one control (T1) without and floc, and four treatments (T2)-With floc (C:N-10:1), (T3)-With floc (C:N-15:1), (T4)-With floc (C:N-20:1) and (T5)-With floc (C:N-25:1). Molasses was taken as carbon source. Stocking density of mono-sex tilapia frys (4.9±0.8 g) were stocked @ 40 fish $0.4m^{-3}$ in each tank. First of all, tank was cleaned up, disinfected by Potassium Permanganate @ 10 mg/L and the next day water was filled up with artisanal water with twenty four hour aeration system connected with aeroxy tube which was supplied by the 1 Hp ring blower.

Water preparation

The water culture was done by adding 10g Probiotics (Provet AQUABAC), 50 g Molasses, 2.5 g Calcium carbonate and 500 g raw salt as Fermented Carbon Oxygen (FCO) in small plastic container with vigorous aeration up to 24-36 hour based on the temperature. When the FCO was ready, it was poured in each tank except control. After applying FCO, molasses was applied in the tanks based on C:N ratio up to one week except control for the multiplication of bacteria in the culture water. Then after ammonia was checked and on the basis of ammonia level, we had to decide whether to apply molasses or not. Based on the Total Dissolved Solids (TDS), raw salt was applied on the basis of water volume in the tanks to reach the optimum level required for biofloc. For this process, raw salt was washed up to 2-3 times to remove the impurities and other contamination and then apply in each tank. After 3 hours later, Total Dissolved Solids (TDS) was measured by TDS meter (Techtonics Company) whether TDS level is OK or not. From the next day, floc was observed by imhoff cone. This process continued throughout the culture period. When the floc was found from 15 to 30 ml, then additional application carbon sources was stopped. And it was started based on the density of floc and the level of Ammonia in the respective tanks.

Procurement and stocking of fish seeds

After the preparation of water, healthy Mono-Sex Tilapia seeds were stocked in each tank according to Avnimelech (1999) @ 100 fish per

m^{-3}. The fish seeds were purchased from Center for Aquaculture Agriculture Research and Production (CAARP), Chitwan, Nepal.

Calculation of C:N ratio

Total Ammonium Nitrogen (TAN) is a combination of unionized ammonia (NH3) and ionized ammonium (NH4+). Ammonia (NH_3) is a highly toxic chemical to fish and changes from ammonia to ammonium and back again relative to the pH level and temperature. Most ammonia test kits are actually testing TAN (Total Ammonia Nitrogen). In water, when NH_3 is < 0.5 mg/L, it is not needed to add carbon source in water. But when NH_3 level is >1 ppm, then the amount of carbon source in biofloc water based on the C:N ratio is calculated. In this experiment, I calculated the amount carbon source (i.e. molasses) based on the formula:-

Basically, if ammonia reading is 1ppm (1 mg/L =1 gram)

- If NH_3 reading is 2 ppm in 10,000 L water = 2 x 10,000 i.e. 20,000 ppm (or 20 gram NH_3).
- If we have to maintain C:N-10:1, then to diffuse this 20 gram (20 x 10 - 200 gram) of Carbon source is added
- Furthermore, if molasses has 25% Carbon, then it is = 200 x 25%= 800 gram molasses is required.
- If NH_3 is 1 ppm, then molasses needs to be added400 gram
- In the next day, if NH_3 reading is <0.5 ppm, then C:N ratio is working. (Avnimelech, 2009)

In this experiment, carbon content of molasses was examined in Lumbini Agro Environment Lab Pvt. Ltd., Sunwal, Nawalparasi, Nepal.)

Table 1. List of commonly used carbon source and their percentage carbon content

Carbon Source	Percentage of Carbon Content (Approx.)	Reference
Molasses	28 %	Sujeet Kumar *et.al*., 2017
Sugar	40 %	Serra *et.al*., 2015
Dextrose	40.89 %	Serra *et.al*., 2015
Rice Flour	40 %	Sujeet Kumar *et.al*., 2017
Rice Bran	43 %	Romano *et.al*., 2018
Tapioca	46 %	Silva *et.al*., 2017
Jaggery	28.8 %	Sakkaravarthi *et.al*., 2015

Calculation of raw salt application in total dissolved solids (TDS)

TDS is the number of dissolved salts, minerals and metals in water.. Normally TDS of freshwater is 400-500 ppm. The optimum TDS level in biofloc fish farming should be between 1000 to 1500 ppm to increase the TDS level one can add additional salt to increase the TDS of water. Adding 1kg raw salt (unionized salt) in 1000 liter water can increase the TDS level of water. If we put 1 g/L raw salt, it will exceed TDS 500-800 ppm. Based on the TDS value, I had calculated the amount of raw salt and put into the tank and maintained the TDS level. For this process, first I observed the TDS level in respective tanks, calculated the amount of raw salt, washed it 2-3 times with fresh water to remove the iodine coating and other impurities and then applied to the tank. Normally, putting 1 g/L raw salt in water equals to 1001.14 ppm and it is safe from nitrite poisoning.

Cleaning and water exchange

The sludge from the rearing tank was cleaned up every week interval by draining the sludge from the output. Based on the abundance of sludge, new water was re-filled in the tank partially. And water aeration system was regularly check the flow rate and adjusted the entry cap accordingly. Furthermore, Aeroxi tube was also cleaned as to wipe out the excess clogging of floc and other debris. And in case of control with no floc, weekly partial draining the water was also done.

Plankton count

For plankton count, 1 liter sample water from respective tanks was passed through the sample water by Plankton Net. And, contents accumulated in the plankton net were collected and preserved in 5% formalin solution. All samples bottles were labelled. After two days, the counting was done by pouring 1 ml aliquot water in Sedgwick-Rafter chamber following the method described by Guillard *et. al*. (2005).

$$\text{Phytoplankton count} = \frac{\text{Total no. of Count in 5 cell}}{5}$$

=…….× 1000 ml sample water

=…….× volume of rearing tank

$$\text{Total no. of bacteria (cfu)} = \frac{\text{No. of colonies} \times \text{Total dilution factor}}{\text{Volume of culture plated in ml}}$$

(cfu = colony forming bacterial units)

Bacterial count

Bacterial count includes following steps:

(a) Sanitation
(b) Sterilization
(c) Preparation of media
(d) Preparation of Petri plate

(a) Sanitation

First of all, I washed the petriplates with detergent powder and let them dried up. Then after cleaned it up with spirit.

(b) Sterilization:

Wrapped petri plates and conical flask with aluminum foil. Then sent it to oven dry at 140 °C for one hour.

(c) Preparation of media

Took 100 ml of distilled water in conical flask. And then put 14 g of nutrient agar into the conical flask. Slightly heated the conical flask with gas flame. Then after autoclaved it up to 15 minutes at 15 psi 121 °C and sent it for incubation at 37°C.

(d) Preparation of petriplate

In laminar airflow, Transferred petriplate and nutrient media from incubator. Poured nutrient media into petriplate covering the whole surface and gently covered it with lid. Then after let it cooled down upto 5 minutes and put it in incubator at 37 °C upto 24 hour. Then applied 100 µl of sample to the prepared petriplate. And after 24 hour, bacteria counting were started dividing the petriplate into quarter. Number of bacteria x 4=Total number of bacteria in petriplate.

Finally, clean up incubator and surface of laminar airflow with spirit before and after work.

Feed preparation

Supplementary Pellet Feed was prepared in IAAS, Aquaculture Lab using automatic feed machine (Model YL100L-4, Shanghai Jiesu Motor Co. Ltd.). The supplementary pellet feed was prepared based on the proximate analysis of all ingredients used in Central Fisheries Promotion and Conservation Center, Balaju, Kathmandu. All the Five diets with the same levels of 25% of dietary protein for tilapia as proposed by Hamilton *et al*. (2019). The preparation of basal diet included rice bran, wheat flour, mustard oil cake, soybean meal, vegetable oil and vitamin-minerals premix. Feed formulation was done using the hit and trial method in the programmed MS-Excel sheet. The ingredients were grinded by mixer grinder (BALTRA, Model-BMG-153/DUKE-3) in dust form and sieved it. Vegetable oil and Additives as vitamin-mineral premix was mixed by sprayer homogenously. The prepared pellet diet was sun dried over one week and then stored in a plastic container.

Feeding

Pellet feeds were provided @ 3% of their body weight during the culture period. The feed was delivered on morning time at 9 AM-10 AM in all controls and treatments.

Table 1. Proximate composition of basal diet (% on dry matter basis)

SN	Proximate composition (Estimated Crude protein %)	Ingredients	Percentage
1	11	Rice bran	40
2	12	Wheat Flour	3
2	38	Mustard Oil cake	20
3	49	Soybean Meal	35
4	-	Vegetable oil	1
5	-	Vitamin and Mineral Premix *(Agrim Fort)	1
		Total	**100**

*Vitamin mineral premix /Kg contains the following: Vitamin A 7,00,000 I.U, Vitamin D3 70,000 I.U, Vitamin E 250mg, Cobalt 250mg, copper 1200mg, Iodine 325mg, Iron 1500mg, Magnesium 6000mg, Potassium 100mg, Sodium 5.9mg, Manganese 1500mg, Sulphur 0.72%, Zinc 9600mg, DL-Methionine 1000mg, Calcium 25.5%, Phosphorus 12.75% (Gurung *et al*, 2024)

Fish growth

For growth measurement, about 20% of fish was sampled randomly on a monthly basis using a portable electronic balance (PHOENIX Model: WT150001XJ Precision: 0.1 g). The growth ***of fish was measured in weight gain by deducting the average initial weight from the*** corresponding weight recorded in each month. The measurement of weight (g) of individual fish was done separately. At the end of the experiment, all fishes was harvested and counted to assess the survival and production.

Data collection

Data was collected on the basis of following growth parameters.

Growth parameters

$$\text{Total Initial Weight (g/tank)} = \text{No. of fish stocked} \times \text{Initial mean weight}$$

$$\text{Total Final Weight (g/tank)} = \text{No. of fish harvest} \times \text{Final mean weight}$$

$$\text{Daily Weight Gain (g.fish/day)} = \frac{\text{Mean final weight-Mean initialweight}}{\text{Culture period}}$$

$$\text{Total Weight Gain (g)} = \text{Total harvest weight (g) -Total initial weight (g)}$$

$$\text{Total Harvest weight (g)} = \text{Final harvest weight (g) - Initial stock weight (g)}$$

$$\text{Gross Fish Yield (GFY) (kg/m}^3\text{/y)} = \frac{\text{Total harvest weight (kg)}}{\text{Culture period (days)} \times \text{culture unit (m}^3\text{)} \times 1000} \times 365$$

$$\text{Net Fish Yield (NFY) (kg/m}^3\text{/y)} = \frac{\text{Total harvest wt. (g)} - \text{total stocked wt. (g)}}{\text{Culture period (days)} \times \text{Culture area (m}^3\text{)} \times 1000} \times 365$$

$$\text{Apparent Feed Conversion Ratio (AFCR)} = \frac{\text{Total weight gain}}{\text{Total feed given}}$$

$$\text{Survival Rate \%} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100$$

$$\text{Gross Margin (NRs.)} = \text{Gross Revenue (NRs.)-Total Variable Costs (NRs.)}$$

Water quality analysis

Water Temperature, Dissolved Oxygen, pH and Total Dissolved Solids (TDS) was observed on daily basis (8AM-10AM) whereas Ammonia, Nitrite and Total Suspended Solids (TSS) and floc was observed on weekly basis. Water Temperature and Dissolved Oxygen was measured by Lutron (PDO-519). pH was measured by Hanna HI 98107. Total Dissolved Solids was measured by TDS-3, India. Ammonia, Nitrite and Nitrate was measured by BIONIX Freshwater Master Test Kit, India. Floc was measured by Imhoff cone. Total Suspended Solids was measured by gravimetric analysis. A water sample is filtered through a pre-weighed glass fiber filter, capturing the solids larger than 2 um. Then, the filter is dried in an oven to remove remaining water and weighed again. The weight difference over the sample volume provides the TSS concentration in mg/L.

Proximate analysis

Quadrant sampling was done for experimental pellet feed during experiment period to draw representative diet sample for proximate analysis. Diet proximate analysis of sample was done according to AOAC (1990) at Central Fisheries Promotion and Conservation Center, Balaju, Kathmandu.

Harvesting

Final harvesting of fishes was done after 150 days by draining each tank completely on termination of research. Harvested fish weight was measured using PHOENIX electronic balance (Model-WT60001X). Fish were counted and their batch weight (g) was recorded.

Statistical analysis

On the basis of individual fish observations, the population means for each growth parameter was computed. The analysis of variance was used to compare different growth parameters using SPSS version 3.6.3. The mean and standard errors was calculated for each treatment. The data entry was done through MS Excel 2016. The accepted level of significance was $p<0.05$.

Results

Growth parameters

The Initial Mean Weight, Total Initial Weight, Initial Stock Number, Apparent Feed Conversion Ratio (AFCR) of five different treatments are presented in Table 2. There was no significant ($p<0.05$) difference among the treatments in case of Initial Mean Weight, Total Initial Weight and Initial Number. But there was significantly ($p<0.05$) different among the treatments in Apparent Feed Conversion Ratio among the treatments. Similarly, Daily Weight Gain (DWG) and Survival (%) have beengiven in Table 2. The initial mean weight of Tilapia fry in different treatment was 5.0±0.4g, 5.3±1.1g, 5.4±1.1g, 4.8±0.4g, 4.4±0.7g in T1, T2, T3, T4 and T5 respectively which were not significantly different with each other ($p>0.05$). But, final mean weight of tilapia was

significantly difference among the treatments ($p>0.05$). The highest final mean weight was found in T5 (58.8±7.6g) and the lowest final mean weight was found in T1 (13.0±0.4g).

Along with it, there was significantly different in final stock number among the treatments ($p<0.05$). Result showed final harvest number was found highest in T5 (35.3±1.7) and the lowest in T1 (23.3±0.9). Similarly, there was significant difference ($p<0.05$) in final harvest weight among the treatments where highest final harvest weight was found in T5 (2104.8±68.1 g/tank) whereas the lowest final harvest weight was found in T1 (304.4±5.0 g/tank). And there was significantly difference in survivability of fishes among different treatments. In Daily Weight Gain, there was significantly different among other treatments ($p>0.05$) where highest DWG was found in T5 (0.4±0.0 g/fish/day) and the lowest was found in T1 (0.1±0.0 g/fish/day). Moreover, Gross Fish Yield in T5 (12.8±0.9 kg/m^3/y) was significantly ($p<0.05$) higher than T4, T3, T2 and T1 with the value of 6.9±0.3, 4.8±0.2, 4.7±0.3 and 1.0±0.0 kg/m^3/y respectively. Furthermore, T5 (12.4±0.5 kg/m^3/y) was also significantly higher in Net Fish Yield than T4, T3, T2 and T1 with the value of 6.3±0.3, 4.3±0.3, 4.2±0.3 and 0.7±0.3 kg/m^3/y respectively.

Table 2. Growth and yield of Nile tilapia in different treatments in 150 days experimental period (Mean±SE).

Growth Parameters	Treatments				
	T1 (Control)	T2	T3	T4	T5
Stocking					
Initial Stock Number (No./tank)	40±0.0	40±0.0	40±0.0	40±0.0	40±0.0
Initial Mean Weight (g/fish)	5.0±0.4^{a}	5.3±1.1^{a}	5.4±1.1^{a}	4.8±0.4^{a}	4.4±0.7^{a}
Total Initial Weight (g/tank)	173.6±39.2^{a}	211.1±43.3^{a}	217.8±43.5^{a}	264.7±7.6^{a}	177.2±29.8^{a}
Harvesting					
Final Harvest Number (No./tank)	23.3±0.9^{b}	31.0±3.0^{a}	29.0±4.0^{a}	34.0±2.1^{a}	35.3±1.7^{a}
Final Mean Weight (g/fish)	13.0±0.4^{d}	24.8±1.8^{d}	28.6±1.6^{c}	33.8±2.6^{b}	58.8±7.6^{a}
Final Harvest Weight (g/tank)	304.4±5.0^{e}	774.8±55.1^{d}	795.1±32.6^{c}	1138.2±43.2^{b}	2104.8±68.1^{a}
Daily Weight Gain (g/fish/day)	0.1±0.0^{c}	0.2±0.0^{b}	0.1±0.0^{c}	0.2±0.0^{b}	0.4±0.0^{a}
Gross Fish Yield (kg/m^3/y)	1.0±0.0^{d}	4.7±0.3^{c}	4.8±0.2^{c}	6.9±0.3^{b}	12.8±0.9^{a}
Net Fish Yield (kg/m3/y)	0.7±0.3^{d}	4.2±0.3^{c}	4.3±0.3^{c}	6.3±0.3^{b}	12.4±0.5^{a}
Apparent Feed Conversion Ratio (AFCR)	0.13±0.3^{d}	0.30±0.5^{b}	0.24±0.3^{c}	0.31±0.6^{b}	0.56±1.9^{a}
Survival (%)	58.3±2.2^{e}	77.5±7.5^{c}	72.5±10.1^{d}	85.0±5.2^{b}	88.3±4.2^{a}

(T1= Without floc (Control); T2= With floc (C:N-10:1); T3= With floc (C:N-15:1); T4= With floc (C:N-20:1) and T5= With floc (C:N-25:1). Mean value with different superscript letter within same row are significantly different at $p < 0.05$)

Table 3. Mean and Range of Water quality parameters of different treatments during the experimental period of 150 days (Mean±SE)

Growth parameters	Treatments				
	T1(Control)	T2	T3	T4	T5
Temp (^{0}C)	34.7±4.3 ^{a}	37.0±3.3 ^{a}	38.4±2.6 ^{a}	38.6±2.2 ^{a}	38.8±2.6 ^{a}
	(16.3-38.2)	(20.5-37.4)	(21.7-38.6)	(23.0-38.9)	(21.1-38.4)
pH	8.0±0.0 ^{a}	8.1±0.1 ^{a}	8.1±0.1 ^{a}	8.2±0.13 ^{a}	8.3±0.1^a
	(6.3-8. 7)	(7.2-8.9)	(7.4-8.8)	(7.4-8.8)	(7.3-9.3)
DO (mg/L)	5.5±2.4 ^{a}	5.8±2.6 ^{a}	5.7±2.4 ^{a}	5.8±2.4 ^{a}	5.1±2.6 ^{a}
	(0.5-8.7)	(0.5-8.9)	(0.5-8.7)	(0.5-8.9)	(0.5-8.9)
NH_3 (mg/L)	0.4±0.3 ^{a}	0.1±0.1 ^{a}	0.2±0.1 ^{a}	0.5±0.2 ^{a}	0.4±0.2 ^{a}
	(0.0-1.0)	(0.0-0.5)	(0.0-0.5)	(0.0-1.3)	(0.0-1.3)
NO_2 (mg/L)	0.3±0.1 ^{a}	0.3±0.2 ^{a}	0.5±0.2 ^{a}	0.4±0.0 ^{a}	0.6±0.1 ^{a}
	(0.0-0.5)	(0.0-1.0)	(0.0-1.0)	(0.0-1.0)	(0.0-1.3)
Floc (mg/L)	2.7±0.2 ^{e}	10.78±0.44^d	11.67±0.51^c	12.11±0.91^b	15.11±1.25^a
	(1.0-4.0)	(10.33-13.00)	(11.00-12.67)	(10.33-13.67)	(13.00-18.00)
TDS (mg/L)	659.11±68.1^e	1225±7.0^d	1415.22±30.8^c	1562.11±37.4^b	1678.67±77.2^a
	(523.0-892.0)	(910.0-1345.0)	(1088.0-1503.0)	(1226.0-1679.0)	(1179.0-1791.0)
TSS (mg/L)	373.78±13.5^a	397.33±20.8^a	361.44±14.91^a	384.56±10.72^a	385±22.01^a
	(305.0-423.0)	(321.0-456.0)	(256.0-432.0)	(238.0-456.0)	(274.0-473.0)

(T1= Without floc (Control); T2= With floc (C:N-10:1); T3= With floc (C:N-15:1); T4= With floc (C:N-20:1) and T5= With floc (C:N-25:1). Mean value with different superscript letter within same row are significantly different at $p < 0.05$)

Water quality parameters

Mean values of water quality parameters of different treatments is shown in Table 3.. There was a significant difference in Dissolved Oxygen, Floc level and TDS at $p < 0.05$. TDS in T5 (1678.67±77.2 mg/L) was significantly ($p < 0.05$) higher than T4, T3, T2 and T1 with the value 1562.11±37.4, 1415.22±30.8, 1225±7.0 and 659.11±68.1 mg/L respectively. The floc level was found highest in T5 (1678.67±77.2mg/L) and the lowest was found in T1 (659.11±68.1mg/L). The highest temperature was found in T5 (38.9°C) whereas the lowest was found in T1 (16.3°C) in Figure 1. Similarly, the highest Dissolved Oxygen was found in T5, T4 and T2 (8.9 mg/L) and the lowest was found 0.5mg/L in all the treatments (Figure 2). The highest pH was found in T5 (9.3) and the lowest pH was found in T1 (6.3) in Figure 3. In case of Ammonia, the highest value was found in T5 and T4 (1.3 mg/L) and the lowest was found with 0.0 mg/L in all the

treatments (Figure 4). In figure 5, the highest nitrite was found in T5 (1.3 mg/L) and the lowest was found with 0.0 mg/L in all the treatments. Moreover, TSS was found highest in T5 (473 mg/l) and lowest in T3 (256. mg/L) (Figure 8).

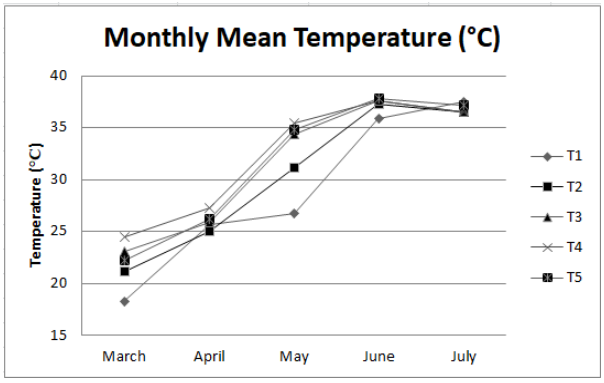

Figure 1. Monthly Mean Temperature (°C) among the treatments during the experimental period

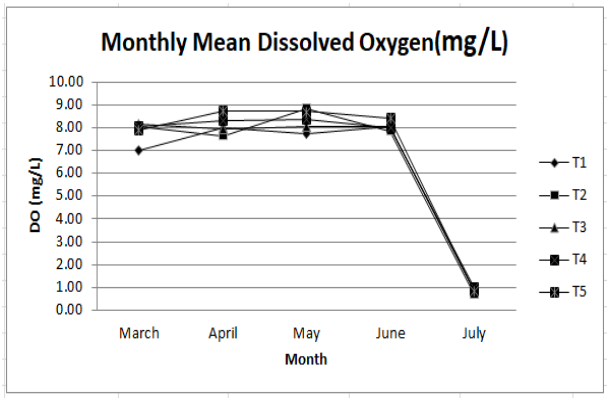

Figure 2. Monthly Mean Dissolved Oxygen (mg/L) among the treatments during the experimental period

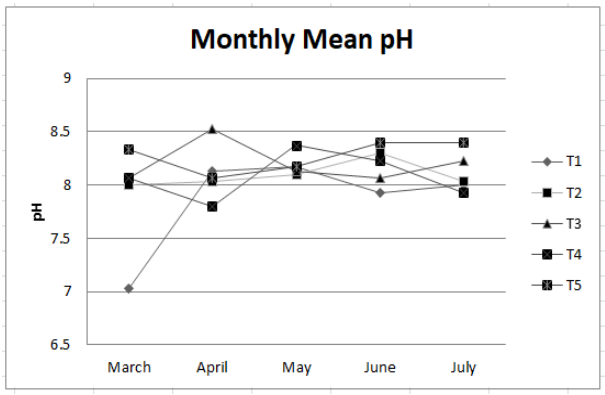

Figure 3. Monthly Mean pH among the treatments during the experimental period

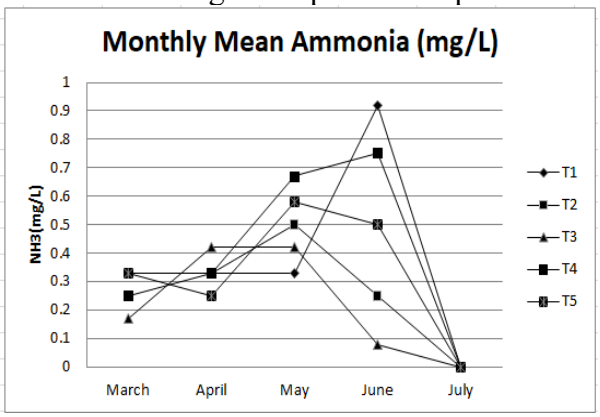

Figure 4. Monthly Mean Ammonia (mg/L) among the treatments during the experimental period

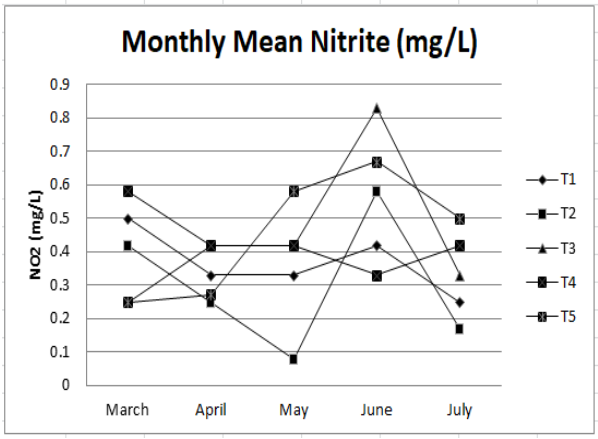

Figure 5. Monthly Mean Nitrite (mg/L) among the treatments during experimental period

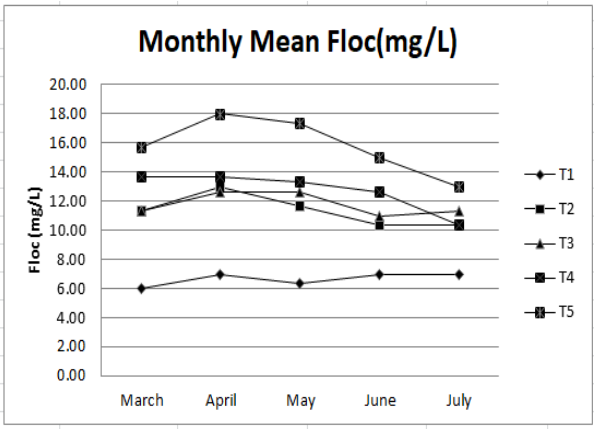

Figure 6. Monthly Mean Floc (mg/L) among the treatments during the experimental period

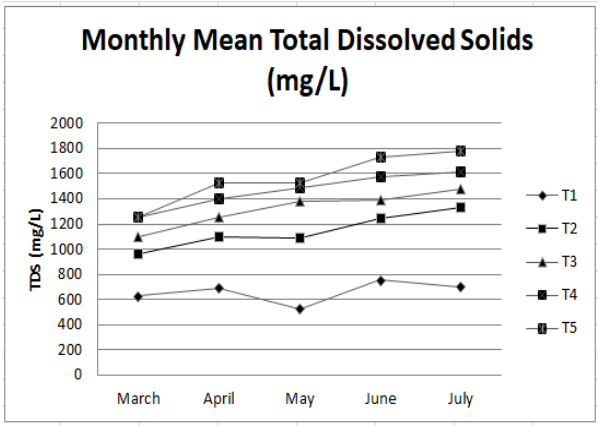

Figure 7. Monthly Mean TDS (mg/L) among the treatments during the experimental period

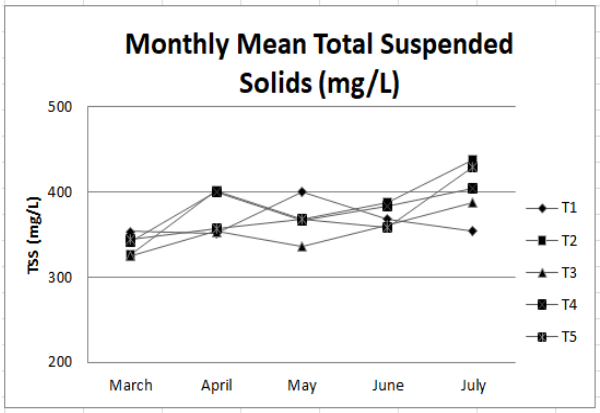

Figure 8. Monthly Mean TSS (mg/L) among the treatments during the experimental period

Table 4. Mean value of abundance ($\times 10^5$ cfu/ml) of bacteria in different treatments during the experimental period of 150 days (Mean±SE)

Abundance of bacteria (cfu/100 ml)	Treatments				
	T1 (Control)	T2	T3	T4	T5
	6.89 ±2.92 ^{e}	20.26 ±8.21 ^{c}	20.03 ±6.1^{d}	24.20 ±2.74^{b}	35.82± 15.33^{a}

(T1= Without floc (Control); T2= With floc (C:N-10:1); T3= With floc (C:N-15:1); T4= With floc (C:N-20:1) and T5= With floc (C:N-25:1). Mean value with different superscript letter within same row are significantly different at p <0.05)

Abundance of Heterotophic Bacteria:

Mean value of abundance of bacteria (cfu/100 ml) in different treatments during the experimental period of 150 days is shown in Table 4. There was no significant difference among the treatments in the mean value of abundance of bacteria at p <0.05. Among the treatments, T5 (35.82±15.33 cfu/100 ml) was significantly higher than T4, T2, T3 and T1 with the value 24.20±2.74, 20.26±8.21, 20.03±6.1 and 6.89±2.92 cfu/100 ml, respectively.

Table 5. Mean value of abundance ($\times 10^2$ cells/L) of phytoplankton in different treatments during the experimental period of 150 days (Mean±SE)

Abundance of phytoplankton (cells/L)	Treatments				
	T1(Control)	T2	T3	T4	T5
Chlorophyceae	0.03±0.01^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
Bacillariophyceae	0.02±0.01^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.02±0.01^{a}	0.02±0.00^{a}
Cyanophyceae	0.04±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.03±0.00^{a}	0.04±0.01^{a}
Euglenophyceae	0.03±0.01^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}

(T1= Without floc (Control); T2= With floc (C:N-10:1); T3= With floc (C:N-15:1); T4= With floc (C:N-20:1) and T5= With floc (C:N-25:1). Mean value with different superscript letters within same row are significantly different at p <0.05)

Abundance of phytoplankton

Mean value of abundance of phytoplankton ($\times 10^2$ cells/L) in different treatments during the experimental period of 150 days is shown in Table 5. There was no significant difference among the treatments in the mean value of abundance of phytoplankton (Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae) at p <0.05.

Table 6. Mean value of abundance ($\times 10^2$ cells/L) of zooplankton in different treatments during the experimental period of 150 days (Mean±SE)

Abundance of zooplankton (cells/L)	Treatments				
	T1(Control)	T2	T3	T4	T5
Rotifers	0.04±0.01^{a}	0.06±0.01^{a}	0.04±0.01^{a}	0.05±0.01^{a}	0.05±0.00^{a}
Copepoda	0.04±0.00^{a}	0.05±0.01^{a}	0.04±0.00^{a}	0.05±0.00^{a}	0.05±0.01^{a}
Cladocera	0.02±0.00^{e}	0.11±0.02^{c}	0.06±0.01^{d}	0.12±0.01^{b}	0.18±0.00^{a}
Protozoa	0.02±0.00^{e}	0.12±0.02^{d}	0.20±0.03^{b}	0.15±0.01^{c}	0.21±0.01^{a}

(T1= Without floc (Control); T2= With floc (C: N-10:1); T3= With floc (C:N-15:1); T4= With floc (C:N-20:1) and T5= With floc (C:N-25:1). Mean value with different superscript letter within same row are significantly different at $p < 0.05$)

Abundance of zooplankton

Mean value of abundance of zooplankton ($\times 10^2$ cells/L) in different treatments during the experimental period of 150 days is shown in Table 6. There was no significantly different among the treatments in the mean value of abundance of Rotifers and Copepoda at $p < 0.05$. But there was significantly different among the treatments in the mean value of Cladocera and Protozoa at $p < 0.05$. In Cladocera, T5 (0.18±0.00 10^2 cells/L) was significantly different than T4, T2, T3 and T1 with the value of 0.12±0.01, 0.11±0.02, 0.06±0.01 and 0.02±0.00 10^2 cells/L respectively. In Protozoa, T5 (0.21±0.01 10^2 cells/L) was significantly different than T3, T4, T2 and T1 with the value of 0.20±0.03, 0.15±0.01, 0.12±0.02 and 0.02±0.00 10^2 cells/L respectively.

Discussion

The present study demonstrated positive results on the production performance of Nile Tilapia in biofloc system. The growth rate of tilapia in different treatments within 150 days period showed that treatment having the highest C/N ratio supported the highest growth in Tilapia. Comparing to control, the growth of Tilapia in the treatment T5 maintained with highest C/N ratio attained higher yield than the control. Final mean weight of tilapia was found significantly difference among the treatments ($p > 0.05$) where final mean weight in T5 (58.8±7.6g) was found higher. Our results support the findings of Bhattacharya *et al*. (2023) who showed that the value of 41.73 ± 6.0 g in C:N ratio 20:1.). Furthermore, there was significant difference in final harvest weight among the treatments where highest final harvest weight was found in T5 (2104.8±68.1 g/tank) which is higher than the final harvest weight of 2006 ± 51g/tank with the outcome of Bhattacharya *et al*. (2023).

Although production performance did not show satisfactory results among the treatments on total biomass. But it can increase the efficiency of feeding. This can be found from the combination of total feed and overall AFCR, which is seen better in C / N ratio treatments as compared to control without biofloc treatment. This might be due to the bioflocs generated from the addition of carbon sources increased the availability of additional food in the culture tank. It confirms with the previous study showing that the addition of carbon sources in biofloc systems will increases fish production through the increased microbial protein produced in the system that can be used as an additional source of nutrition for the cultured fishes (Zhao *et al*., 2014). The higher efficiency of feed utilization in biofloc treatments may be observed in the lower total feed in biofloc treatments.

The low total feed in biofloc treatments indicates that tilapia fish consume various types of feed available in the culture media, including bioflocs suspension. Tilapia is known as omnivorous species that consume various types of feed including algae and detritus (Wang and Lu, 2015). Besides it, biofloc may contain major nutrients such as protein and lipids that will contribute to the growth of the cultured fishes (Crab *et al*., 2010). A previous study by

Ekasari *et al*. (2010) notifies that the biofloc system produced proteins that can be easily absorbed, which might be contribute to the lower FCR. The results of other studies showed that the culture of tilapia in biofloc system has decreased FCR and reduce feed consumption by 20% compared to tilapia culture with a water exchange system (Azim and Little, 2008; Emerenciano *et al*., 2013). The low FCR to the availability of biofloc microorganisms that will reduce the requirement for external feeding thereby decreases the FCR (Ekasari *et al*., 2010; Pérez-Fuentes *et al*., 2016). In case of DWG and AFCR, it was found 0.4±0.0 g/fish/day and 0.56±1.9 in T5 which is quite similar to the findings of Dilmi *et al*. (2022). During the study, abundance of phytoplankton was also studied where Chlorophyceae and Cyanophyceae was found highest in biofloc system among others reveals similar result with the findings of Aboseif *et al*. (2022). Similarly, protozoa were found the most dominant species of zooplankton which resembles the result of Aboseif *et al*. (2022).

The levels of Ammonia and nitrite in the culture media were within a normal range for tilapia culture (Cavalcante *et al*., 2014; Caldini *et al*., 2015; Dagne *et al*., 2013). The lowest average Ammonia and nitrite concentrations during the culture period were observed in the biofloc treatment with a C/N ratio of 15 and 20. The low concentration of Ammonia in biofloc tanks compared with that of the control may be due to the presence of heterotrophic bacteria in BFT systems which accelerated the rate of ammonia reduction in the media (Widanarni *et al*., 2012). The values of pH and temperature did not differ significantly among the treatments and they were found within the optimal conditions for the normal growth of tilapia. High microbial activity was also resulted which may be a gradual reduction in the dissolved oxygen concentration in all treatments (Fleckenstein *et al*., 2018; Avnimelech, 2009). Furthermore, the floc volume in all the biofloc treatments was significantly higher than that in the control. This may be due to the addition of carbon sources in these treatments, by which increased the growth of biofloc (bacterial biomass) (Pérez-Fuentes *et al*., 2016)

Conclusion

Biofloc treatment with a C/N ratio of 25 gave the highest total biomass values and improves the water quality among treatments.

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