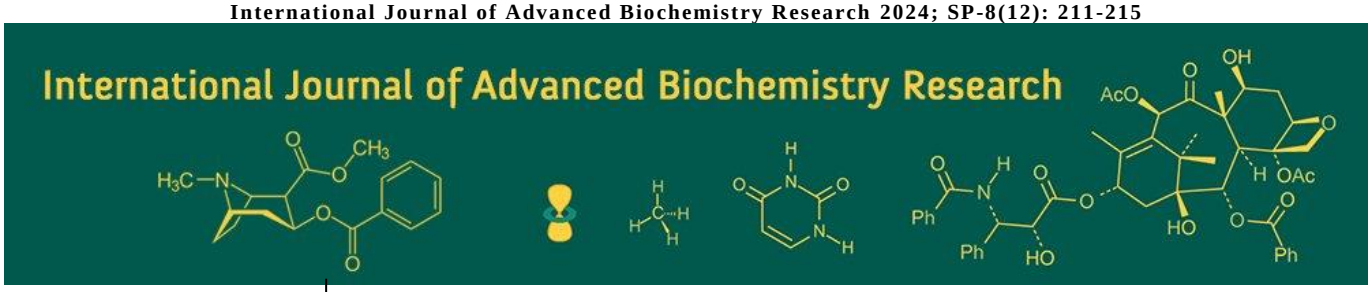

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Effects of probiotic on fry rearing of rohu in biofloc system

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Abstract
This experimental study was conducted with addition of probiotic at different time intervals on the fry rearing of Rohu (*Labeo rohita*) to observe the growth rate, Fishes in control and 1st month of treatment tanks were fed with commercial feed @4% body weight and @ 2% body weight of 2nd month of treatment tanks. The experiment was made up of 4 treatments and each having 3 replicates. The Control was without biofloc, T_1 was with only once application of probiotic at the starting time of biofloc system, T_2 with daily application of probiotic, T_3 with application of probiotic in 7 days interval and T_4 with application of probiotic in 15 days interval. The experiment was conducted for 60 days only. Probiotic was applied as FCO (Fermented carbon organic) method**.** In each tank stocking density of fry was 30/150 ml with initial weight of each fry was 0.3±0.02 g. C:N ratio was 10:1 maintained with jaggery as carbon source. Various water parameters and growth parameters of fish were recorded during the experiment at 15 days interval. Highest body weight gain was 4.22±0.22 g, daily weight gain was 0.07 g in T_2. The survival of biofloc tank in T_2 and T_3 was found to be 100%. It was concluded that a biofloc system with daily probiotic addition as FCO helps in increasing the growth and immunological parameters of Rohu fry.

Highlights: The study recommended that the biofloc with daily addition of probiotic shows the highest growth of Rohu fry. This research will be helpful for the fish farmers those who are going for rearing of fry to fingerlings of Rohu as in this case the survivability is 100%, whereas normal survivability of fry to fingerling is 60 to 70%.

Keywords: Biofloc, probiotic, fry of *Labeo rohita*, growth parameters, water parameters

Introduction
The primary tenet of biofloc technology is the recycling of waste nutrients. Nitrogenous waste transforms into microbial biomass that the cultured animals can consume right away or that can be recovered and utilised as a feed element (Kuhn *et al*., 2010) [11]. Heterotrophic microflora is encouraged to flourish by modifying the carbohydrate amount of the feed or by adding an external carbon resource to the water (Avnimelech, 2009) [3]. This makes the bacteria to take the waste ammonium for the production of new biomass. In contrast to standard methods like the administration of antibiotics, antifungals, exogenous probiotics, and prebiotics, Biofloc has recently been proposed as a potential innovative way for illness treatment with a "natural probiotic impact" (Emerenciano *et al*., 2013) [7]. Biofloc is a heterogeneous mixture of extracellular polymeric compounds and beneficial microorganisms suspended in organic particles (Ray *et al*., 2010) [18]. The culture system can be supplemented with various organic carbon resources (such as tapioca, corn, wheat, glycerol, glucose, acetate, molasses etc.) or the feed composition can be changed by increasing the carbon content of the feed (Avnimelech, 1999) [2].

In biofloc system farmers are using probiotic daily and also at different intervals of time period. So there is a need of research on the application of probiotic in different intervals of time period to study the growth, survivability rate and immunological parameters of Rohu fry reared in biofloc system. In fry rearing of Indian major carps the normal survivability is 60 to 70%. Therefore there is also a need of research in fry rearing in biofloc system to increase the survivability percentage. Keeping these in view, the current study was attempted to study the use of probiotic in fry rearing of Rohu in biofloc system. The study has formulated with

different objectives like to study the growth and survival percentage of Rohu fry reared in biofloc system and changes in the water pararameters of the tank. Vitamin E plays important role for growth of rohu (Sau *et al*, 2004; Sau and Paul, 2004 and Karjee *et al*., 2023) [19, 20, 10].

Materials and Methods

Design of the experiment

Experiment was done for 60 days in the aquaculture laboratory of the College of Fisheries (OUAT), Rangailunda, using 200l FRP tanks from 4th October to 4th December, 2022 for P.G.research only. Animals used for experimental purpose were fry of *Labeo rohita* (Rohu) which were obtained from the Government Fish Farm at Kausalyaganga, Bhubaneswar, Odisha. These fish were transported to the laboratory in ambient temperature with oxygen packing, disinfected with potassium permanganate solution (1 ppm) to get rid of any ectoparasites that might be present and stocked in three numbers of 500 L FRP tanks with borewell water. These fish were reared 15 days in an aerated environment in order to acclimatize to laboratory conditions. During this phase fishes were fed with commercial diet @ 4% of biomass twice a day. During the period of acclimatization, the biofloc tanks were prepared as detailed below.

A completely randomized design (CRD) pattern was selected for the experiment. The investigation was designed with 1 control and 4 treatments with 3 replicates for every treatment with a stocking density of 30 numbers of fishes per each tank. Biofloc was established in four treatment tanks T_1, T_2, T_3 and T_4 having same carbon sources i.e. jaggery, each having triplicates with same stocking densities. The control was taken without biofloc. Continuous aeration was provided in all tanks. The experimental tanks are like:

Control: Stocking of fry of Rohu without biofloc, T_1: Stocking of fry of Rohu with only once application of probiotic at the starting time of biofloc system, T_2: Stocking of fry of Rohu with daily application of probiotic in biofloc system, T_3: Stocking of fry of Rohu with application of probiotic in 7 days interval in biofloc system, T_4: Stocking of fry of rohu with application of probiotic in 15 days interval in biofloc system.

Preparation of biofloc tanks

Thirteen tanks, *i.e.* four treatments (T_1, T_2, T_3 and T_4) each with three replications, were filled with seasoned water for the preparation of biofloc tanks. 10g of probiotics (UniEcosense) and 50g of jaggery (Carbohydrate source) were then added to each. The tanks were kept on vigorous aeration. Next morning, 200g of common salt was added to those tanks to speed up the flocculation process and to maintain the salinity of the water at 1ppt. 12.5g jaggery was added to each tanks for next two days. The tanks were left for one day before being stocked with fry of Rohu. Three tanks were filled with clean borewell water and kept ready for use as control tank. After stoking the fry of Rohu, 5 L of water was placed in an airtight container with constant aeration. It will be fortified with 10g of probiotic and 100g of jaggery. After 48 hours, FCO was prepared. Then a dose of 60-100 ml FCO was added to the tank water according to the treatment. The fishes were fed commercial feed on a daily basis. The feeding ration was divided into two equal portions and administered twice daily, in the morning and evening @ 4% body weight to control and 1st month of treatment tanks and @ 2% body weight in 2nd month of treatment tanks.

Fish biomass sampling

Periodic sampling was undertaken out every 15 days interval to monitor growth and health of fish species. Ten numbers of fishes were selected at random for the purpose and feeding ration was adjusted as per the computed biomass.

Harvesting

After 60 days of experimentation, all the fish were harvested. Individual weight of fishes, total biomass, number of fishes survived *etc*. were recorded for each treatment.

Water quality monitoring

Water samples were collected from biofloc and control tanks in every 15 days interval to record and monitor selected physicochemical parameters such as temperature, pH, dissolved oxygen, total alkalinity, TAN (Total Ammoniacal Nitrogen), nitrite, and nitrate following the protocol mentioned in APHA (2017) [1].

Assessment of growth parameters

A digital mono pan balance with 0.01 g precision was used to measure the fishes' weight (Wenser, IND/09/08/466). These data were used to calculate the average initial weight and average ultimate weight of each fish raised in each tank. Other growth parameters, including average bodyweight gain (BWG), daily bodyweight gain (DBW), percentage bodyweight gain (PBW %) and survival (%) were calculated using this data using the below methods and formulas.

Body weight gain (BWG)

Body weight gain (g) = [Average final weight (g) – Average initial weight (g)]

Percentage weight gain (PWG)

$$\text{Percentage weight gain} = \frac{[\text{Final weight of fish (g)} - \text{Initial weight of fish (g)}]}{\text{Initial weight of fish (g)}} \times 100$$

Daily body weight gain (DBW)

$$\text{Daily weight gain (g)} = \frac{[\text{Final weight of fish (g)} - \text{Initial weight of fish (g)}]}{\text{Experimental days}}$$

Survival percentage

The given formula was used to calculate it.

$$\text{Survival (\%)} = \frac{F}{I} \times 100$$

Where, F = Final no of harvested fish species
I = Initial no of stocked fish species

Statistical analysis

Statistical tool for Social Science (SPSS 25.0 for windows) was used for computing statistical analysis. One-way analysis of variance (ANOVA) was used to look at the significant differences in water quality and growth characteristics between the control and biofloc treated tanks. Post-hoc analysis was done by using Duncan's Multiple Range Test (DMRT) to compare means. The significance level of 0.05 was used for analysis.

Results

The results found in this experiment are described below.

Physicochemical Parameters: The Table-1 represents the values of physiological parameters in different tanks. Sampling was done in each 15 days interval. Throughout the period, the Temperature was found to be in between 24°C and 26°C in all treatments, including the control. The recorded values were brought to one tabular form from all the control tanks and treatment tanks and then one-way ANOVA was done. There is significant difference in Temperature values between the control and treatments was investigated (using a one-way ANOVA) at a level of 5% significance.

The pH value in the current study ranged from 7.3 to 7.6. The data from all of the control and treatment tanks was compiled into a single tabular form, and a one-way ANOVA was performed. There is no significant difference in pH value between the control and treatments was investigated (using One-way ANOVA) at a level of 5% significance. Throughout the study, the DO was from 6.8 ppm to 7.8 ppm. A One-way ANOVA was performed on the data from all of the control and treatment tanks, which was put into a single tabular form. There is no significance difference in dissolved oxygen value between the control and treatments were determined using One-way ANOVA at a level of 5% significance.

Throughout the study period, alkalinity in the control tanks ranged from 160 ppm to 200 ppm. During the culture period, the alkalinity in treatment tanks ranged from 160 ppm to 180 ppm. The data from all of the control and treatment tanks was combined into a single tabular form and subjected to a one-way ANOVA. There is significant difference in alkalinity values between the control and treatments was investigated (using a one-way ANOVA) at a level of 5% significance.

Throughout the culture period, total ammoniacal nitrogen in control tanks is 0.06 ppm to 0.54 ppm. During the experimental period, total ammoniacal nitrogen in T_1 is 0 ppm to 0.36 ppm, while total ammoniacal nitrogen in T_2 is in between 0 ppm to 0.23 ppm, total ammoniacal nitrogen in T_3 is 0 ppm to 0.3 ppm and in T_4 is 0 ppm to 0.32 ppm. The data from all of the control and treatment tanks was combined into a single tabular form, and a one-way ANOVA was performed on it. There is significant difference in total ammoniacal nitrogen value between the control and treatments were investigated using one-way ANOVA at a level of 5% significance.

The nitrite level in control tanks ranges from 0.04 ppm to 0.21 ppm. Nitrite levels in T_1 range from 0 ppm to 0.19 ppm, T_2 from 0 ppm to 0.14 ppm, T_3 from 0 ppm to 0.15 ppm and T_4 from 0.02 ppm to 0.14 ppm. The data from all of the control and treatment tanks was compiled into a single tabular form and analysed using a one-way ANOVA. The significant difference in nitrite value between the control and treatments was investigated at a level of 5% significance. The estimated value suggests that the control and treatments with replications during the study period have a significant difference.

Nitrate concentration is between 0.5 ppm to 2.3 ppm in control tanks. The nitrate level in T_1 was between 0.4 ppm to 2.4 ppm, 0.5 ppm to 3.1 ppm in T_2, and 0.5 ppm to 2.7 ppm in T_3 and T_4 was 0.5 ppm to 2.6 ppm. Using a one-way ANOVA, the data from all the control and treatment tanks were pooled into a single table. There is a significant difference in nitrate value between the control and treatments was investigated at a level of 5% significance.

Growth Parameters

The growth parameters are presented in Table-2. When the growth of the biofloc treated group is compared with control group the weight gain of fishes reared under biofloc T_1, T_2, T_3 and T_4 was 3.29 ±0.22 g, 4.22±0.22 g, 3.63± 0.04 g and 3.35±0.27 g respectively grow relatively higher than the control group (C) as 2.63±0.06 g.

The biofloc treated groups were compared with control group the weight gain percentage (%) of biofloc reared fishes T_1 (839.29%), T_2 (1218.61%), T_3 (1026.97%) and T_4 (945.83%) showing a higher value than the respective control group C (701.56%). When the weight gain percentage (%) among the various biofloc reared species was compared T_2 (1218.61%) shows a better result.

The biofloc treated groups were compared with control group T_2 had the highest average daily growth (0.07 g) followed by T_3 (0.06 g) and T_1 and T_4 was (0.05 g) where C0ntrol was 0.04g. When the daily weight gain among the various biofloc reared treatments was compared T_2 (0.07 g) shows a better result than the other.

The biofloc treated groups were compared with control group T_2 (4.30%) had the greatest SGR, followed by T_3 (4.03%), T_4 (3.91%) and T_1 (3.73%) than the respective control group 3.46% respectively. When the specific growth rate among the various biofloc reared treatments compared T_2 (4.30%) shows a better result.A one-way ANOVA with a significance level of 5% was used to analyze the significant difference in SGR between the control and treatment groups. The estimated value suggests a significant difference between the control and replication treatments during the study period.

The survival of biofloc tank (T_2 and T_3) was found to be 100%, while in T_1 was 95.56%and T_4 was 94.44% and in C it was 91% respectively. When the Survival among the various biofloc reared species was compared, where in T_2 and T_3 the survival rate was higher than other biofloc tanks.

Table 1: Water quality parameters of Rohu fry in control and biofloc tanks with ANOVA and DMRT

Parameters	Control	T_1	T_2	T_3	T_4
Temperature (°C)	25.9^{b}±0.15	24.2^{a}±0.09	24.2^{a}±0.25	24.5^{ab}±0.31	24.4^{a}±0.21
DO (ppm)	7.2^{b}±0.11	7.1^{ab}±0.00	7.0^{a}±0.04	7.1^{ab}±0.04	7.1^{ab}±0.05
pH	7.5^{b}±0.01	7.4^{ab}±0.10	7.4^{ab}±0.02	7.3^{a}±0.13	7.4^{ab}±0.06
Alkalinity (ppm)	182.7^{b}±0.64	172.2^{a}±2.23	173.3^{a}±1.20	173.6^{a}±1.74	173.8^{a}±0.94
Ammonia (ppm)	0.32^{b}±0.02	0.17^{a}±0.05	0.12^{a}±0.06	0.16^{a}±0.07	0.15^{a}±0.05
Nitrite (ppm)	0.1^{a}±0.02	0.08^{a}±0.01	0.11^{ab}±0.23	0.13^{b}±0.08	0.10^{ab}±0.01
Nitrate (ppm)	1.25^{a}±0.11	1.3^{a}±0.14	1.62^{a}±0.19	1.42^{ab}±0.08	1.44^{ab}±0.08

Note: The values are presented in Mean ± Standard Deviation and in a row values having ANOVA and DMRT

Table 2: Growth performance parameters of Rohu fry in control and biofloc tanks with ANOVA and DMRT

Parameters	C	T_1	T_2	T_3	T_4
Initial Weight (g)	0.33^{a}± 0.03	0.35^{a} ± 0.02	0.32^{a} ± 0.01	0.32^{a} ± 0.03	0.32^{a}± 0.01
Final Weight (g)	2.63^{c}± 0.06	3.29^{b} ± 0.22	4.22^{a} ± 0.22	3.63^{b}± 1.01	3.35^{b} ± 7.64
Bodyweight Gain (g)	2.30^{d} ± 0.07	2.94^{c} ± 0.21	3.90^{a} ± 0.21	3.31^{b} ± 0.04	3.03^{bc} ± 0.27
Percentage weight Gain (%)	701.56^{d}±68.76	39.29^{c}±41.95	1218.61^{a}±49.27	1026.97^{b}±83.93	945.83^{bc}±79.39
Daily Weight Gain (g)	0.04^{d} ± 0.001	0.05^{c} ± 0.003	0.07^{a} ± 0.003	0.06^{b}±0.0005	0.05^{bc} ± 0.004
Survival (%)	91.11^{c}±1.92	94.44^{b}±1.92	100^{a}±0.00	100^{a}±0.00	95.56^{b}±1.92

Note: The values are presented in Mean ± Standard Deviation and in a row values having ANOVA and DMRT

Discussion

Water quality parameters

Throughout the trial, the Temperature in the control and treatment groups was found to be in between 24 °C to 25.9 °C. Within the treated tanks, there was no discernible difference. During the study period, the one-way ANOVA revealed significant differences between the control and treatment tanks. Fish can withstand Temperature ranging from 3 to 35 °C (Pauly and Froese, 2012) [17]. Temperature has a direct impact on the oxygen content in water as well as the metabolism and proliferation of microbial fauna. Temperature is linked to the metabolism of cultured organisms and microbes. Temperature has a significant impact on the biofloc culture (De Schryver *et al.*, 2008) [6]. According to Wilen and Balmer (1999) [21], the ideal Temperature for biofloc formation is between 18 °C to 32 °C degrees. Temperature has an impact on floc properties as well as the metabolism of floc microbials. The majority of floc breakdown occurs around 40 °C due to a substantial fall in bacterial biomass. According to the above-mentioned scientists' findings, temperature has a direct impact on floc formation in the tank.

All flocculation characteristics are influenced by pH, and the metabolism of cultured organisms is dominated by pH (Mikkelsen and Nielsen, 2001) [15]. The pH balance between harmful ammonia and non-toxic nitrate is maintained by this factor. The physiological functioning of farmed organisms is also affected by pH and this is a critical aspect to control in a biofloc aquaculture system. The pH of the water within the control and biofloc treated tanks is between 7.3 and 7.6. During the study period, the one-way ANOVA revealed no significant differences between the control and treatment tanks. pH influenced the rate of nitrification and the production of non-autotrophic microbe. They also proposed that the pH in the biofloc system should be between 6.5 and 9, depending on the cultivated species, for the biological cycle to function properly. According to Chen *et al.* (2005) [4], pH values of 7.0-9.0 are the most suitable.

It has a greater impact on productivity and growth. Many species can be harmed by low levels of dissolved oxygen (DO). The presence and amount of DO in an aquatic habitat are critical for bacteria metabolism, as well as floc size, shape, and pattern, according to Crab (2010) [5]. When DO goes below 1.1 ppm, he observed a huge filamentous bacterium aggregation, since these bacteria have been demonstrated to thrive better in anaerobic conditions. It has a significant effect on all physicochemical parameters, including dissolve oxygen limitations. As a result, the concentration of fish oxygen consumption is a significant aspect to consider when choosing a carbohydrate. According to Olah *et al.* (1987) [16], bacterial biomass accounts for 77% of total oxygen consumption in ponds. He also observed that the nitrification requirement is approximately 4 ppm for oxidising 1 mg TAN. DO is an unavoidable occurrence for cellular metabolism and floc production, according to Wilen and Balmer (1999) [21]. DO limit over 5 ppm obtained acceptable range of floc that would have a good impact on cultured aquatic animal growth (Martins *et al.*, 2003) [14]. The dissolved oxygen levels in the control and treatment tanks ranged from 6.8 to 7.8 ppm. As a result, the DO level in this study was well within the permitted range for floc maintenance, fish survival, and growth.

Ideal range of alkalinity of water for better growth performance is below 300 ppm. During the study period, the one-way ANOVA revealed there is a significant difference between the control and treatment tanks. Furtado *et al.* (2015) [8] says the pH and alkalinity have a negative association with research days, and that this link can be corrected by applying lime to the water and cultured organisms in pond. Lower alkalinity is caused by a lack of inorganic carbon synthesis by nitrifiers, which improves water quality by lowering pH, ammonia levels, and increasing nitrate-nitrogen content in the system, as well as a carbon shift from bicarbonates to CO_2. Alkalinity levels in control and biofloc tanks ranged from 160 ppm to 200 ppm which falls in the ideal range and also improves the water quality.

Ammonia is thought to be the last product of protein metabolism. Unlike the ionized form (NH_4^+); ammonia has a negative influence on cultured organisms when present in unionized form. Unionized ammonia is a harmful type of nitrogen that has an adverse effect on growth and survival (Lin and Chen, 2003) [12]. Ideal level of ammonia concentration is 0.3 ppm to 1 ppm. In the control tank, ammonia levels ranged from 0.06 to 0.54 ppm, while in the biofloc tank, it was 0 to 0.36 ppm. In the current study

ammonia level falls in the range of ideal level. In fact, in the biofloc tanks the ammonia concentration is much lower than the control tanks indicating the better microbial activity of converting toxic ammonia to nontoxic nitrate. During the study period, the one-way ANOVA revealed there is a significant difference between the control and treatment tanks.

Nitrite is formed when ammonia is oxidised which means that more nitrite is formed than ammonia during shellfish production. In the nitrification cycle, nitrate is the final product. Although nitrate is much less toxic than either ammonia or nitrite, concentrations of up to 75 mg/L of NO_3^--N will compromise the welfare and growth of the farmed animals. growth of the farmed animals (Furtado *et al*., 2015) [8]. Nitrate levels in the control tank ranged from 0.5 to 2.3 ppm, while they ranged from 0.4 to 3.1 ppm in the biofloc tank. During the study period, the One-way ANOVA revealed no significant differences between the control and treatment tanks.

Growth parameters

In this study, *Labeo rohita* fry in biofloc treatment exhibited a significant difference in average body weight gain and daily body weight gain in comparison with the control group. While the T_2 with daily probiotic addition shows higher growth (4.22±0.22 g). The results are comparable to the observations found in Rohu (Mahanand *et al.*, 2013) [13] when they were cultured in a biofloc-based system.

The continual availability of in situ floc created in the system is responsible for the increase in weight gain in biofloc raised fish. Biofloc provides extra protein (EAA), polyunsaturated fatty acid (PUFA), minerals, and vitamins in addition to commercial feed supplements.The catfish *Clarias gariepinus* juveniles shows higher growth in fermented biofloc when compared to non-fermented biofloc (Hapsari, 2016) [9]. The prospective of biofloc technology is where Rohu get the benefit from food's high nutritional value. The maximum net yield was about 87% higher with the biofloc treatment than with the control treatment (Mahanand *et al*., 2013) [13]. Rohu cultivated in the biofloc system (105%) experienced a weight growth that was substantially higher than Rohu cultivated in the control (65.8%). Similar to that, the biofloc system showed a daily weight gain that was 1.40g higher than the control (0.68 g).

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