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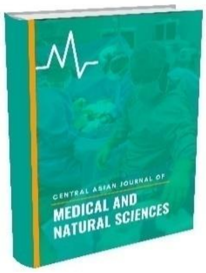

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The Impact of Promix Probiotics on the Growth and Survival of Pangas (*Pangasius Pangasius*) In the Biofloc System at 40% CP Feed

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Abstract: Biofloc is an environmentally and socially acceptable super intensive system that overcomes the problem of limited water, land and feed causing increase in aquaculture productivity. In the present study 200 fingerlings of yellow catfish, (Pangasius pangasius) with average body weight (5.17±0.09g, 5.10±0.06g) and length (2.52±0.0cm, 2.54±0.01cm) in control and biofloc were cultured in indoor circular cemented tanks respectively for 10 weeks. Water exchange system as a control group (C) and biofloc (BF) with two carbon sources (probiotics of Big fish Company and molasses added had a C/N rate of 15:1 to form the floc) under limited water exchange at 5% feed of 40% CP feed. Several water quality parameters were maintained in a specific range such as dissolved oxygen (6.5-7.4 mg/L), temperature (25-29°C), and nitrate (1.1-1.7mg/L). pH (6.9-8), total dissolved solids (1.4-2.3mg/L), total ammonia nitrogen (0.4-0.8mg/L) and nitrite (0.4-1.3mg/L) and on weekly basis the assessment of survival rate and growth parameters in term of length gain, weight gain, specific growth rate and feed conversion efficiency along with water quality variables consisting of temperature, dissolved oxygen, pH, nitrates, total ammonia nitrogen and total dissolved solids was done during 10 week experiment in control and biofloc tanks of P. pangasius. The survival rate of the Pangas fingerlings was not significantly different in both control and biofloc groups. However survival was more in biofloc based culturing system as compared to control. The specific growth rate (SGR) of P.pangasius fingerlings in biofloc groups was significantly ($p < 0.05$) higher than that of control group. The feed conversion ratios (FCR) of the fish was not significantly different in both groups but was less in biofloc (0.48±0) than control

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(0.56±0.01) in last, as well as almost every week of the experiment. Therefore, this study demonstrated the suitability of probiotics use in aquaculture as wastewater purifying, growth-promoting, and survival-enhancing technology for culture of P.pangasius.

INTERODUCTION

According to (FAO, 2020) world's human population should increase up to 9 billion peoples on Earth up to 2050. Due to this increased global population 60% more food will be needed hence, increase, expansion and amplification of aquaculture production are extremely required. The fish usage is much higher than the other type of white meat sources since 1961 to 2018 (Golovina et al., 2019). It is necessary to increase the production of animal protein which is the main nutritional source of human's diet; and the main source of animal protein is aquaculture which could produce protein with faster rate at low cost (Ahmed et al., 2017). According to FAO it is estimated that world aquaculture production will increase from 82.1M tones in 2018 to 109M tones in 2030 (FAO, 2020).

Aquaculture as a food producing industry offers plentiful opportunities to improve poverty, reduce hunger and malnutrition, produces economic growth and ensures the best use of natural resources (FAO, 2017). But from the last decade the global climate is quickly changing due to industrialization and urbanizational wastes, acid rain and other water pollutants that had negative effects on the aquatic environment (Yuslan et al., 2021). Supportable aquaculture industry development should concern on system that provide high yield and profit with the utilization of fewer resources like space, water, energy, as well as lesser impact on the environment. Aquaculture production is improved by focusing on the utilization of feed nutrients and it can be done by two various types of approaches firstly by improving the quality of feed and feeding strategies and secondly by re-utilization of wastes through various modifications in aquaculture (FAO, 2017).

Biofloc fish farming is the basic technique and most fast-moving industry today which has produced a desirable fish yield. This system was developed in 1990s to utilize the wastes of fish and supplementary feed. Firstly, this technology was originated in Indonesia then into South Asian countries through fish farmers. During the past 8 years it was flourished in India, Malaysia, Thailand and Japan (Emerenciano et al., 2017). It plays a vital role for providing food to the increased global population (FAO, 2020). This technology enhances the production and productivity by its contribution to the supply of good quality fish juveniles. In addition, it contributes to the improvement of the fish production. Biofloc technology could support the supply of good quality fish seeds by improving the reproductive performance of aquaculture animals and by enhancing the larval immunity and robustness (Ekasari et al., 2016).

In recent years the application of probiotic in fish production culture systems by using Biofloc technology increased many folds. Probiotic are the live microorganisms with specific quantities which are used for the benefit actions on host and environment wellness that surrounds them (Olmos and Paniagua-Michel, 2014). The central advantages of probiotics are the nutrient assimilation increase, stimulation of immune system (Balcazar et al., 2006) potential pathogen exclusion, nitrogen compound degradation, to prevent infections, growth promoters (Seenivasan et al., 2016) and improve water quality (Ringo and Song, 2016). The natural probiotic in biofloc aquaculture system reduces

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30% water treatment costs because this system depends on low water exchange process. Due to the use of biofloc in this technique 10 to 20% less feed is used consequently 40 to 50% reduced the costs feed (Hargreaves, 2013), furthermore, the biofloc has a protein content of between 25 and 50% and the fat content ranges from 0.5 to 15% (Emerenciano et al., 2013). Survival of fish increases up to 83% with the use of probiotics (De Paiva et al., 2016).

Pangasius pangasius known as yellowtail catfish is widely distributed in natural water bodies of Indian sub-continent. Its abundance is also felt in the major east coast flowing major river system of India. It is bottom dweller and is omnivorous in nature (Nguyen and Jolly, 2018). Hence it accepts any form and type of feed containing little more than 30% protein for good performance in confined rearing. It is also considered as a good candidate species for controlling Molluscan population in the pond system. In the wild the fish grows to 20-25 kg. This fish is considered as an ideal species for aquaculture due to its high growth. The decline of its population from the natural waters has been observed during last two decades due to over exploitation, habitat degradation, water pollution, destruction of the breeding grounds etc. Hence it becomes essential to concentrate on its captive production for its future aquaculture production to satisfy the consumers demand as well as to re-establish its natural population (Sahoo et al., 2018; Chattopadhyay, 2019).

MATERIALS AND METHODS

The objective of the current research was to evaluate the impact of promix probiotics on the growth and survival of *P. pangasius* in biofloc system at 40% CP feed. The probiotics were mixed with the molasses with continuous supply of aeration for the experimental group of fish.

3. 1. Experimental Conditions

The research work based on biofloc system was conducted at the Saline Water Aquaculture Research Centre Muzaffargarh, Punjab, Pakistan.

P. pangasius was collected from Tawakal Fish Hatchery, Muzaffargarh, Punjab, Pakistan (Figure 3.2). Netting was performed to collect experimental samples (30) of different size that were transported to wet laboratory in oxygenated double-layered plastic (Polythene) bags filled with brackish water. All collected seeds were strong, healthy and active with dark bright grey coloration. Water quality parameters were checked that were approximately similar to water used in circular tanks. Twelve hundred *P. pangasius* at an average weight of 5.1g and length of 2.5cm were stocked into six circular tanks each having equal number of fish on 01-2-2022 as an experimental fish.

The fish was fed by a commercial diet (Sind Aqua Feed, Figure 3.3) manufactured by Sind Feed and Allied Products, Plot #19, Sector # 16, Korangi industrial Area Karachi (Pakistan) with 40% crude protein. All fishes were fed with 5% of their body weight (g) for three times a day at 9:00am,1:00pm and 5:00pm in all tanks of fishes either controlled or experimental.

3.2. Apparatus/Chemicals

The experiment was conducted in six cemented indoor circular tanks by using brackish water with salinity ranging 3-5 while other apparatus and chemicals that were used during the study period include probiotics of Big Fish Company (CFU ≥ 27 Billion/g), molasses, salt, aeration blowers, air pipes, filter pipe (22cm diameter with 0.8 cm pore size in the center of tank), $CaCO_3$, plastic tub, polythene bags, WSFSWL (NEOBALL_E) of 40W that produces 1500 lumens of light, ring blower of one hours power (air pump), fully submersible automatic aquarium heater of Rs (150-300W) electrical, suction pump of 60 W, plastic covering sheet, net for tank covering, portable DO(YSI DO200), pH (YSI pH100) and other meters for temperature and TDS, HACH fish farming test kit (Model FF-1A, Cat No. 2430-02, Lot A2146) for TAN, NO_2 and NO_3, measuring Scale, weight electronic machine and Sind Aqua Feed with 40% crude protein.

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3.3. Experimental Design

Experiment consisted of two treatments one as a control (without biofloc) and second as an experimental (biofloc) in triplicate form at feeding rate of 5% of their body weight. Six circular cemented tanks each with diameter of 182.88 cm having capacity of 2424 L filled with 2000L water were used to run the experiment. The brackish water was originally taken SWARC research center. Before culturing the experimental fish in circular cemented tanks, the tanks were treated with $CaCO_3$ and then dried for next 2 days, to avoid any fungal infection. These tanks are located in the closed area at research centre with a central pipe of 5 cm diameter is present for the drainage of water and sludge when necessary. A complete aeration and thermoregulation system was used to control the oxygen deficiency and temperature respectively in water tanks. Probiotics of Big fish Company were used to check the growth and survival rate of *P. pangasius.*

3.4. Preparation of Biofloc

Probiotics of Big fish Company having following bacteria and enzymes were used for biofloc formation (Figure 3.4). Biofloc was prepared by the addition of probiotics (150g) of big fish Company (CFU ≥ 27Billion/g) and molasses (500g) in 12-liter water for one experimental (2000L) water tank. These ingredients were mixed thoroughly into the plastic tub with complete aeration before 24 hours of addition into the experimental tank. Total dissolved solids and pH of this biofloc were maintained at 1800 (mg/L) and 8.5 respectively.

Bacillus Subtilis 27 x 10^9 CFU/g Protease 500 U/g

Bacillus Licheniformis 27 x 10^9 CFU/g Cellulose 200 U/g

Enterococcus Faecalis 2 7x 10^9 CFU/g Pectinase 100 U/g

Bacillus Pumilus 27 x 10^9 CFU/g Xylanase 100 U/g

Bacillus Megaterium 2 7x 10^9 CFU/g

3.5. Assessment of Water Quality Parameters of *P. pangasius*

Water replacement was conducted only in control tank at a level of 5% on each day before feeding. The water quality parameters consisting of temperature ($^{\circ}$C), pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), total dissolved solid (TDS), Nitrite (NO_2) and Nitrates (NO_3) were measured on the daily bases prior to first feeding (9:00am) in the all tanks by using the scientific instruments. DO and pH were measured by using portable (YSI DO 200 and YSI pH 100) respectively. TAN, NO_2 and NO_3, were measured by HACH Fish Farming Test Kit (Model FF-1A, Cat No. 2430-02, Lot A2146).

A ring blower of one K hours power (Air pump) and fully submersible automatic aquarium heater (150-300W) used to maintain aeration and temperature respectively in all circular tanks either control or experimental of fish (24 hrs) throughout the duration of the experiment (Figure 3.5). Aeration system contains one black color pipe which was made by connecting three equal sized pipes in circular shape with the help of screws. This ring was connected with air pipe of 1cm diameter that was connected with plastic pipe of 2cm diameter over which brass valve was present to control proper aeration and that was connected to a common pipe of three feet with 8 cm diameter that was connected with a motor of 1K hp which was supplied with three phase connection of electricity. And a plastic cover was also used when temperature was declining. There was no exchange of water instead fresh water addition to replace the losses of water by evaporation in biofloc tanks and a submersible suction pump of 60W was used to remove the debris when necessary. And a filter pipe of 22 cm diameter with 0.8 cm filter pore was used for filtration. The system was checked at least two times daily. Special

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effort was made to observe the tanks and checked the signs of unconventional behavior of fish and any other problem.

3.6. Assessment of Survival Rate of *P. pangasius*

Dead fish were removed from the experimental tanks without being replaced and recorded to calculate the survival rate (SR) on each day. SR was calculated using equation by (Yusuf et al., 2015; Meritha et al., 2018).

$$\text{Survival rate (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of Fish stocked}} \times 100$$

3.7. Assessment of Growth Parameters of *P. pangasius*

Sampling was conducted on weekly basis to measure various growth parameters of the fish such as length gain in cm (LG), weight gain in gram (WG), feed conversion ratio (FCR) and specific growth rate (SGR).

3.7.1. Length Gain

The growth of *P. pangasius* was observed by the measuring the body length from the base of the caudal spine to the anterior edge of the head with the help of measuring scale. The length of fish was measured by the using of the following formula:

$$\text{Length gain (cm)} = \frac{\text{Mean final length (cm)} - \text{Mean lnitial Length (cm)}}{\text{Mean initial length (cm)}} \times 100$$

3.7.2. Weight Gain

The fish weight was measured by the using of the following formula:

$$\text{Weight gain (g)} = \frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{\text{Mean initial weight (g)}} \times 100$$

3.7.3. Specific Growth Rate (SGR)

Immediate change in fish weight is called SGR. It was callculated by using equation (Khanjani et al., 2020; Popoola et al., 2021) given below:

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Ln Final Mean Weight} - \text{Ln Final Mean Weight)}}{\text{Length of Feeding Trials (days)}}$$

3.7.4. Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was determined by using equation given below (Yusuf et al., 2015; Meritha et al., 2018):

$$\text{FCR} = \frac{\text{Amount of dry feed (Kg)}}{\text{Weight gain (Kg)}}$$

3.8. Statistical Analysis

Experiment was layed out as two factor factorials under Randomized Complete Block Design (RCBD) and the data was analyzed through SPSS software (version 26) by using ANOVA and Duncan Multiple Range test (DMRT). Microsoft Excel-2007 was used to draw graphs. All data was presented as Mean±SE while Kaplan meier survival analysis was used to analyze the survival rate of fish.

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RESULTS

Experimental was performed for 10 weeks to examine "The impact of probiotics on *P. pangasius* growth and survival in biofloc based system" with one control group at constantly maintained water quality parameters like temperature (25-29℃), DO (6.5-7.4mg/L), pH (6.8-8), TDS (1.4-2.3mg/L), TAN (0.4-0.8mg/L), NO_2 (0.4-1.3mg/L) and NO_3 (1.1-1.7mg/L). Fingerlings of *P. pangasius* were cultured in biofloc based system with the use of 150g probiotics of (CFU≥27 Billion/g) and molasses 500g. The results for survival rate and growth parameters like in growth parameters of fish like length gain, weight gain, specific growth rate and and feed conversion ratio in biofloc as compared to control with equal (5%) feeding rate are presented in tables 4.1-4.10 and their graphs are shown in Figure 4.1-4.5. All water quality parameters were recorded on weekly basis in control and biofloc groups and this data is represented in Tables 4.11-4.24 and their graphical representation is shown in Figure (4.6.1-4.12).

4.1. Survival Rate of *P. pangasius*

The survival rate of the Pangas fingerlings was not significant but higher in biofloc group as compared to control (Table 4.1-4.2, Figure 4.1).

4.2. Assessment of Growth Parameters of *P. pangasius*

4.2.1. Length Gain

P. pangasius length gain in biofloc groups was significantly ($p< 0.05$) higher than that of control group. At the end of experiment mean length of Pangas in control and biofloc was 24.01±0.29cm and 33.80±0.06cm, respectively (Table 4.3-4.4, Figure 4.2).

4.2.2. Weight Gain

Significantly ($p<0.05$) higher weight gain of Pangas was found in biofloc as compared to control. At the end of experiment mean length of Pangas in control and biofloc was 48.63±0.66g and 66.53±0.90g, respectively (Table 4.5-4.6, Figure 4.3).

4.2.3. Specific Growth Rate

The specific growth rate (SGR) of *P. pangasius* fingerlings in biofloc groups was significantly ($p<0.05$) higher than that of control group. The minimum and maximum SGR values observed from 2st to 10th week were 5.33±0.03 and 12.07±0.03, respectively (Table 4.7-4.8, Figure 4.4).

4.2.4. Feed Conversion Ratio

The feed conversion ratios (FCR) of the fish were not significantly different in control and biofloc groups but was less in biofloc (0.48±0.00) than control (0.56±0.01) in last, as well as almost every week of the experiment. The mean weekly values of FCR observed in both groups on weekly base are presented in Tables 4.9-4.10, Figure 4.5.

4.3. Assessment of Water Quality Parameters of *P. pangasius*

4.3.1. Temperature (°C)

The experiment was conducted in indoor systems, uniform temperature conditions were maintained throughout the study period by using thermostats and no significant difference observed in both control and biofloc tanks. The minimum and maximum water temperatures maintained in control and biofloc from 1^{st} to 10^{th} week were 26.50±0.06, 29.43±0.23 and 25.87±0.48, 28.90±0.21, respectively (Table 4.11-4.12, Figure 4.6).

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4.3.2. pH

On 1st and 4th week average pH values in control and biofloc groups have shown no significant difference. Significantly ($p<0.05$) higher mean pH values were recorded in control than the biofloc groups from 2nd week to 10th week. The weekly mean pH values fluctuated in control and biofloc group. The mean pH values on 1st and 8th week of experiment in control group and biofloc group were 7.53±0.03, 7.93±0.067and 7.5±0.00, 7.5±0.00 respectively. The minimum and maximum water pH maintained in control and biofloc from 1st to 10th week were 7.53±0.03, 8±0 and 6.87±0.67, 7.57±0.88, respectively (Table 4.13-4.14, Figure 4.7).

4.3.3. Dissolved Oxygen (DO)

Dissolved Oxygen (DO) was not significantly ($p< 0.05$) different in control and biofloc based system. The minimum and maximum DO levels observed in control and biofloc based system from 1st to 10th week was 6.8±0.58, 7.4±0.58 and 6.53±0.03, 7.2±0.58, respectively. Overall DO contents in both groups were within the optimum range for survival and growth of *P. pangasius* (Table 4.15-4.16, Figure 4.8).

4.3.4. Total Dissolved Solids (TDS)

The weekly mean TDS values fluctuate in both control and biofloc group. The average minimum and maximum TDS values on 1st and 10th week of experiment in control group and biofloc group were 1.7±0, 1.767±0.03 and 1.7±0, 2.03±0.03 respectively. Significant differences were observed in TDS values throughout the experimental duration except 1st week in both control and biofloc group ($p<0.05$). TDS values in biofloc group were significantly higher ($p<0.05$) than control (Table 4.17-4.18, Figure 4.9).

4.3.5. Total Ammonia Nitrogen (TAN)

The weekly mean TAN values fluctuate in control and biofloc group. The mean minimum and maximum TAN values from 1st to 10th week of experiment in control group and biofloc group were 0.4±0-0.8±0and 0.4±0-0.6±0, respectively. No significant differences were observed in TAN values on 1st, 2nd, 7th, and 8th week of experiment in control group. TAN values were significantly higher ($p<0.05$) in 1st, 4th, 6th, 7th, and 10th week of experiment in control than the biofloc group (Table 4.19-4.20, Figure 4.10).

4.3.6. Nitrite (NO_2)

The nitrite levels gradually decreased from 1st week to 10th week in the biofloc group, while fluctuates in control. Throughout the experimental period the nitrite levels of control were significantly higher ($p<0.05$) than biofloc. The average weekly values of nitrite observed in all the experimental tanks are presented in the Table 4.22. The minimum and maximum nitrite values observed from 1st to 10th week were 0.6667±0.23333, 1.3±0 and 0.4±, 0.7±0 respectively (Table 4.21-4.22, Figure 4.11).

4.3.7. Nitrate (NO_3)

Throughout the experimental period the average nitrate level fluctuates in both control and biofloc group and no significant differences were found. However the average nitrate level was more in control group in comparison to control group from 1st -10th week of experiment. The minimum and maximum nitrite values observed from 1st to 10th week were 1.3±0, 1.7±0 and 1.1±0, 1.2±0 respectively (Table 4.23-4.24, Figure 4.12).

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Table 4.1: Mean survival rate (%) of *P. pangasius* in control and biofloc tanks on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	200±0.10	200±0.10	200±0.10
Week 2	198±0.13	199±0.13	198±0.13
Week 3	198±0.13	198±0.15	198±0.14
Week 4	196±0.15	198±0.17	197±0.16
Week 5	196±0.15	197±0.18	196±0.17
Week 6	194±0.17	196±0.16	195±0.17
Week 7	193±0.18	196±0.19	195±0.19
Week 8	192±0.15	1960.15	194±0.15
Week 9	191±0.04	196±0.04	1940.04
Week 10	190±0.06	196±0.07	193±0.07
Mean±S.E.	195±0.02	197±0.04	196±0.03

Table 4.2: Kaplan Meier survival analysis of *P. pangasius* in control and biofloc tanks on weekly basis.

VAR00003 Conc	Mean[a]			
	Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
.00	42.00	6.39	29.48	54.52
650.00	51.33	7.04	37.53	65.14
Overall	46.256	4.85	36.75	55.76

Table 4.3: Mean length gain (cm) of *P. pangasius* in control and biofloc tanks on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	2.52±0.00m	2.54±0.01m	2.53±0.01h
Week 2	4.30±0.00lm	4.87±0.03lm	4.58±0.13gh
Week 3	6.71±0.05klm	8.51±0.06jklm	7.61±0.40fg
Week 4	9.20±0.06ijkl	11.93±0.15hijk	10.57±0.62ef
Week 5	11.56±0.10hijk	15.50±0.12fghi	13.53±0.89de
Week 6	14.20±0.20ghij	19.40±0.06defg	16.80±1.17cd
Week 7	16.83±0.31efgh	22.93±0.09cde	19.88±1.37bc
Week 8	19.50±0.46defg	27.03±0.09c	23.27±1.70b
Week 9	21.73±0.50cdef	39.70±8.95a	30.72±5.68a
Week 10	24.01±0.29cd	33.80±0.06b	28.91±2.19a
Mean±S.E.	13.06±1.31	18.62±2.30	15.84±1.36

Mean with different letters represents statistically significant differences (p<0.05).

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Table 4.4: Analysis of variance on length gain of *P. pangasius* in control and biofloc tanks on weekly basis.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	23.53	2	11.76	0.97	0.389
Tanks	464.71	1	464.71	38.25	0.000
Weeks	5215.03	9	579.45	47.61	0.000
Tanks*Weeks	384.79	9	42.75	3.52	0.003
Error	461.64	38	12.15		
Total	6549.69	59			

Table 4.5: Mean weight gain (g) of *P. pangasius* in control and biofloc tanks on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	5.17±0.09j	5.10±0.06j	5.13±0.05g
Week 2	8.63±0.07j	9.87±0.04j	9.25±0.28g
Week 3	17.33±3.83i	16.70±0.06i	17.02±1.72f
Week 4	18.23±0.12i	23.10±0.40i	20.67±1.10f
Week 5	23.23±0.19i	29.93±0.52h	26.58±1.52e
Week 6	37.40±9.31fg	37.17±0.32fg	37.29±4.17d
Week 7	34.00±0.12gh	44.70±0.21de	39.35±2.39d
Week 8	38.83±0.83efg	52.41±0.26c	45.62±3.06c
Week 9	43.97±0.37def	59.33±0.59b	51.65±3.45b
Week 10	48.63±0.66cd	66.53±0.90a	57.58±4.03a
Mean±S.E.	27.54±2.79	34.49±3.72	31.01±2.35

Mean with different letters represents statistically significant differences ($p<0.05$).

Table 4.6: Analysis of variance on weight gain of *P. pangasius* in control and biofloc tanks on weekly basis.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	40.20	2	20.10	1.302	0.284
Tanks	722.73	1	722.73	46.82	0.000
Weeks	17519.59	9	1946.62	126.09	0.000
Tanks*Weeks	666.17	9	74.02	4.71	0.000
Error	586.64	38	15.44		
Total	19535.32	59			

Table 4.7: Mean specific growth rate of *P. pangasius* in control and biofloc tanks on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	0.00±0.00g	0.00±0.00g	0.00±0.00e
Week 2	5.33±0.03f	7.43±0.07de	6.38±0.47d
Week 3	7.37±0.34de	10.63±0.03bc	9.00±0.75bc
Week 4	7.6±0.32de	9.98±0.73c	8.78±0.64c

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Week 5	7.8±0.46de	10.63±0.73bc	9.22±0.74bc
Week 6	8.03±0.52de	11.27±0.75abc	9.65±0.83abc
Week 7	8.2±0.10de	11.67±0.27ab	9.93±0.79ab
Week 8	8.57±0.17d	12.07±0.03a	10.32±0.79a
Week 9	7.47±0.61de	10.80±0.81abc	9.13±0.87bc
Week 10	7.00±0.21e	10.57±0.09bc	8.78±0.80c
Mean±S.E.	6.74± 0.45	9.50±0.64	8.12±0.43

Mean with different letters represents statistically significant differences (p<0.05).

Table 4.8: Analysis of variance on specific growth rate of *P. pangasius* in control and biofloc tanks on weekly basis.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.813	2	0.407	0.759	0.475
Tanks	114.817	1	114.817	214.361	0.000
Weeks	499.733	9	55.526	103.666	0.000
Tanks*Weeks	16.080	9	1.787	3.336	0.004
Error	20.354	38	0.536		
Total	651.796	59			

Table 4.9: Mean feed conversion ratio of *P. pangasius* in control and biofloc tanks on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	0.00±0.00	0.00±0.00	0.00±0.00
Week 2	0.12±0.00	0.10±0.00	0.11±0.00
Week 3	0.14±0.01	0.12±0.00	0.13±0.00
Week 4	0.18±0.01	0.18±0.01	0.18±0.00
Week 5	0.23±0.01	0.22±0.01	0.23±0.01
Week 6	0.29±0.02	0.26±0.02	0.27±0.01
Week 7	0.32±0.00	0.30±0.00	0.31±0.06
Week 8	0.39±0.02	0.33±0.00	0.36±0.02
Week 9	0.46±0.06	0.43±0.03	0.45±0.03
Week 10	0.56±0.01	0.48±0.00	0.52±0.02
Mean±S.E.	0.27±0.03	0.2417±0.02651	0.26±0.02

Table 4.10: Analysis of variance on feed conversion ratio of *P. pangasius* in control and biofloc tanks on weekly basis.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.00104	2	0.00104	1.106	0.341
Tanks	0.01	1	0.01262	13.396	0.001
Weeks	1.37	9	0.15262	162.077	0.000
Tanks*Weeks	0.01	9	0.00093	0.983	0.469
Error	0.04	38	0.00094		
Total	1.43	59			

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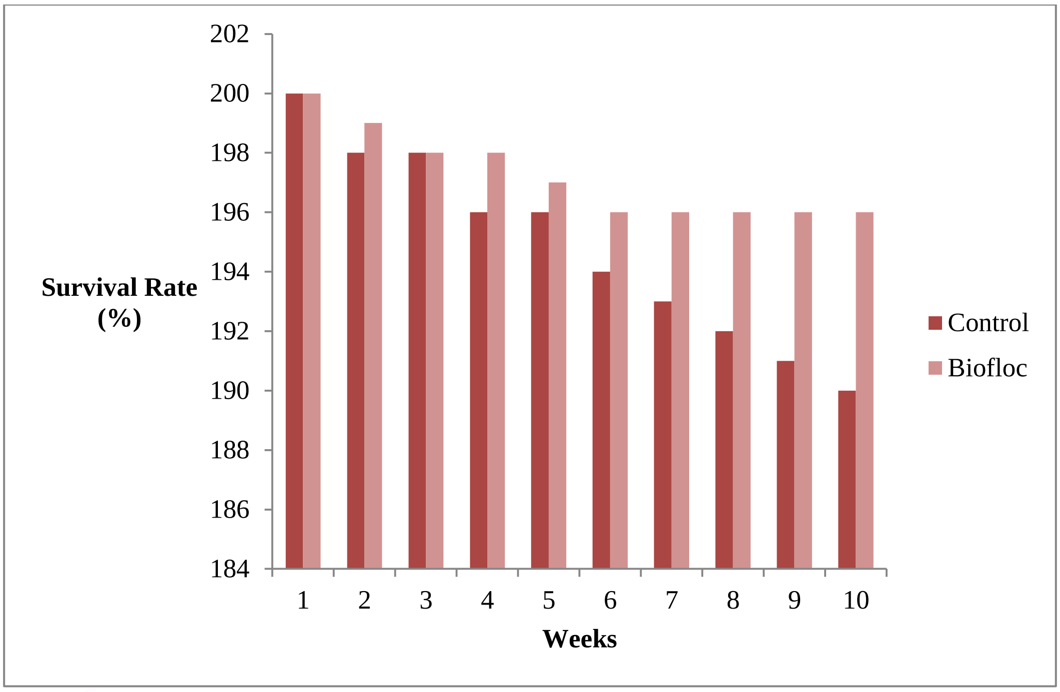

Figure 4.1: Graphical representation of mean survival rate (%) of *P. pangasius* in control and biofloc tanks on weekly basis.

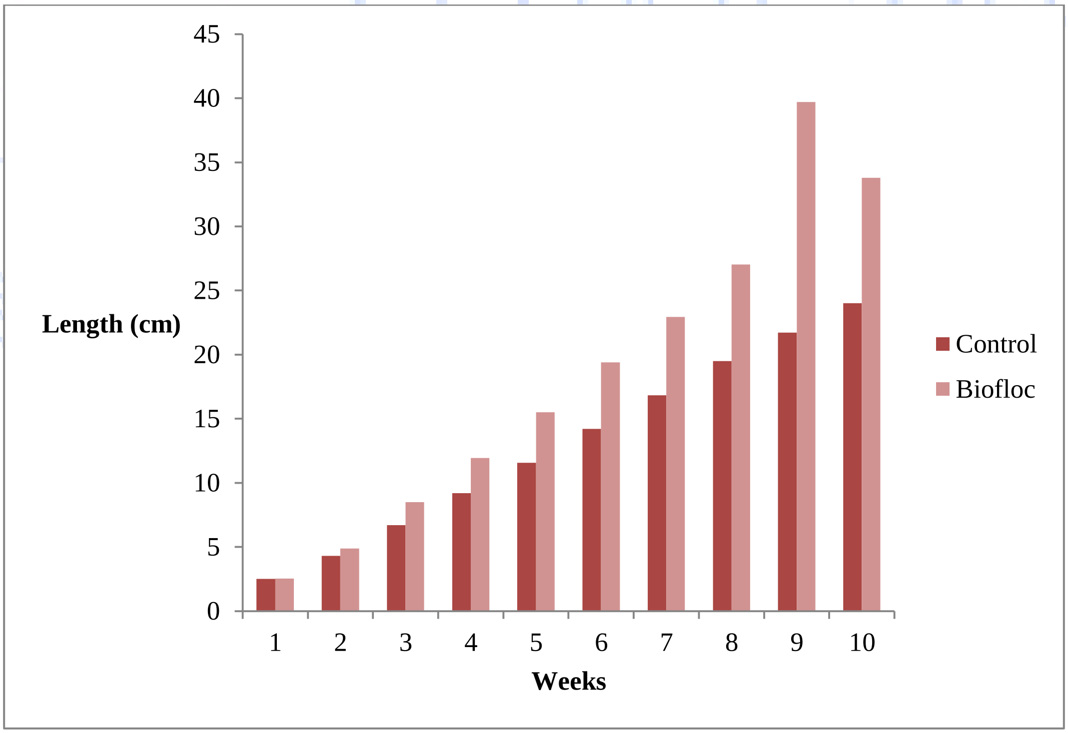

Figure 4.2: Graphical representation of mean length gain (cm) of *P. pangasius* in control and biofloc tanks on weekly basis.

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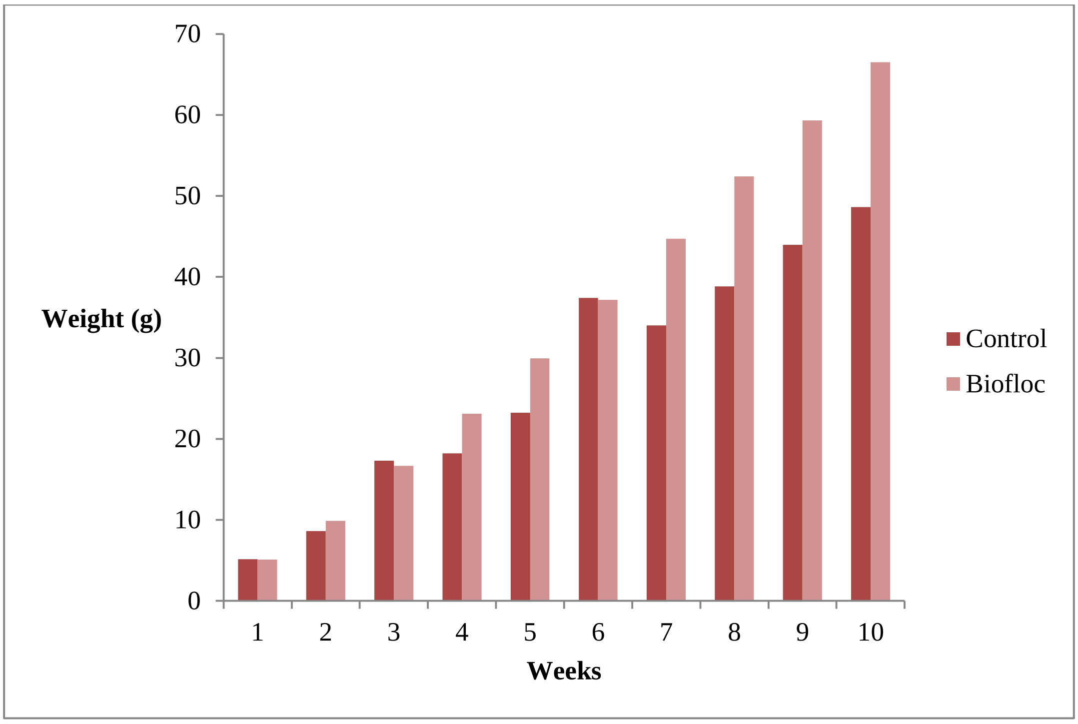

Figure 4.3: Graphical representation of mean weight gain (g) of *P. pangasius* in control and biofloc tanks on weekly basis.

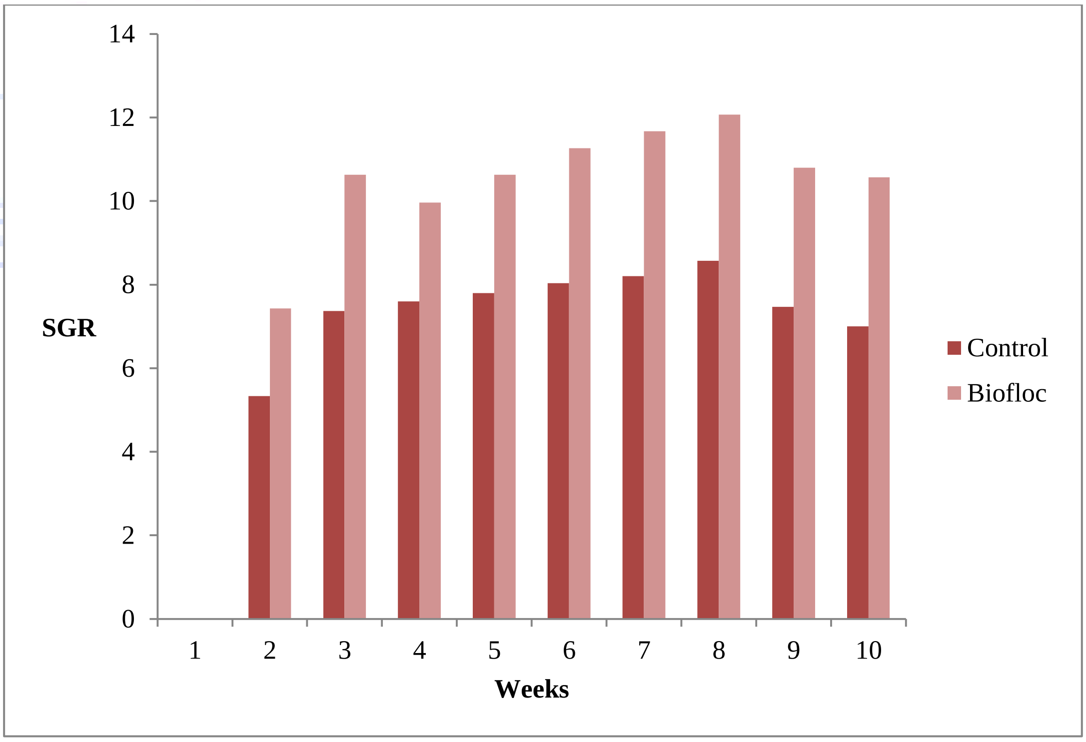

Figure 4.4: Graphical representation of mean specific growth rate of *P. pangasius* in control and biofloc tanks on weekly basis.

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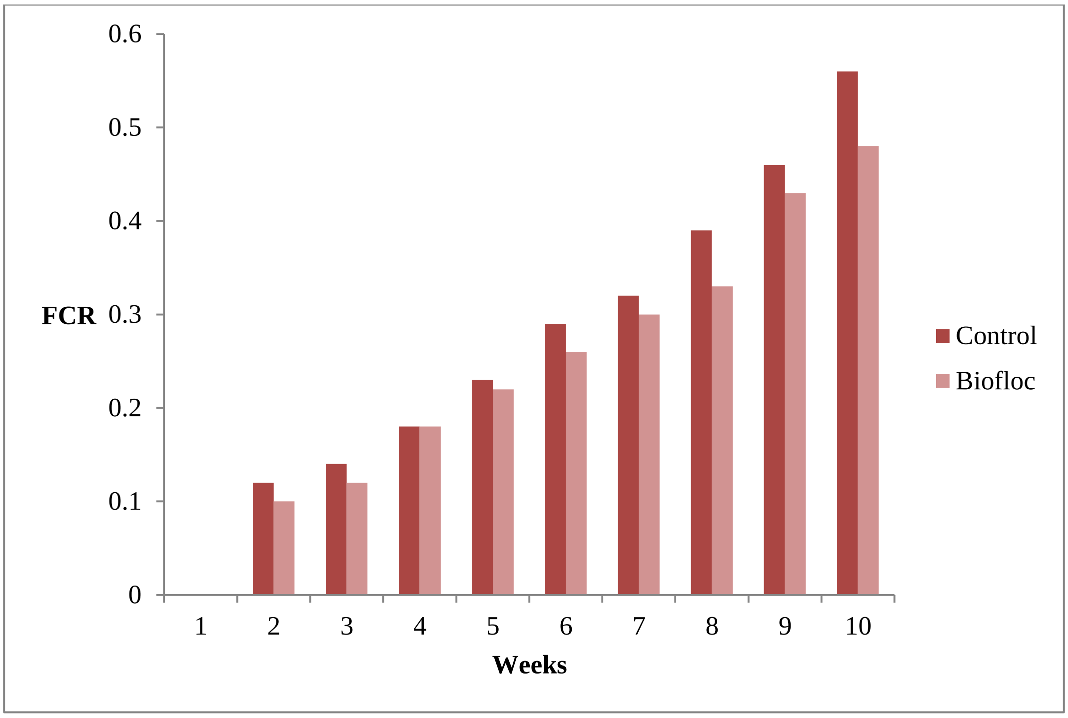

Figure 4.5: Graphical representation of mean feed conversion ratio of *P. pangasius* in control and biofloc tanks on weekly basis.

Table 4.11: Mean water temperature (°C) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	27.03±0.03	27.13±0.03	27.08±0.03
Week 2	29.00±0.05	28.20±0.81	28.60±0.40
Week 3	28.10±0.06	27.83±0.33	27.97±0.16
Week 4	27.23±0.67	26.63±0.64	26.93±0.43
Week 5	26.50±0.06	25.90±1.15	26.20±0.53
Week 6	26.77±0.32	25.87±0.48	26.32±0.33
Week 7	28.23±0.15	27.47±0.38	27.85±0.25
Week 8	27.70±0.25	27.30±0.15	27.50±0.16
Week 9	29.43±0.23	28.90±0.21	29.17±0.18
Week 10	27.97±0.24	28.23±0.07	28.10±0.13
Mean±S.E.	27.71±0.18	27.35±0.23	27.57±0.15

Table 4.12: Analysis of variance (ANOVA) on water temperature in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	1.47	2	0.73	1.34	0.273
Tanks	49.33	9	5.48	10.06	0.000
Weeks	3.04	1	3.04	5.57	0.023
Tanks*Weeks	1.99	9	0.22	0.41	0.924
Error	20.71	38	0.55		
Total	76.54	59			

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Table 4.13: Mean water pH measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E
	Control	Biofloc	
Week 1	7.53±0.03efgh	7.50±0.00efgh	7.52±0.02cd
Week 2	7.87±0.07abc	7.40±0.10gh	7.63±0.12abc
Week 3	8.00±0.00a	7.57±0.09defg	7.78±0.10a
Week 4	7.53±0.03efgh	7.37±0.03gh	7.45±0.04d
Week 5	7.57±0.07defg	7.30±0.21h	7.43±0.11d
Week 6	7.67±0.03cdef	6.87±0.07i	7.27±0.18e
Week 7	7.93±0.03ab	7.37±0.07gh	7.65±0.13abc
Week 8	7.80±0.06abcd	7.50±0.00efgh	7.65±0.07abc
Week 9	7.73±0.15bcde	7.45±0.03fgh	7.60±0.09bcd
Week 10	7.93±0.07ab	7.50±0.00efgh	7.72±0.10ab
Mean±S.E	7.76±0.04	7.38±0.04	7.57±0.04

Mean with different letters represents statistically significant differences ($p<0.05$).

Table 4.14: Analysis of variance (ANOVA) on water pH in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.00	2	0.00	0.08	0.920
Tanks	1.28	9	0.14	7.88	0.000
Weeks	2.09	1	2.09	116.21	0.000
Tanks*Weeks	0.63	9	0.07	3.91	0.001
Error	0.68	38	0.02		
Total	4.69	59			

Table 4.15: Mean water dissolved oxygen (mg/L) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E
	Control	Biofloc	
Week 1	7.17±0.03	7.17±0.03	7.17±0.02
Week 2	7.40±0.58	7.20±0.58	7.30±0.57
Week 3	7.27±0.19	7.07±0.19	7.17±0.13
Week 4	7.27±0.09	7.03±0.03	7.15±0.07
Week 5	7.37±0.03	7.20±0.58	7.28±0.05
Week 6	7.37±0.03	7.10±0.58	7.28±0.07
Week 7	6.97±0.03	6.70±0.10	6.83±0.08
Week 8	7.33±0.12	7.10±0.58	7.23±0.08
Week 9	7.00±0.58	6.80±0.10	6.90±0.07
Week 10	6.80±0.58	6.53±0.03	6.67±0.07
Mean±S.E	7.19±0.04	6.99±0.46	7.09±0.03

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Table 4.16: Analysis of variance (ANOVA) on water dissolved oxygen in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.12	2	0.06	2.91	0.062
Tanks	2.49	9	0.28	14.25	0.000
Weeks	0.62	1	0.62	31.98	0.000
Tanks*Weeks	0.08	9	0.01	0.49	0.875
Error	0.74	38	0.02		
Total	4.05	59			

Table 4.17: Mean water TDS (mg/L) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E
	Control	Biofloc	
Week 1	1.70±0.00de	1.70±0.00de	1.70±0.00c
Week 2	1.53±0.03gh	1.60±0.00fg	1.57±0.02e
Week 3	1.47±0.03hi	1.60±0.00fg	1.53±0.03e
Week 4	1.57±0.03g	1.70±0.00de	1.63±0.03d
Week 5	1.47±0.03hi	1.60±0.00fg	1.53±0.03e
Week 6	2.13 ±0.03b	2.33 ±0.07a	2.23±0.06a
Week 7	1.40±0.00i	1.67±0.03ef	1.53±0.06e
Week 8	1.70±0.00de	2.13±0.03b	1.92±0.01b
Week 9	1.47±0.03hi	1.60±0.00fg	1.53±0.03e
Week 10	1.77±0.03d	2.03±0.03c	1.9±0.06b
Mean±S.E	1.62±0.04	1.71±0.05	1.71±0.03

Mean with different letters represents statistically significant differences ($p<0.05$).

Table 4.18: Analysis of variance (ANOVA) on water TDS in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.01	2	0.00	1.152	0.327
Tanks	3.02	9	0.34	145.099	0.000
Weeks	0.47	1	0.47	202.163	0.000
Tanks*Weeks	0.20	9	0.02	9.604	0.000
Error	0.09	38	0.00		
Total	3.79	59			

Table 4.19: Mean water TAN (mg/L) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E
	Control	Biofloc	
Week 1	0.6±0.00c	0.50±0.00d	0.55±0.02bc
Week 2	0.60±0.00c	0.60±0.00c	0.60±0.00b
Week 3	0.50±0.00d	0.50±0.00d	0.50±0.00c

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Week 4	0.80±0.00a	0.50±0.00d	0.65±0.07a
Week 5	0.40±0.00e	0.40±0.00e	0.40±0.00d
Week 6	0.70±0.00b	0.40±0.00e	0.55±0.07bc
Week 7	0.60 ±0.00c	0.50±0.00d	0.55±0.02bc
Week 8	0.60±0.00c	0.60±0.00c	0.60±0.00b
Week 9	0.50±0.00d	0.50±0.00d	0.50±0.00c
Week 10	0.80± 0.00a	0.50±0.10d	0.65±0.08a
Mean±S.E	0.61±0.02	0.5±0.01	0.555±0.01

Mean with different letters represents statistically significant differences ($p<0.05$).

Table 4.20: Analysis of variance (ANOVA) on water TAN in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.00	2	0.00	1.000	0.377
Tanks	0.31	9	0.03	23.222	0.000
Weeks	0.18	1	0.18	121.000	0.000
Tanks*Weeks	0.25	9	0.03	18.778	0.000
Error	0.06	38	0.00		
Total	0.81	59			

Table 4.21: Mean water NO_2 (mg/L) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E.
	Control	Biofloc	
Week 1	0.90±0.00bc	0.70±0.00de	0.80±0.04abc
Week 2	1.00±0.00b	0.70±0.00de	0.85±0.07bc
Week 3	1.20±0.00a	0.60±0.00ef	0.90±0.13a
Week 4	1.30±0.00a	0.50±0.00fg	0.90±0.18a
Week 5	1.20±0.00a	0.50±0.00fg	0.85±0.16bc
Week 6	0.90± 0.00bc	0.50±0.00fg	0.70±0.89cde
Week 7	1.00±0.00b	0.50±0.00fg	0.75±0.11bcd
Week 8	1.30±0.00a	0.40±0.00g	0.85±0.20bc
Week 9	0.77±0.13cde	0.40±0.00g	0.58±0.10e
Week 10	0.87±0.23bcd	0.40±0.00g	0.63±0.15de
Mean±S.E	1.04±0.04	0.52±0.02	0.78±0.04

Mean with different letters represents statistically significant differences ($p<0.05$).

Table 4.22: Analysis of variance (ANOVA) on water NO_2 in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.04	2	0.02	1.950	0.156
Tanks	0.67	9	0.07	7.178	0.000
Weeks	4.11	1	4.11	397.227	0.000
Tanks*Weeks	0.68	9	0.08	7.307	0.000

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Error	0.39	38	0.01		
Total	5.89	59			

Table 4.23: Mean water NO_3 (mg/L) measured in control and biofloc tanks of *P. pangasius* on weekly basis.

Duration	Tanks		Mean±S.E
	Control	Biofloc	
Week 1	1.30±0.00	1.20±0.00	1.25±0.02
Week 2	1.40±0.00	1.30±0.00	1.35±0.02
Week 3	1.50±0.00	1.30±0.00	1.40±0.04
Week 4	1.50±0.00	1.30±0.00	1.40±0.04
Week 5	1.60±0.00	1.10±0.00	1.35± 0.11
Week 6	1.60±0.00	1.20±0.00	1.40±0.09
Week 7	1.50±0.00	1.10±0.00	1.30±0.09
Week 8	1.70±0.00	1.20±0.00	1.45± 0.11
Week 9	1.50±0.00	1.20±0.00	1.35± 0.07
Week 10	1.60±0.00	1.10±0.00	1.35±0.11
Mean±S.E	1.52±0.02	1.20±0.01	1.36±0.02

Table 4.24: Analysis of variance (ANOVA) on water NO_3 in control and biofloc tanks of *P. pangasius* within 10 weeks.

Source	Sum of Squares	Df	Mean Square	F	Significance
Replications	0.00	2	0.00	1.57	0.063
Tanks	0.17	9	0.02	11.07	0.000
Weeks	1.54	1	1.54	5.62	0.024
Tanks*Weeks	0.35	9	0.04	0.38	0.782
Error	0.00	38	0.00		
Total	2.06	59			

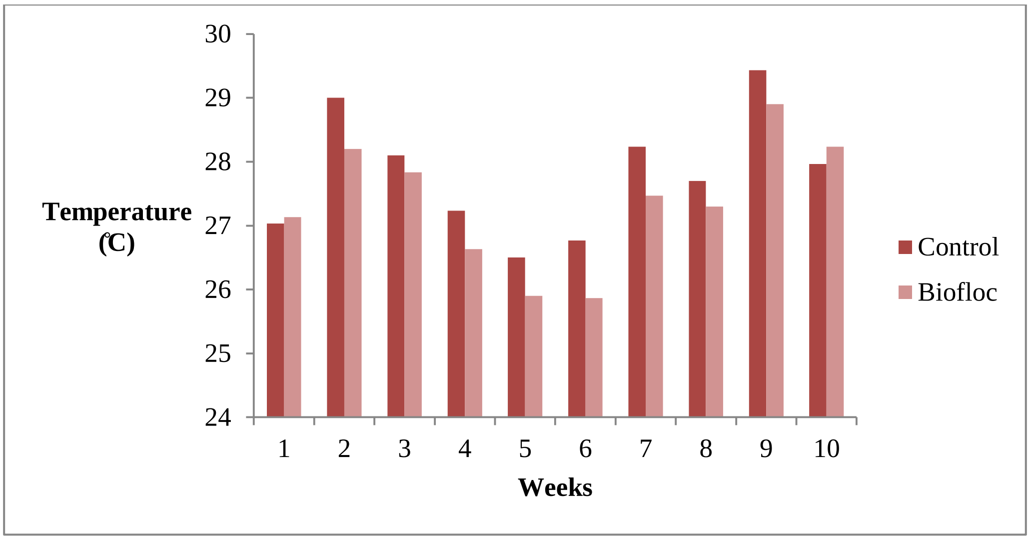

Figure 4.6: Graphical representation of mean water temperature (℃) of *P. pangasius* in control and biofloc tanks on weekly basis.

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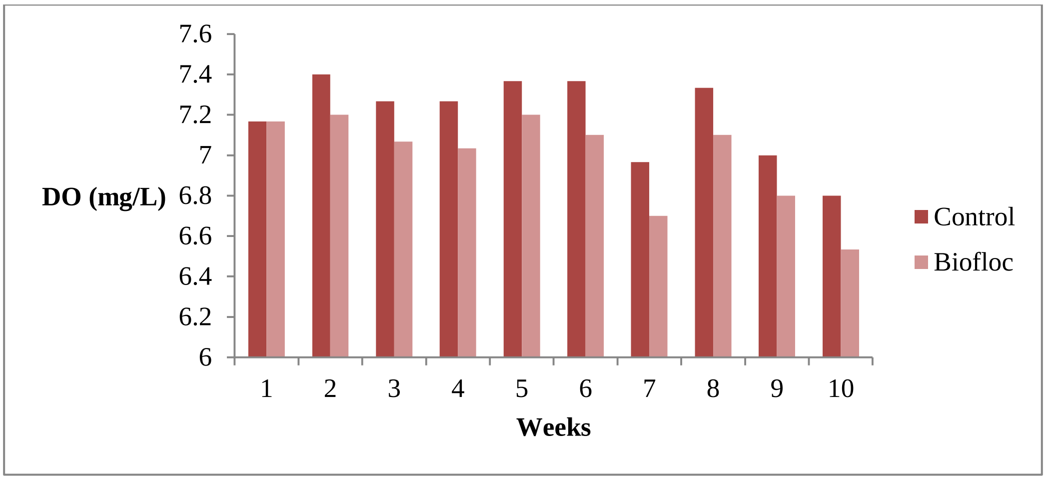

Figure 4.7: Graphical representation of mean water dissolved oxygen (mg/L) of *P. pangasius* in control and biofloc tanks on weekly basis.

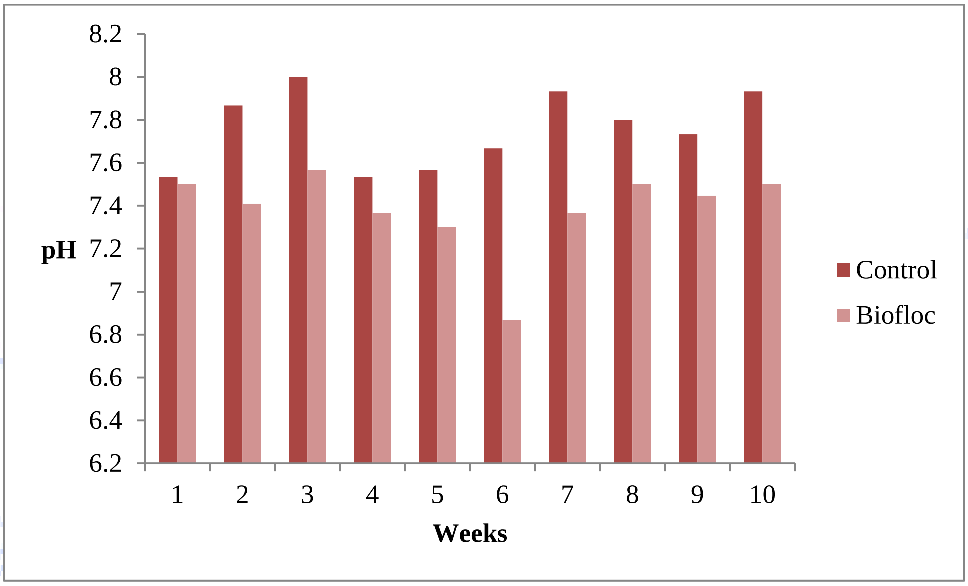

Figure 4.8: Graphical representation of mean water pH of *P. pangasius* in control and biofloc tanks on weekly basis.

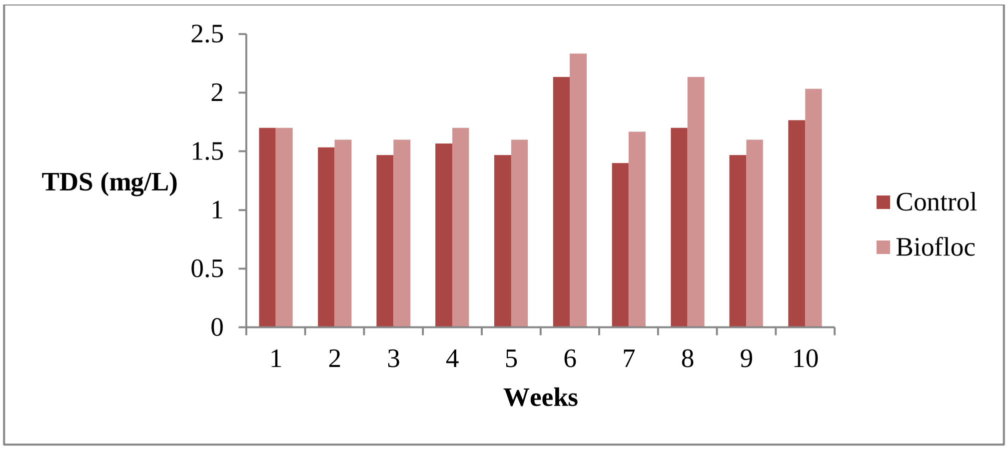

Figure 4.9: Graphical representation of mean water TDS (mg/L) of *P. pangasius* in control and biofloc tanks on weekly basis.

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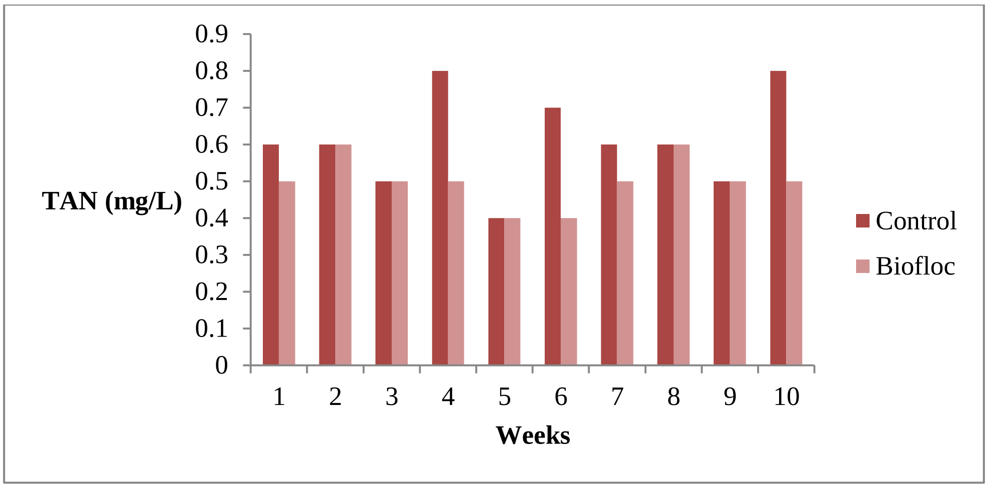

Figure 4.10: Graphical representation of mean water TAN (mg/L) of *P. pangasius* in control and biofloc tanks on weekly basis.

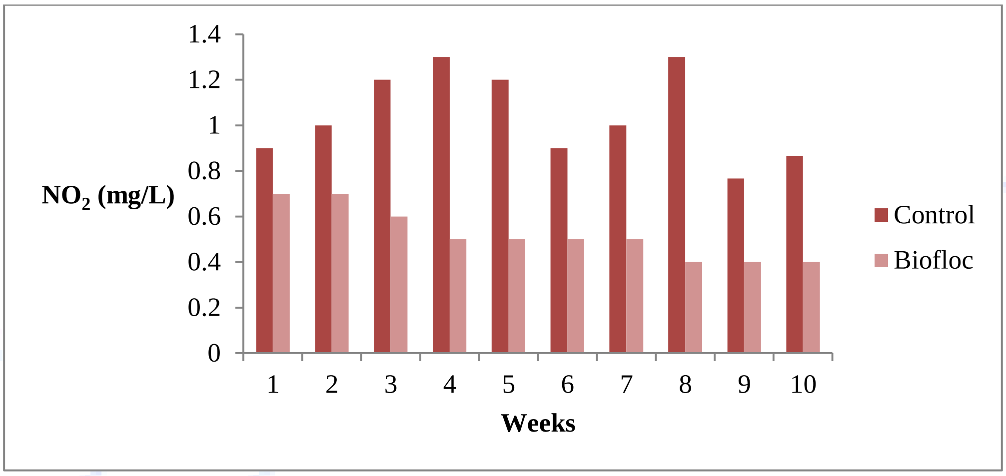

Figure 4.11: Graphical representation of mean water NO_2 (mg/L) of *P. pangasius* in control and biofloc tanks on weekly basis.

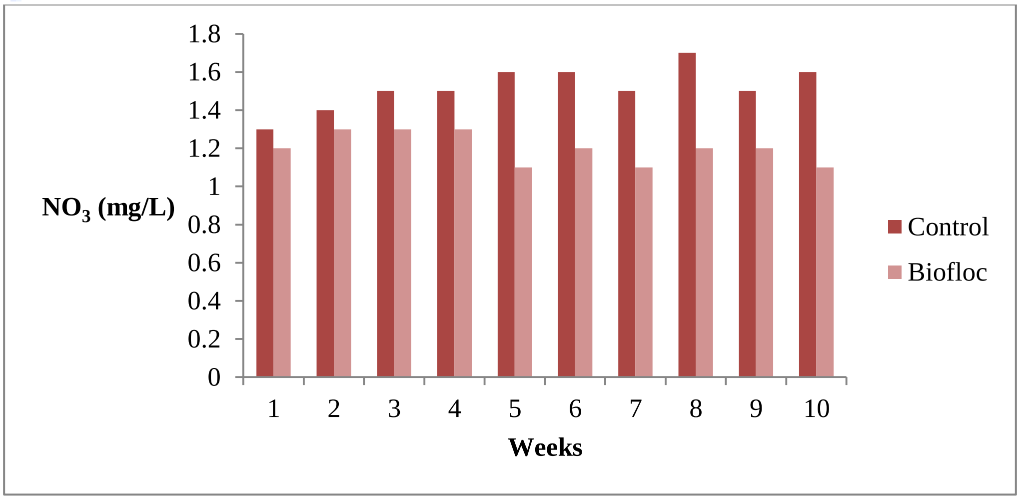

Figure 4.12: Graphical representation of mean water NO_3 (mg/L) of *P. pangasius* in control and biofloc tanks on weekly basis.

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CENTRAL ASIAN STUDIES

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