Original Research Article

Comparative Study of Biofloc, Bio-Phyton and Aquamimicry System Using Millet as Carbon Source in Catla catla

Abstract :

A comparative study was conducted for 65 days of three treatment groups - Biofloc (T1), Biophyton (T2), and Aquamimicry (T3) - alongside a control group (T4) to investigate the growth performance, water quality parameters, proximate composition and total plate count of *catla catla* fingerlings(0.69±0.02A) . In this millet serve as carbon source in treatment and in control no carbon source is added. At the end of experiment biofloc technology (T1) exhibited superior results across various parameters, highlighting the robustness of this established approach. In results T1 (Biofloc) outperformed compare to other treatments in growth performance metrics, showcasing higher body weight gain percentage (39.49±4.35B),Specific growth rate (2.30±0.00b), feed efficiency ratio(0.88±0.07F),protein efficiency ratio(2.20±0.17F),lipid efficiency ratio(14.67±1.18e) and survival rates(90.21±1.77C) and lower feed conversation ratio (1.15±0.09F). Biofloc technology (T1) maintained optimal water quality parameters, indicating effective nutrient cycling and microbial activity. The consistent performance in water quality aligns with the positive outcomes in growth performance, emphasizing the interdependence of these factors. Microbial analyses, including TPC confirmed the presence of more beneficial microbes in Biofloc (T1) compared to T2, T3 and control respectively. The microbial profile in T1 may have contributed to the overall health and growth performance observed in this treatment. Based on result acquired from this study, millet used as a carbon source for biofloc to augment the growth and productivity of *C. catla* under farming.

Keywords: *Growth Performance, Water Quality, Microbial Load, Catla, Biofloc, Bio-Phyton, Aquamimicry*

1. INTRODUCTION

Aquaculture stands out as an economically promising source of food commodities, offering a pathway towards enhanced food and nutritional security for the global population in the future, while reducing reliance on capture fisheries. Achieving sustainable growth in aquaculture production hinges on two key objectives: implementing high stocking density aquaculture systems with modern technologies and introducing new species (FAO,2022). The intensification of aquaculture has significantly contributed to the heightened generation of toxic nitrogen metabolite waste from aquaculture systems. The nutrient-rich aquaculture effluent has had a detrimental impact on the ecosystem, triggering public outcry. Consequently, the long-term viability of aquaculture production systems is at risk (Dauda et al., 2018). Yet, intensifying aquaculture systems brings its own set of difficulties. Maintaining water quality becomes a crucial worry as densely populated aquatic environments are prone to pollution. Diseases can spread more easily in such conditions, demanding careful management and monitoring. Additionally, intensified aquaculture often results in high feed consumption, adding pressure to global fisheries for feed ingredients. The substantial waste produced from these systems presents an environmental challenge, affecting both water ecosystems and the nearby land. The considerable water demand of intensified aquaculture further worsens the strain on already limited water resources.It is crucial to adapt aquaculture practices to withstand these changes for the sustainable growth of the industry. Striking a balance between the demand for more protein production and the environmental and resource challenges linked to intensified aquaculture is essential to tackle the intricacies of global food security in the future.

About fifty percent of the total expenses in aquaculture output stem from feed costs, primarily due to the expensive nature of protein in commercial diets. A crucial step in enhancing aquaculture productivity and sustainability involves optimizing the utilization of feed nutrients.Providing a greater supply of natural food to farmed animals is considered an alternative to the intensive use of artificial feed in fish farming. The use of artificial substrates and biofloc technology, highlighted as two current options, is suggested as potential solutions (BFT) (Cavalcante et al., 2016).

Biofloc technology fosters a dense community of microorganisms within aquaculture systems, reducing reliance on external water sources. Described as "the application of bacterial, algae, or protozoa aggregates in a matrix with organic matter to enhance waste treatment, disease control, and water quality,"Biofloc Technology (BFT) is seen as a new "blue revolution" (FAO, 2022). It is a low-cost, eco-friendly method with large-scale adoption, promoting minimal water exchange (Cavalcante et al., 2016). This sustainable approach utilizes microorganisms to absorb nitrogenous substances, generate microbial proteins, and maintain water quality economically. It emphasizes aqua mimicry, providing a stress-free environment and discouraging pathogen proliferation (Bossier and Ekasari, 2017). BFT focuses on in-situ microbe processing, widely employed in aquaculture for effective water filtration and floc production (Jung et al., 2020). Anticipated to ensure human food security, BFT alters the water's C:N ratio to remove harmful substances, utilizing bioflocs to regulate water quality and serve as a natural food supply (Kumar et al., 2021). The flocs also act as natural bioremediation, preserving water quality and serving as significant nutrient sources, promoting growth, immunity, and disease tolerance in aquaculture animals. To encourage periphyton growth, enhance water quality, and increase feed efficiency, a variety of underwater substrates can be utilized in fish and shrimp tanks.

Aquamimicry, an innovative approach in aquaculture, utilizes fermented carbon sources to boost copepod populations. Copepods, small crustaceans, play a crucial role in aquatic ecosystems as a natural food source for various aquatic organisms. (Bossier and Ekasari, 2017).

The effective implementation of the mentioned technology hinges significantly on the C:N ratio, a crucial factor ultimately determined by the exogenous carbon source employed. The selection of an appropriate carbon source is vital, considering factors such as the percentage availability, the presence or absence of any anti-nutritional factors, structural complexity for easy accessibility, and digestibility. While various carbon sources have been utilized in Biofloc Technology (BFT) and Aquamimicry systems over the years, the exploration of millet as a potential carbon source has been relatively limited. Also 2023 is celebrated as international year of millet, we use millet as carbon source. Further research and investigation into the utilization of millet in these systems could offer valuable insights into its effectiveness and potential benefits.

As dietary preferences shift towards healthier and sustainable options, millets are gaining popularity as a vital element of a balanced diet. The embrace of millets not only contributes to agricultural sustainability but also enhances food security and supports human health, establishing millets as a valuable and multifaceted crop in the quest for a resilient and well-nourished global population

2 MATERIALS AND METHODS

2.1 Experimental design and set-up

The research experiment was carried out in the wet laboratory at the College of Fisheries Science (C.F.Sc) of Kamdhenu University, located in Rajpur (Nava), Himmatnagar. Water quality analysis was conducted in the biochemistry laboratory, while microbiological analysis was performed at the central biotechnology laboratory at Kamdhenu University, Himmatnagar.

C: N was calculated by method described by Avnimelech, 1999. *Catla catla* were stocked at a rate of 1.5 g/L of water. After 7 days of acclimatization period, fish fry with an average initial body weight of Catla (0.88±0.03) gm were distributed in treatment. The bore well water was filled in each circular tank. Water refilled when the evaporation loss to compensate the water level, and in control daily 10% water was changed. The fry were fed at the rate of 10% body weight to satiety, twice a day with a commercial pellet of 0.6mm for 30 days (40% protein, 6% fat, 3% crude fiber, 11% moisture).

2.2 Preparation of inoculum

To set up a biofloc system, begin by utilizing a 1000-liter capacity water tank and filling it with 300 liters of water. Ensure aeration is provided. Next, incorporate 30 grams of ammonium sulfate as a nitrogen source. Subsequently, transfer a portion of water into a separate bucket and combine it with millet as the carbon source. Introduce probiotics into the millet-water mixture, ensuring thorough mixing. Reintroduce this mixture back into the main tank and continue aeration. After 5 days, observe a decrease in ammonia levels. Once the floc volume reaches 10-15 ml per liter of water, the tank is prepared for fish stocking (Avnimelech, 2014).

2.3 Bio-phyton tank set-up

Based on the previous instructions, the biofloc was established in triplicate within 300 liters of water. Periphytic substrate was incorporated vertically to enhance surface area for periphyton development. Agriculture netting and bamboo were utilized as substrates. Additionally, artificial light was supplied to foster periphyton growth. Across all three replicates, a consistent C:N ratio of 20:1 was maintained by adding millet.

2.4 Aquamimicry set-up

To initiate fermentation, the following facilities are required: 100 kg of millet and 300 liters of pond water. Additionally, 20 kg of $NaHCO_3$ and 10 g of probiotics are added, and the mixture is left to ferment for at least 48 hours, with occasional stirring. Once fermented, this solution is broadcasted throughout the tank while aeration is on. It's crucial to avoid dragging the solution through the tank on a daily basis, as this can significantly reduce biofilm development. To maintain copepod density in the system, fermented millet juice is added at a rate of 150 ml every three days.

2.5 Assessment of physico-chemical water quality parameters

A standardised Cole parmer(PC100)pH/Conductivity meter was used to test the pH of experimental tank water at weekly. Total dissolved solids(TDS), electric conductivity(EC) and temperature were determined weekly using a HANNA instruments EC/TDS/ Temperature portable meter. Whereas dissolve oxygen (DO) and total ammonium nitrogen (TAN) were analysed weekly using standard method (APHA, 1998). Floc volume was measured weekly by using imhoff cone.

2.6 Determination of fish growth parameters and survival

Fishes (n=15) of different treatment groups were weighed at every 10 days interval and assessed for the growth parameters like body weight gain(%) (BWG), Feed conversion ratio(FCR), feed conversion efficiency (FCE), specific growth rate(%) (SGR), Protein efficiency ratio (PER), Hepatosomatic Index (HIS), Lipid Efficiency ratio(LER), Intestinal Somatic Index (ISI) and Survival rate (%). Fish growth characteristics were measured using the following formulas:

Survival rate (%) =(Number of fish at the end of the experiment / Number of fish at the beginning of experiment) x 100

SGR (%) = [ln(FW)-ln(IW)/N]x100

BWG (%) = (FW- IW) x100/IW

FCR = Feed intake/ weight gain

FCE = 1/FCR

LER = Wet weight gain / lipid intake on dry matter basis

PER = Wet weight gain / protein intake on dry matter basis

HIS (%) = (Wet weight of liver/wet weight of whole fish)x100

ISI(%) = (Wet weight of intestine/ wet weight of whole fish)x100

(Where, FW= Final weight, IW= Initial weight, N = Number of days of the experiment, ln= Natural log.)

2.7 Proximate analysis

At the end of experiment proximate composition of floc was measured according to standard protocol of AOAC, (1995).Crude protein and lipid was evaluated by Kjeldahl method and Soxhlet apparatus, respectively. Moisture was analyzed by Automatic moisture analyzer.

2.8 Total plate count

Sample (water, floc, gut) were collected in sterile tube and performing the serial dilution using 0.85% saline. 100µl sample were taken for the spreading on nutrient agar by using the sterile spreader. Total number of colonies were counted by LABWAN microprocessor colony counter and calculated as per following formula.

CFU/ml=total no. of colonies x dilution factor/volume of sample

2.9 Microscopic Observation

Collect the developed floc and examine the live feed / floc development under the compound microscope with magnification of 10X and 40X.

2.10 Statistical analysis

The experiment was conducted using completely randomized design and the analysis of data was analysis using one-way ANOVA (analysis of variance), with descriptive values mean ± standard error, and homogeneity of subsets by using Tukey’s method for different superscripts.

3 RESULT AND DISCUSSION

3.1 Physico-Chemical Water Quality

Over the course of the 65-day experiment, water quality parameters were evaluated at distinct intervals (1-7, 8-14, 15-21, 22-28, 29-35, 36-42, 43-49, 50-56, 57-63 days respectively) across four treatments viz. Bio-floc (T1), Bio-phyton (T2), Aqua-mimicry (T3), and Control (C) respectively. The dissolved oxygen (DO) levels were systematically evaluated and revealed that during the initial week (1-7 days), (T3) exhibited significant lower ($p<0.05$) mean DO compare to rest of the treatments including control. Whereas, on 8-

14th and 36-42nd interval mean DO values was non-significant among treatments. On the 15-21st and 43-49th days interval the average mean value of DO found significant difference ($p<0.05$) in control compared to T1, T2, and T3 respectively. In the later period of experiment, it was observed that on the 50-56th and 57-63rd days interval the DO values were significant higher ($P<0.05$) compared to T1 respectively. The increasing trend of DO in the control followed by T3, T2 and the lowest DO level was noticed in T1 across the experimental period. The average mean values of temperature (^{o}C) were notably high in the initial phase of the experiment whereas decrease in the later part of the experiment among all the treatments. Further there is slight fluctuation in temperature across the treatment during the experimental period. The average mean values of temperature on 1-7th, 14-21st, 22-28th, 29-35th and 35-42nd day interval found non-significant ($P>0.05$) among the treatments respectively whereas, on 7-14th days T1 and control shows significant ($p<0.05$) difference with T2 and T3 respectively. However, on 42-49th and 56 -63rd day interval T2 shows significant difference ($P<0.05$) with T1 and control respectively.

The values of total ammonium nitrogen during the experiment al period shows initial highest in T2, followed by T3 and control and least in T1 for 2 week (1-14th days interval) afterward there was a decreasing trend was observed particular in T1 , T2 and T3 respectively. On 1-7th, 8-14th, 22-28th,36-42nd, 43-49th days interval the values of TAN found non-significant($P>0.05$). Whereas, on the 15-21st day interval T1 shows significant difference with T3, however, on 50-56th and 57-63rd day of interval T1 shows significant difference($p<0.05$) with T2, T3 and control respectively. The decreasing trends in total ammonia nitrogen (TAN) concentration in the Biofloc system may be attributed to the availability of heterotrophic bacterial community growth. Similar observations were noted by Avnimelech (1999), Ebeling et al. (2006), Xu et al. (2016), and Zhu et al. (2021). They concluded that an increase in the carbon-to-nitrogen (C/N) ratio prompts a shift in the biofloc community to a heterotrophic system, effectively maintaining TAN and nitrite nitrogen (NO2-N) at lower concentrations even at higher stocking densities in the culture water. Our findings, supported by Solanki et al. (2013), regarding water parameters, showed that increasing the C/N ratio from 10 to 20 significantly ($p < 0.05$) reduced TAN and NO2-N while increasing nitrate nitrogen (NO3-N) in the water.

In the present experiment the values of Total Dissolved Solids (TDS) show decreasing trends among the treatments including control particularly in the later part of the experiment. On the 1-7th and 8-14th days of experiment the average values of TDS found significant ($p<0.05$) in T2 and T3 compared to T1 and control respectively. Further, on 15-21stday T3 shows significant ($p<0.05$) difference with control and T1 respectively. However, on 29-35th, 36-42nd, 43-49th and 50-56th day of experiment the values of control show significant ($p<0.05$) difference with rest of the treatments. The values of TDS on 57-63rdday of interval control and T1 shows significant ($p<0.05$) with T2 and T3 respectively. The level of total dissolved solids (TDS) can have both positive and negative effects on fish growth. Khanjani et al. (2016) proposed that in zero water exchange tanks, total suspended solids (TSS) tend to increase over time due to reduced water exchange, high organic substance content, and an increase in microbial biomass, leading to high TDS levels over time. However, Avnimelech (2014) and Hosain et al. (2021) suggested that excessive TSS levels can be harmful, especially for certain fish species.

During the experiment, The Electrical Conductivity (EC) values exhibited distinct trends across various treatments and intervals. Notably, a declining pattern in EC values was observed among the treatments, including the control, particularly in the later stages of the experiment. During the initial intervals (1-7 days and 8-14 days), significant differences ($p<0.05$) in mean EC values were noted. T2 showed significantly lower EC than T1 and Control during the 1-7thday interval, while T3 exhibited significantly lower EC than T1 and Control during the 8-14th day interval. Further distinctions emerged during the 15-2nd day interval, with T3 displaying significant differences ($p<0.05$) in EC values compared to Control and T1. However, in the subsequent intervals (22-28 days and 29-35 days), the Control consistently demonstrated significantly lower EC values compared to the other treatments. As the experiment progressed into the later intervals (36-42 days, 43-49 days, 50-56 days, and 57-63 days), significant differences ($p<0.05$) in EC values were consistently observed, with the Control showing higher or lower values compared to the rest of the treatments. The increase in total dissolved solids (TDS) level in T1 may be due to a higher conglomeration of the microbial community resulting from the availability of waste. Perez-Fuentes et al. (2016) and Mugwanya et al. (2021) suggested that the carbon source and the carbon-to-nitrogen (C/N) ratio influence the nutritional content and quality of biofloc.

Further the values of pH in the present study exhibited notable variations across different treatments and interval. During the initial intervals (1-7 days and 8-14 days), significant differences ($p<0.05$) in mean pH values were observed. T1 exhibited a significantly higher ($p<0.05$) pH compared to T2, T3, and T4 during the 1-7th day interval. Similarly, in the 8-14th day interval, T4 displayed a significantly lower pH compared to the other treatments. As the experiment progressed to the 15-21st day interval, T3 demonstrated a significantly higher pH compared to T4, T2 and T1 respectively. However, in subsequent intervals (22-28 days T3 shows significant differences ($p<0.05$) in T1 and T4 respectively. In the later stages (36-42 days, 43-49 days, 50-56 days, and 57-63 days), significant differences ($p<0.05$) in pH values was recorded in T3 compared to rest of the treatment. T3 exhibited a significantly higher pH compared to T1 and T4 during the 36-42nd day interval. The finding supported by Das et al (2017) who conducted experiment on *Catla catla* and revealed that the average pH was in the suitable range (7.40–7.70)

3.2 Growth performance of *Catla catla*

The average initial and final weight performance among different treatments (Bio-floc - T1, Bio-phyton - T2, Aqua-mimicry - T3, Control - T4) was analysed. In the experiment average initial and final weight was measured and illustrated in table 1. Form Biofloc, bio-phyton, aqua-mimicry, control decline trend was found. Highest final weight was found in biofloc (4.45±0.14a) and minimum was found in control (2.30±0.14c). In final weight significance difference was found as $p<0.05$. There was a decreasing trend observed in the average final weight from T1 to T4 respectively. The treatment T1 shows significant ($p<0.05$) higher average weight compared to T3 and T4 respectively. The finding was supported by Solanki et al (2023) conducted an experiment on *Gibelion catla* spawn rearing in the indoor biofoc system using rice bran used carbon source for manipulating C/N ratios .The analysis of the growth performance across different intervals (1-10, 11-20, 21-30, 31-40, 41-50, 51-60) for various treatments (Bio-floc - T1, Bio-phyton - T2, Aqua-mimicry - T3, Control - T4) reveals distinctive trends in the growth performance of the experimental subjects.

The calculated Body Weight Gain (BWG) during the initial stages 1-10th and 11-20th days interval shows an increasing trend in T1 followed by T3, T2 and T1 respectively, the data found non-significant ($p>0.05$) among treatments. Further, similar trend was observed during the 31-40th day of experiment where T2 shows maximum BWG % followed by T1, T4 and T3 respectively, the values of BWG % found significant in T3 compared to rest of the treatments. In the end of the experiment 51-60th days of sampling BWG % shows significant difference in T1 compared to T2, T3 and T4 respectively. The highest BWG % was observed in T1 and the lowest in T4.*Catla catla* primarily feeds on the water's surface, but studies by V.G. (1991) suggest that *Gibelion catla*, a subspecies, is also a surface feeder and can explore the middle and bottom layers of water. Additionally, Catla spawn begin feeding on plankton, mainly zooplankton, from the third day after hatching.

The observed values of Specific Growth Rate (SGR) found non-significant ($p>0.05$)at most of the day's interval viz. 1-10th, 11-20th,41-50th and 51-60th respectively. However, there was a decreasing trend observed in SGR from T1 to T4 respectively. The maximum value of SGR was noted in T1 and minimum in T4. Except on 21- 30thhere, T1 shows significant difference ($p<0.05$) T3 and T4 respectively. Whereas, on 31-40th day interval T4 shows significant difference ($p<0.05$) rest of the treatments, However, in the following intervals, Bio-floc (T1) consistently demonstrated superior SGR compared to the other treatments, suggesting its efficacy in promoting favourable growth rates throughout the experimental period.

The Feed Conversion Ratio (FCR) values show and increasing trends from T1 to T4 respectively. The values of FCR found significant ($p<005$) during the initial day's interval (1-10th, 11-20th, 21-30th day of intervals) in T4 compared to T1 respectively. Further, the values of FCR shows an increasing trend, the maximum FCR was noticed in T4 and minimum in T1 respectively throughout the experimental period. Similar results were observed in FCE the values show a decreasing trend from T1 to T4 throughout the experimental period at different days intervals. The FCE values found significant($p<0.05$) at 1-10th and 12-20th day of interval in T4 compared to T1 respectively whereas, on 2-30th and 51-60th days of interval the values of FCE found significant in T1 compare to T3 and T4 respectively. However, on the 41-50th days of interval FCE values find non-significant ($p>0.05$) across the treatment. The findings suggest that Bio-floc (T1) maintained relatively higher feed conversion efficiency throughout the experiment, indicating its potential for optimizing feed utilization in aquaculture practices.

The observed values of Protein Efficiency Ratio (PER) show a decreasing trend from T1 to T4 throughout the experiment al period at different days of intervals. The values of PER found significant in T4 compared to T1 in 1-10th and 11-20th days of interval respectively. However, the values on 21-30th and 51-60th days of interval shows significant difference in T3 and T4 respectively. The values did not show any significant difference ($p<0.05$) on the 41-50th day of experiment. The findings suggest that Bio-floc (T1) maintained relatively higher protein efficiency throughout the experiment, indicating its potential for optimizing protein utilization in aquaculture practices. Similarly, the value of LER shows significant difference values in T1 compared to T4 (1-10th days interval), with T2 and T4 on (11-20th and 51- 60th days of interval) and with T3 and T4 on (21-30th days of interval) and respectively. On the 41-50th days of interval no significant difference ($p>0.05$) was observed among treatment. The survival (%) observed during the experimental period indicates higher survival in T1 followed by T2, T3 and T4 respectively. The survival (%) found significant in T4 compared to T2 and T3 on 1-10th days and 11-20 days' interval respectively.

The Hepatosomatic Index (HSI) and Intestine Somatic Index (ISI) were assessed for four different treatments. The HSI values during the study period indicated significant differences ($p<0.05$) in T4 compared to rest of the treatment. In the study Bio-floc (T1) had the highest HSI value at 1.72±0.19 followed by Aqua-mimicry (T3) at 1.22±0.19, Bio-phyton (T2) at 1.05±0.35 and least HIS values observed in Control (T4) at 4.04±0.22. Additionally, the Intestine Somatic Index (ISI) showed variations across the treatments, with Control (T4) having the highest ISI at 10.44±0.79), followed by Aqua-mimicry (T3) at 5.41±1.54, Bio-floc (T1) at 5.16±0.27, and Bio-phyton (T2) at 4.87±0.44. The treatment T4 shows significant difference ($p<0.05$) compared to rest of the treatment. These findings suggest distinct effects of different treatments on liver and intestine conditions in the studied aquatic environment.

Our results were consistent with the findings of Solanki et al. (2023), as they found that specific growth rates (SGR) were significantly higher in the C/N 20 treatment. Additionally, the availability of high microbial protein may have contributed to better growth in treatments compared to the control, as justified by Wilen and Balmér. (1999) and Shamsuddin et al. (2022). They observed that higher levels of dissolved oxygen (DO) reduce larger and compact floc sizes into smaller ones, allowing fish to easily consume the floc and thus enhancing fish growth. Furthermore, the microbial community develop in the treatments (T1 to T3 respectively) may enhance the innate/non-specific immune systems of cultured species by providing a wide range of immunostimulatory effects against microbial infections, supported by Kumar et al. (2023). Several bacterial microbial cell walls contain lipopolysaccharides, glucans, or peptidoglycans, known as microbe-associated molecular proteins (MAMPs), which can activate nonspecific immune mechanisms, leading to a significantly enhanced immune response in farmed species (Aguilera-Rivera et al., 2019; Panigrahi et al., 2019, 2020).

Enzymes play a significant role in digestibility, contributing to growth factors. Wang (2015) and Liu et al. (2019) suggested that microbial species contain various nutritional factors and digestive enzymes like amylase and proteases, improving food digestion and absorption. This leads to efficient feed utilization and enhanced growth performance of the host. Kim et al. (2013) found abundant bacterial biomass, with bacterial cell walls containing immunostimulatory agents like bacterial peptidoglycan, lipopolysaccharide and β-1,3-glucans, enhancing non-specific immune activity in shrimp. Additionally, Cerezuela et al. (2013)

proposed that microbes produce signalling molecules that alert the immune system, protecting hosts from pathogenic microbial infection.

The significant growth improvement observed in the present study is supported by several researchers. Ahmad et al. (2016) and Kamilya et al. (2017) found higher growth rates in *Labeo rohita*, while Mansour and Esteban (2017), Mirzakhani et al. (2019), Hwihy et al. (2021), and Shourbela et al. (2021) reported similar results in Oreochromisniloticus. Additionally, Bakhshi et al. (2018) and Aalimahmoudi and Mohammadiazarm (2019) observed enhanced growth in Cyprinus carpio when reared in biofloc water, showing improved feed efficiency ratio (FER), specific growth rate (SGR), and feed conversion ratio (FCR). Moreover, cultured animals displayed an enhanced non-specific immune response, with significantly increased serum protein, serum albumin, total immunoglobulin, lysozyme, respiratory burst, and myeloperoxidase activity. Overall, the presence of beneficial microbes in the system positively influences the growth, immune response, and disease tolerance of cultured animals.

In treatment "T1," the superior growth performance may be attributed to the abundance of microbial communities due to the high C:N ratio of 20:1 with millet as the carbon source. This environment offers high microbial protein and optimal water quality parameters. Additionally, the immunostimulatory benefits from the microbial communities were noted by several researchers, contributing to better growth performances. Xu and Pan (2013), Hostins et al. (2019), suggested that biofloc contains microbe-associated molecular proteins (MAMPs) and microbial bioactive components such as carotenoids, vitamins, glutathione, antioxidants, and minerals, which nutritionally modulate fish health and immune response, resulting in better growth performance and increased resistance against microbial pathogens. Despite the presence of harmful bacterial communities confirmed by total plate count (TPC) and MALDI-TOF analysis, the growth performances were superior under the biofloc system, possibly due to the superior growth of heterotrophic bacteria and associated benefits or interactions with harmful bacteria, supported by the findings of Khanjani et al. (2016). They revealed that the presence of carbonated organic matter enables heterotrophic bacteria to become more active than other bacteria, removing nitrogen and carbon from water through an absorption process and producing microbial biomass/flocs. These biomasses were subsequently combined/fed by other organisms (such as algae, detritus, ciliates, or yeast) and collectively formed biofloc in the culture system. However, in treatment "T2" (bio-phyton system), there was also a significant contribution to the abundance of food, but mostly the composition consisted of primary producers. Generally, in the peri-phyton system, the nibbling habits of fish explore, and the attached peri-phyton ensures continuous feeding attached on the substrate. Furthermore, the selection of suitable substrate enables the attachment site for microbial communities as well. However, the growth of fishes under the aqua-mimicry system was attributed to the availability of zooplanktons, which are the primary feed of *Catla catla,* particularly in the younger stages. Basically, the presence of copepods and other zooplanktons in "T3" are highly nutritious, as supported by Satoh et al. (2009), who revealed that copepods, particularly in terms of LC-PUFA (e.g., eicosapentaenoic, docosahexaenoic, and arachidonic acids), are crucial for growth and development. Additionally, copepods are rich in carotenoids, free amino acids (such as taurine), peptides, vitamins, and minerals (including selenium, iodine, copper, and manganese).

3.3 Total plate count

The Total Plate Count (TPC) in colony-forming units per milliliter (CFU/ml) provides insights into microbial populations in water and fish gut samples across different treatments (T1, T2, T3, T4). In water samples, Bio-floc (T1) and Bio-phyton (T2) exhibited showing highest TPC values (1.2105 and 1.0105 CFU/ml, respectively), followed by Aqua-mimicry (T3) and Control (T4) (2.9104 and 1.9104 CFU/ml, respectively). Similar trends were observed in fish gut samples, T1 had the highest (3.5107CFU/ml), followed T2(3.2107 CFU/ml), then T3 with (2.6105 CFU/ml), and the lowest count was observed in Control (T4) with (3.8105 CFU/ml).

The abundance of microbial communities in biofloc systems can enhance growth, immunity, and disease tolerance in hosts (Kumar et al., 2021). Heterotrophic microbial biomass may suppress harmful microorganisms (Michaud et al., 2006). Kumar et al. (2023) suggested that bacteria in clusters or biofilm mode of life play a crucial role in removing or converting harmful compounds, serving as excellent bio-sorbent materials for remediation of toxic substances.

3.4 Microscopic Analysis of Planktonic Communities during the Experimental Period

In the current investigation, various plankton species were examined under the microscope at magnifications at 10X and 40X across different treatments. The observed plankton species include *Euglyphatuberculata*, *Centropyxis aculeate*, *microalgae, Copepod sp., Synedra sp., nematodes*, *ciliates,* and *rotifers.* This comprehensive microscopic analysis contributes to a detailed understanding of the diverse planktonic community present in the studied environments, shedding light on the ecological dynamics within each treatment. The diverse community of organisms, including *Euglyphatuberculata* and *Centropyxis* aculeate (Testate Amoebae), *microalgae, Copepod sp., Synedra sp. (Diatom), nematodes, ciliates,* and *rotifers,* plays pivotal roles in aquaculture ecosystems.

3.5 Proximate composition of the fish

The Proximate composition of fish culture on dry matter percentage basis under treatment are as follows(Table 2). The protein content among treatment T1, T2, T3 and T4 shows 14.16%±0.07, 18.00%±0.10, 13.85%±0.07 and 11.56±0.29a respectively, In the proximate analysis T2 shows highest protein deposition in fish muscles, The significant difference ($p<0.05$) was noticed in T4 and T2 compare T3 and T1 respectively. Whereas the total fat % was recorded highest in T2 (2.370%±0.03) followed by T1 (2.39%±0.03), T3 (2.21%±0.06) and least in T1 (2.12%±0.29) respectively. There was no significant difference ($p>0.05$) observed among treatments. Further, the moisture % shows significant difference in T1

compared to T3 and T4 respectively. The highest moisture percentage observed in T3 78.52%±0.12) followed by T2 (71.98%±0.28), T4 (71.92%±0.47) and the least moisture % observed in T1 (61.99%±0.27). Our results match with the finding of Solanki et al. (2023) they revealed higher crude protein content with the higher content in C/N 20. In the present experiment the best growth performance was noticed among treatments viz. T1 followed by T2, T3 and least in T4 almost amongst all the selected parameters. The growth performance parameters viz. average final weight gain, BWG %, SGR, FCR, FCE, Survival (%) was showed an increasing trend in T1, followed by T2, T3 and T4 respectively. full potential. These findings contribute to the ongoing efforts to refine and implement sustainable aquaculture practices.

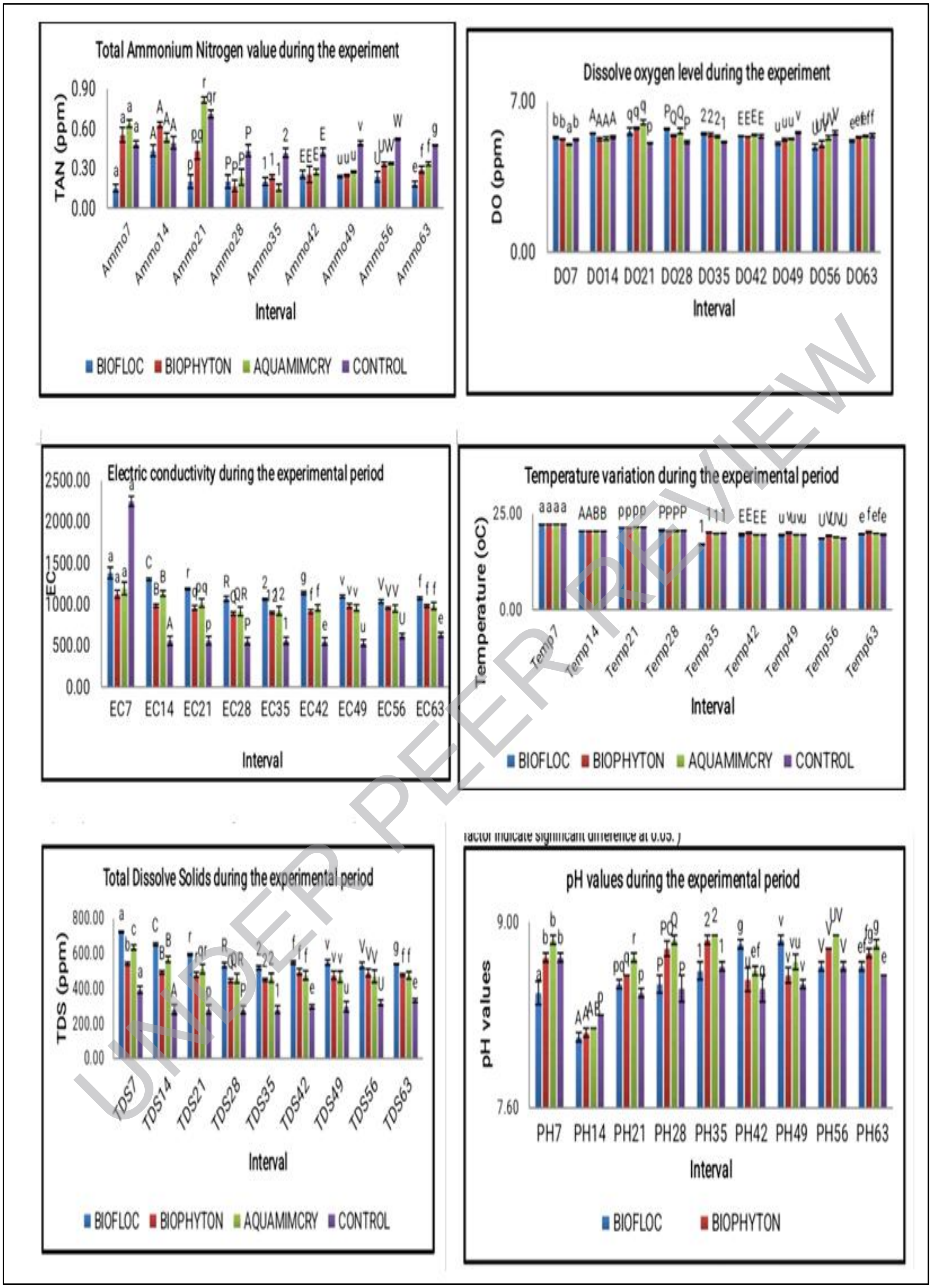


Figure 1: Water quality parameters recorded during the experimental period.

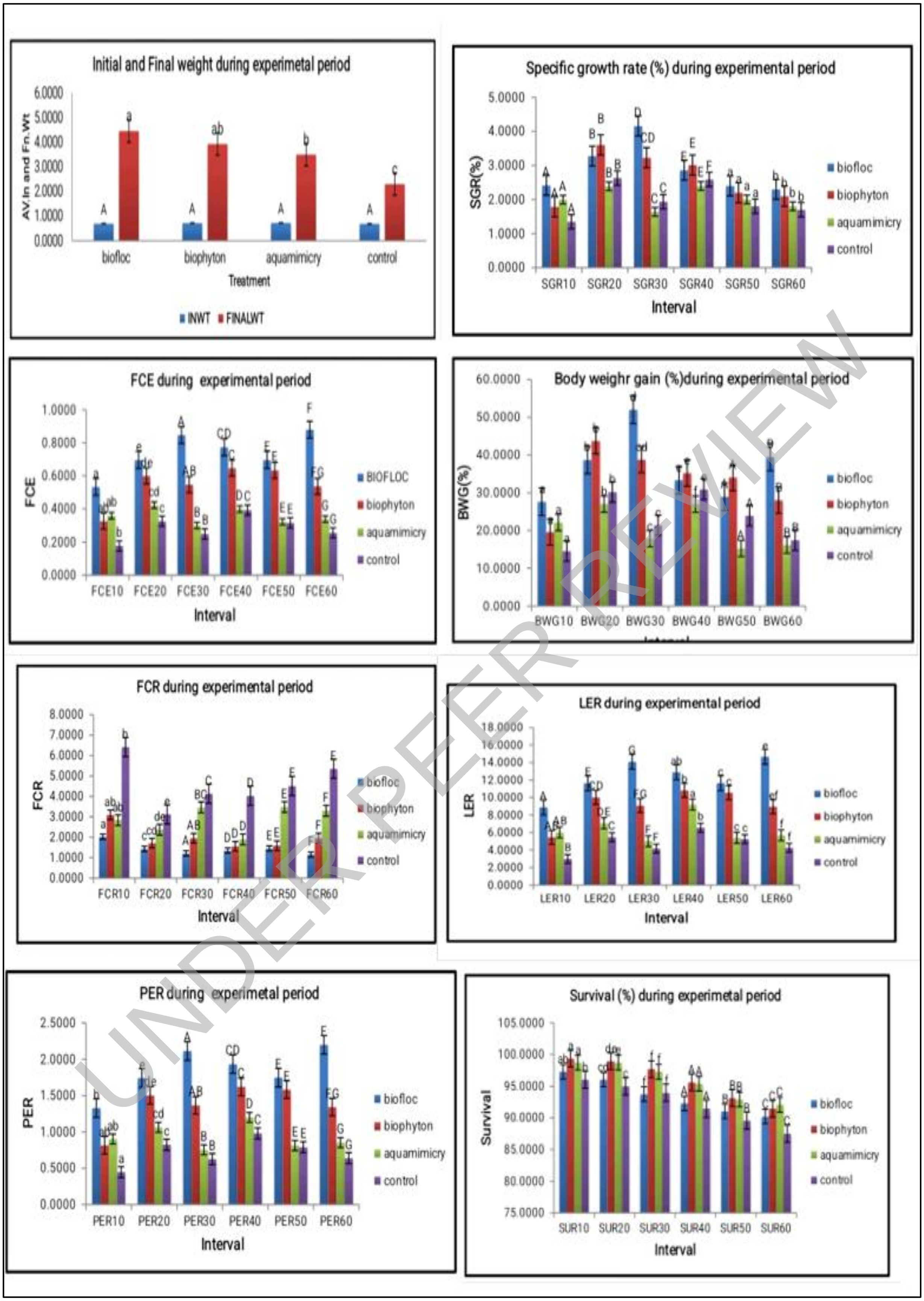
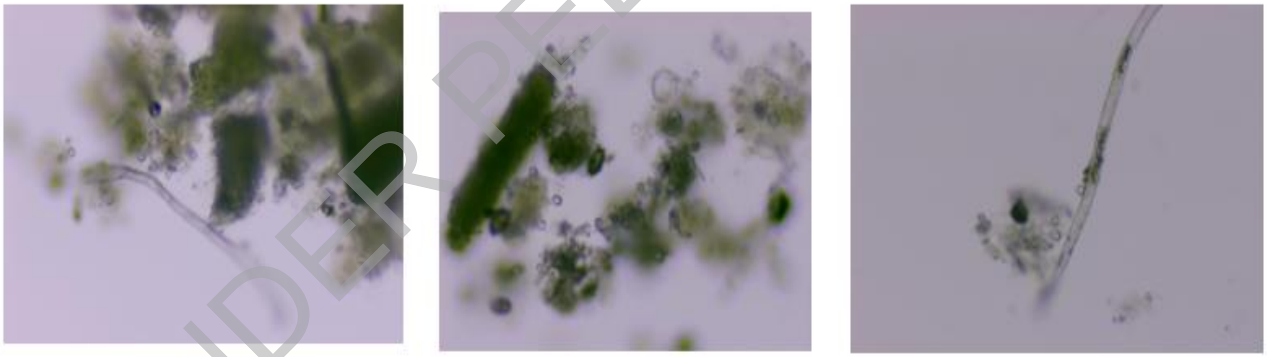


Figure 2: Growth performance of *catla catla* during experimental period

A= *copepod sp.* B=ciliates C = floc

D= *Centropyxis aculeate* E= *euglypha tuberculata* F= *copepod*



G=*Synedra sp.* H= micro algae I= nematodes

Figure 3. (A to I) Plankton and floc observed under microscope (40X and 10X)

Table.1 Average initial and final weight of *Catla catla* recorded during experimental period

Parameter	T1	T2	T3	T4
Initial Wt.	0.69±0.02^{A}	0.71±0.01^{A}	0.72±0.01^{A}	0.68±0.01^{A}
Final Wt.	4.45±0.14^{a}	3.93±0.18^{ab}	3.49±0.14^{b}	2.30±0.14^{c}

***Note(Initial Wt. = Initial weight; Final Wt. = Final weight; C:N ratio= Carbon:Nitrogen ratio;T1= Bio-floc (20:1); T2= Bio-phyton ;T3=Aqua-mimicry ; T4=Control; The mean values followed by the different superscript letters in each factor indicate significant difference at 0.05.)**

Table 2. Proximate composition of the fish collected from different treatments

Composition (% dry matter)	Treatment			
	T1	T2	T3	T4
Protein	14.16 ± 0.07^{b}	18.00 ± 0.10^{c}	13.85 ± 0.07^{b}	11.56 ± 0.29^{a}
Fat	2.370 ± 0.03^{a}	2.39 ± 0.03^{a}	2.21 ± 0.06^{a}	2.12 ± 0.29^{a}
Moisture	61.99 ± 0.27^{a}	71.98 ± 0.28^{b}	78.52 ± 0.12^{c}	71.92 ± 0.47^{b}

***Note(T1= Bio-floc (20:1); T2= Bio-phyton; T3=Aqua-mimicry; T4=Control; The mean values followed by the different superscript letter (a, b, c) in each factor indicate significant difference at 0.05)**

4. CONCLUSION

The growth performance of *Catla catla* was meticulously assessed under varied treatments, aiming to delineate the most conducive environment for optimal growth outcomes. Three distinct treatments were implemented: Biofloc (T1), Biophyton (T2), Aqua-mimicry (T3), alongside a Control (T4). Notably, millet served as the primary carbon source in treatments T1 and T3, a crucial factor under investigation. The experimental results unveiled compelling insights into the efficacy of each treatment regimen. Treatment T1 emerged as the standout performer, exhibiting significantly higher growth parameters across multiple metrics, including biomass weight gain (BWG), survival percentage, and specific growth rate (SGR), accompanied by a lower feed conversion ratio (FCR). Conversely, treatment T4, the control group, demonstrated the least favourable growth outcomes, underscoring the importance of alternative approaches in fostering optimal growth conditions. While Biophyton and Aquamimicry systems hold promise, further optimization and understanding of their microbial dynamics are essential for realizing their

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