

Physiological and Haemato-Biochemical Evaluation of *Pangasius pangasius* Reared in a Biofloc-Based Aquaculture System

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SUMMARY

The objective of this study was to determine the physiological and biochemical reactions of *Pangasius pangasius* grown in a biofloc technology (BFT)-based aquaculture system. The setup time of the experiment took 90 days, and the fish were randomly split and placed into two biofloc treatment tanks (T1, T2) and one control tank. Growth, survival, hematology, and serum biochemistry performances were assessed. The results indicated that biofloc growth rearing was a better way to measure fish growth performance. After 90 days, the biofloc rearing treatment 2 (T2) had the highest absolute growth rate at 449.0 g, while treatment 1 (T1) had 439.8 g, and the control had 412.2 g. The survival rate was higher in biofloc systems (T1: 93%; T2: 91.7%) than in the control group (89.5%). Hematological tests indicated increased red blood cell (RBC) and hemoglobin levels in the biofloc groups, suggesting enhanced oxygen transfer and reduced stress. The substantial decrease in white blood cell (WBC) counts in T2 indicates improved immune function. The serum biochemistry revealed diminished glucose levels (signifying reduced stress) and elevated total protein and albumin levels in fish cultivated in bioflocs, indicating enhanced nutrient absorption. The study shows that biofloc technology improves the growth, health, and feed conversion efficiency of *P. pangasius*, even when external feed is scarce. This makes fish farming more sustainable and productive.

Keywords: *Pangasius pangasius*, Biofloc technology, Hematology, Serum biochemistry

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INTRODUCTION

Biofloc technology (BFT) is a sustainable fish farming technique that optimizes water quality and provides nutrition through microbial bioflocs. These microbes comprise bacteria, protozoa, algae, and organic matter that metabolize toxic nitrogenous waste into biomass, easily consumed by fish (Avnimelech, 2009; Crab et al., 2012). Such systems are beneficial for intensive fish farming with low water exchange, providing both environmental and economic gains (Emerenciano et al., 2013; Hargreaves, 2006). The use of biofloc systems promotes growth, immunity, and overall health in

several species, including shrimp (*Litopenaeus vannamei*), tilapia (*Oreochromis niloticus*), and catfish (*Clarias gariepinus*, *Pangasius* spp.) (Azim and Little, 2008; Ekasari et al., 2014; Kumar et al., 2012).

Pangasius pangasius, also called the Indian Butter Catfish, is an economically and nutritionally significant freshwater species native to the Ganges and Brahmaputra River systems of South Asia (Rathod et al., 2018). It belongs to the genus *Pangasius*, family Pangasiidae, order Siluriformes, class Actinopterygii, phylum Chordata, and kingdom Animalia (Hamilton, 1822). It has high economic value due to rapid growth, disease resistance/strong immunity, efficient/optimized feed conversion, firm white flesh, and low-fat content (Talwar and Jhingran, 1991). *P. pangasius* has become one of the viable species in sustainable aquaculture, supplying the surging global demand for protein-rich aquatic food (FAO, 2018).

The selection of *P. pangasius* for this study was based on its adaptability to intensive aquaculture practices, low dissolved oxygen, high stocking densities, efficient use of alternative nutrients in biofloc technology, and significant economic value in the market (Kumar et al., 2012). Moreover, studies investigating the hematological and serum responses of *P. pangasius* under BFT conditions are limited; therefore, this species is of scientific interest for physiological and biochemical analyses. More extensive studies on its response to BFT could improve culture practices, increase productivity, and promote sustainable fish farming in developing countries.

The growth performance of cultured species is a key component for aquaculture success. The provision of microbial biomass, essential fatty acids, and bioactive components through biofloc systems promotes fish growth by reducing commercial feed requirements and improving feed utilization (Avnimelech, 2009; Ekasari et al., 2014). In *Pangasius pangasius*, biofloc availability enables continuous feeding and enhances nutrient utilization, resulting in higher specific growth rates (SGR) and weight gain (Kumar et al., 2012).

The size of red and white blood cells, in addition to hemoglobin and hematocrit levels, are major indicators of overall fish health and stress tolerance. Biofloc systems positively influence these parameters due to their microbial diversity and immunostimulatory effects (Kumar et al., 2012). Catfish juveniles reared in biofloc systems have shown increased RBC and Hb levels, which enhance oxygen transport and overall health (Ahmad et al., 2017). The WBC levels are also increasing, indicating a stronger immune system and infection resistance. This positive change is attributed to decreased environmental stress, increased nutrient uptake, and stable water conditions in the biofloc system (Azim and Little, 2008; Emerenciano et al., 2013). Serum biochemical parameters such as glucose, total protein, albumin, cholesterol, and liver enzymes (AST, ALT) are keysensitive indicators of metabolic status and stress levels in fish. Biofloc systems help stabilize these parameters by improving metabolic efficiency and reducing oxidative damage (Martins et al., 2011; Xu and Pan, 2012). In *Pangasius pangasius*, cultured in biofloc environments, serum glucose tends to decrease (indicating reduced stress), while total protein and albumin levels increase, showing better nutritional uptake and immune competence. Additionally, the liver enzyme activities remain within normal ranges, reflecting reduced hepatic stress compared to conventional culture methods (Ahmad

et al., 2017). These findings highlight the physiological advantages of BFT for maintaining metabolic homeostasis in high-density aquaculture. The aim of this study is to evaluate the overall performance and physiological health of *Pangasius pangasius* cultured in a biofloc system, focusing on growth performance, hemato-biochemical, and serum parameters. This study also provides valuable insights into whether BFT can serve as an effective alternative to conventional aquaculture by enhancing fish growth, health, and environmental control.

MATERIALS AND METHODS

STUDY DESIGN AND EXPERIMENTAL SETUP

The experiment was carried out in Basti Kacholian (29.407297° N, 71.798414° E), Bahawalpur, Punjab, Pakistan. Thirty healthy *P. pangasius* (initial mean weight \approx 100 g) were randomly distributed into three tanks: two biofloc treatments (Treatment 1, T1; Treatment 2, T2) and one clear-water control (control), at a stocking rate of 10 fish per tank. Each tank received 75 kg of commercial floating feed (30% crude protein) per day. The biofloc tanks were maintained using the carbon-to-nitrogen (C:N) ratio control method presented by (Avnimelech, 2009).

GROWTH MONITORING AND SURVIVAL ASSESSMENT

The study was conducted over 90 days. Total length, fork length, and body weight of experimental fish were recorded weekly. Growth performance was assessed using Absolute Growth Rate (AGR), Specific Growth Rate (SGR), Relative Growth Rate (RGR), and Average Daily Gain (ADG). Survival percentage was evaluated at the end of the study (Manikandan et al., 2020).

WATER QUALITY MONITORING

Portable digital meters (Hanna Instruments) were employed to monitor water quality parameters (temperature, pH, salinity, dissolved oxygen, and total dissolved solids). In the biofloc system, the C:N ratio was adjusted by adding carbon to promote microbial growth, following standard biofloc protocols (Crab et al., 2012; Emerenciano et al., 2013; Xu and Pan, 2012).

HEMATOLOGICAL ANALYSIS

Blood sampling was performed using EDTA-treated syringes. The hematological parameters, namely red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT), were determined using Sahli's method and a hemocytometer under a light microscope, as per reference protocols (Ahmad et al., 2017).

SERUM BIOCHEMISTRY

Blood samples were centrifuged at 3,000 rpm for 15 min to separate the serum. Serum parameters glucose, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, and bilirubin were measured with commercial diagnostic kits.

These biomarkers were used to assess metabolic, liver, and kidney health in the biofloc and control groups (Martins et al., 2011).

RESULTS

ABSOLUTE GROWTH RATE

The absolute growth rate of control group at 45 days was 201.2g while after 90 days it was 412.2g. The absolute growth rate of T1 and T2 after 45 and 90 days were (217.4g, 439.8g) and (227.1g, 449g) respectively. The highest growth rate was observed in T2 (Table 1).

Table 1: Absolute growth rate of *Pangasius pangasius* during the experimental period.

Sr. No	Control	Treatment 1	Treatment 2
Stocking	1000	1000	1000
Weight (g) at stocking time	5.3	5.1	5.7
Weight (g) after 45 days			
Sample 1	200.4	220.2	230.0
Sample 2	205.2	222.2	232.3
Sample 3	207.4	221.8	233.6
Sample 4	209.8	224.6	235.5
Sample 5	210.5	225.3	234.7
Mean Weight (g)	206.2	222.4	232.3
Absolute Growth (g)	201.2	217.4	227.1
Weight (g) after 90 days			
Sample 1	400.7	440.2	445.3
Sample 2	410.0	443.6	450.7
Sample 3	422.5	446.8	456.5
Sample 4	425.3	445.6	459.0
Sample 5	430.3	450.0	460.1
Mean Weight (g)	417.4	444.8	454.9
Absolute Growth (g)	412.2	439.8	449.0

SURVIVAL RATE

The survival rates for control group, T1 and T2 were 89.5%, 93% and 91.7% respectively, with highest survival rate recorded in T1 (Figure 1).

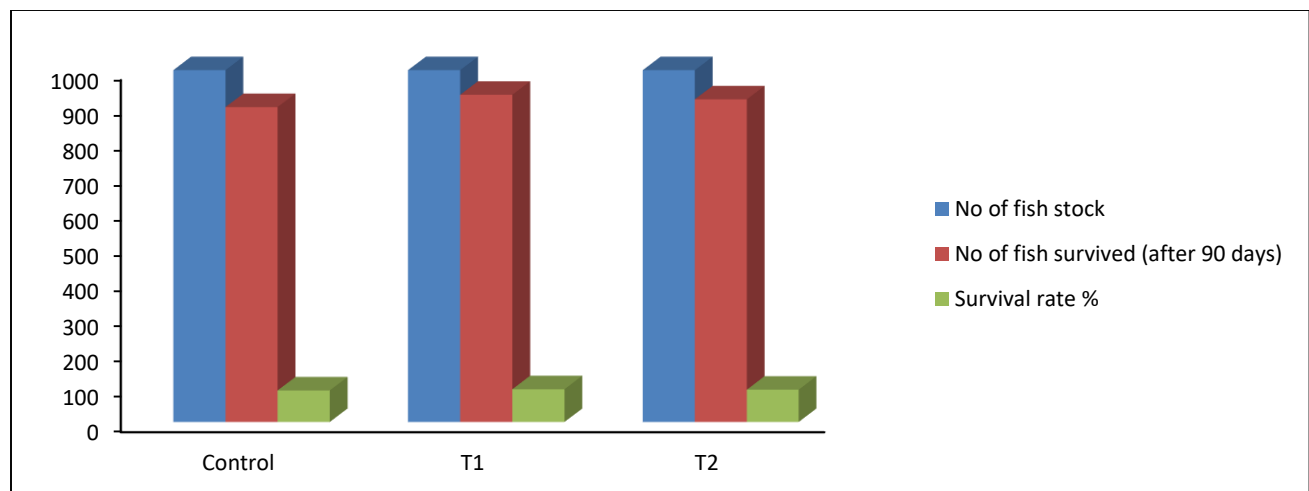


Figure 1: Survival analysis between control and treatment groups after 90 days.

MORTALITY RATE

The mortality rates for control group, T1 and T2 were 10.5%, 7% and 8.3% respectively, with highest mortality measured in control group (Figure 2).

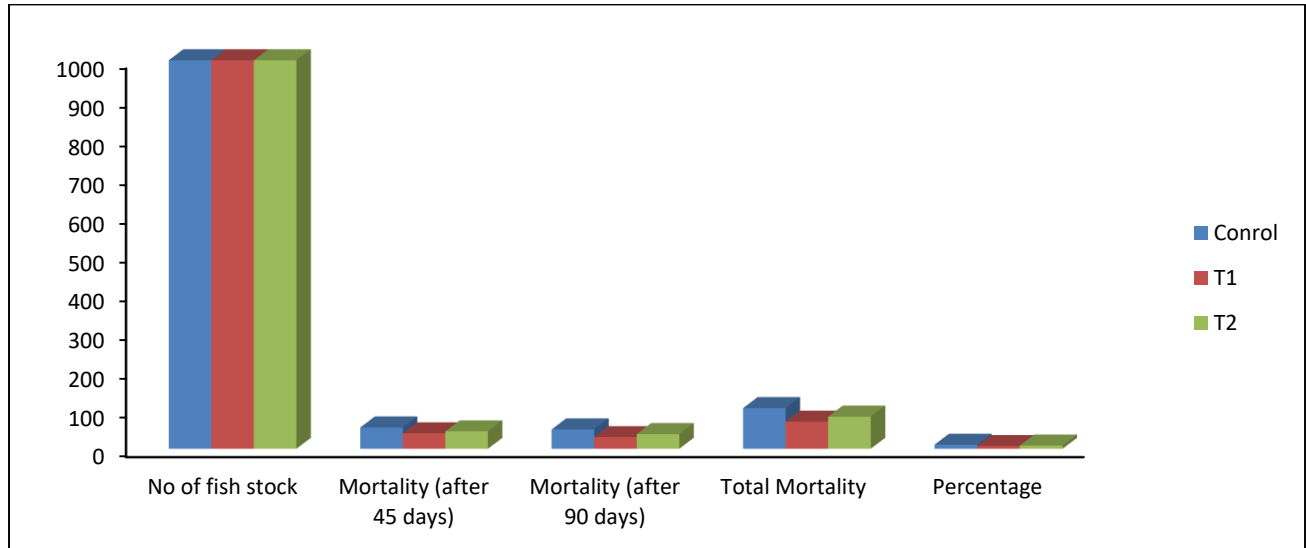


Figure 2: Mortality rates (%) between control and treatment groups on Day 45 and Day 90.

HEMATOLOGICAL ANALYSES

The Mean \pm SE values and statistical significance levels of RBC, Hb, HCT, MCV, MCH, MCHC, WBC, and PLT were assessed in control and treatment groups (T1, T2) of *Pangasius pangasius* during the 1st (45 days) and 2nd (90 days) sampling periods. One-way ANOVA revealed statistically significant ($P < 0.05$) differences among the groups for most parameters in both sampling periods, with variations observed between control and treatment groups as well as between T1 and T2 (Table 2).

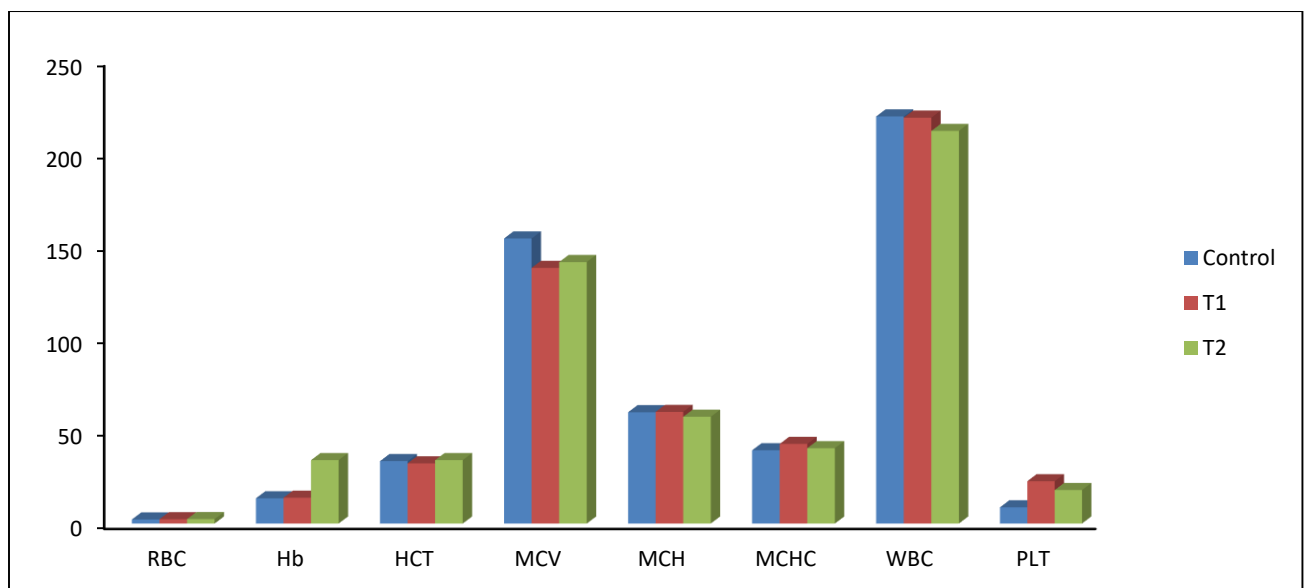


Figure 3: Comparison of hematological parameters of fish after 45-Days sampling.

After the first sampling, higher levels of RBC, Hb, and HCT were observed in T2. MCV was slightly higher in the control group, while MCH showed a similar pattern with minor decreases in T2. MCHC exhibited minimal variation among treatments. WBC counts were lower in T2, while PLT counts were considerably higher in T1 (Figure 3).

After the second sampling, T1 showed higher RBC, Hb, HCT, and MCV values compared to T2, while MCH was slightly lower in T1. MCHC varied minimally. WBC counts were lowest in T2, and PLT counts were highest in T2 (Figure 4).

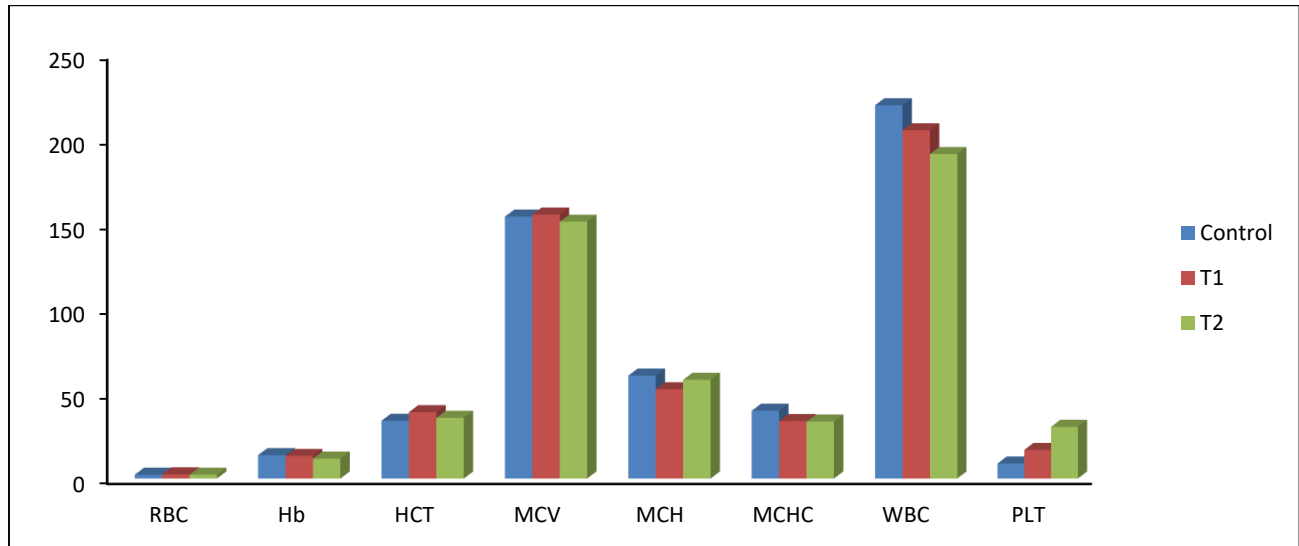


Figure 4: Comparison of hematological parameters of fish after 90-Days sampling.

SERUM ANALYSES

For the serum study, the Mean \pm SE values and statistical significance levels of glucose, total protein, albumin, cholesterol, triglycerides, ALT, AST, ALP, urea, and creatinine were examined in control and treatment groups (T1, T2) of *Pangasius pangasius* during the 1st and 2nd sampling periods. Statistical analysis revealed significant differences ($P < 0.05$) among groups for most serum biochemical parameters, including glucose, total protein, albumin, cholesterol, triglycerides, ALT, AST, ALP, and urea at one or both sampling intervals. Creatinine and bilirubin showed no significant variation ($P > 0.05$) across treatments in both samplings (Table 3).

After the first sampling (45 days), glucose levels were lowest in T1, while total protein and albumin were slightly higher in T2. Cholesterol and triglycerides were highest in the control group. ALT was lowest in T2, while AST peaked in T1. ALP increased notably in T2. Urea was slightly higher in T1, whereas creatinine and bilirubin showed minimal variation among treatments (Figure 5).

After the second sampling (90 days), glucose levels were lowest in T1, while total protein, albumin, and cholesterol were lowest in T2. Triglyceride levels were highest in the control group. ALT was lower in T2, whereas AST, ALP, and urea showed a marked increase in T1. Creatinine and bilirubin showed minimal differences among treatments (Figure 6).

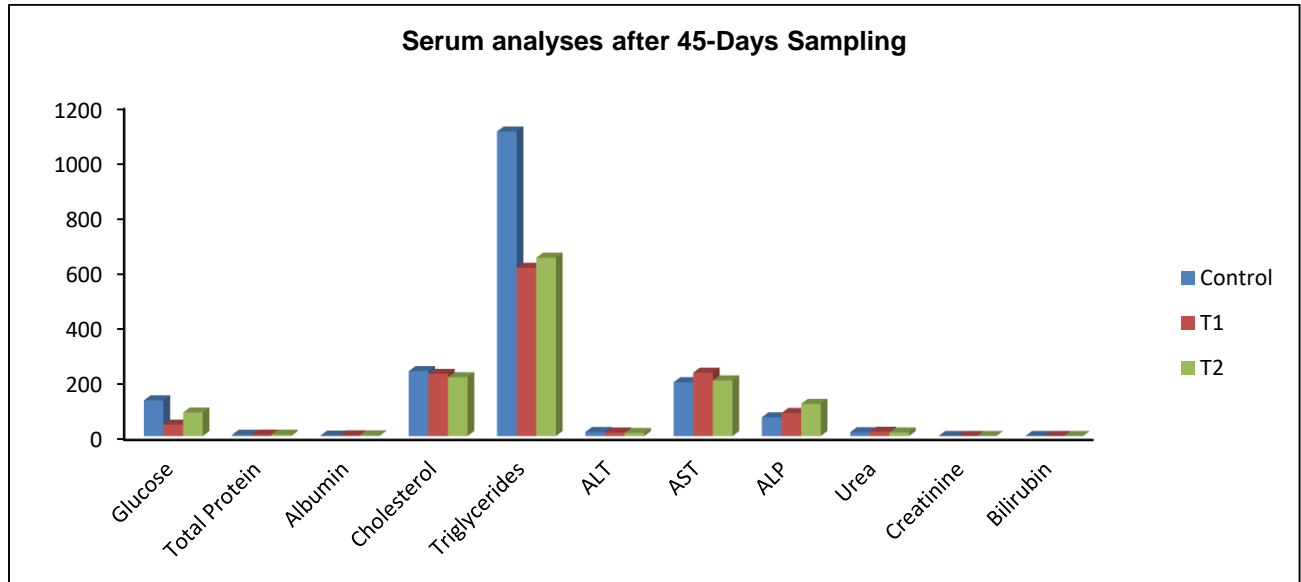


Figure 5: Comparison of serum parameters of fish after 45-Days sampling.

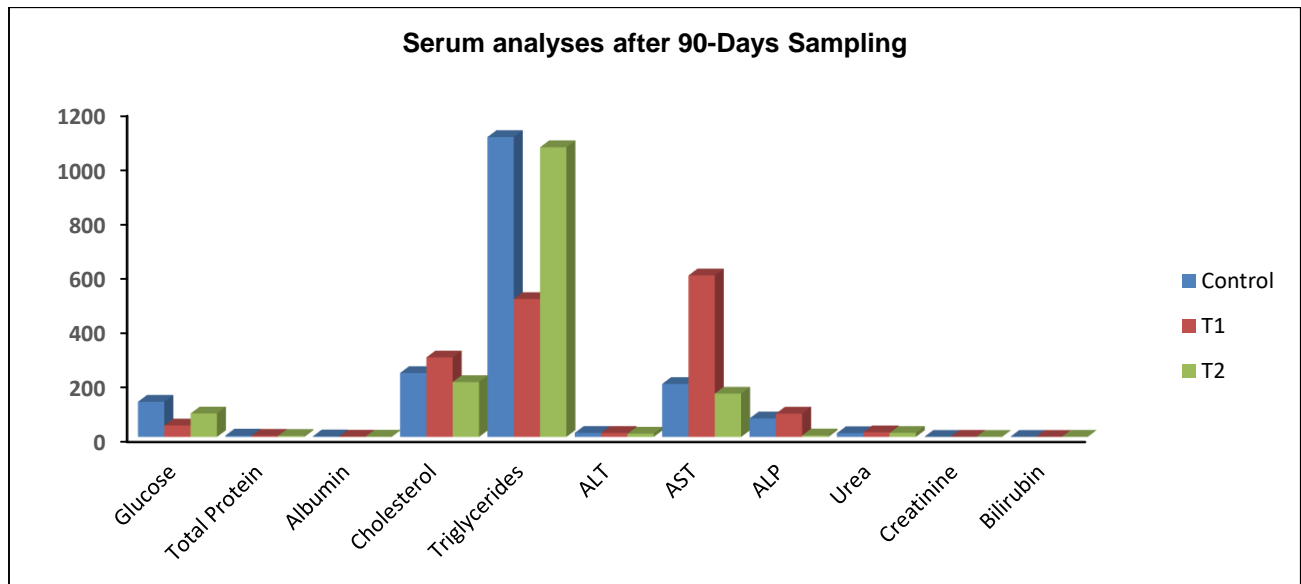


Figure 6: Comparison of serum parameters of fish after 90-Days sampling.

DISCUSSION

The current research provides evidence that the biofloc system has a significantly positive impact on the physiological, hematological, and biochemical profiles of *Pangasius pangasius* compared to the control group, making it a sustainable technique in aquaculture.

Fish cultured in biofloc tanks (T1 and T2) exhibited higher absolute growth rates than the control group, with T2 showing the highest weight gain of 449 g after 90 days. These results support previous findings that biofloc provides a constant supply of microbial protein and bioactive compounds, consequently enhancing

growth in cultured fish (Avnimelech, 2009; Shourbela et al., 2023). Additionally, there were increased rates of survival in groups cultured in biofloc systems, indicating lower environmental stress and enhanced tolerance, as observed in tilapia and catfish farmed using biofloc technology (Nageswari et al., 2023; Popoola et al., 2024).

Although RBC and HCT displayed slightly different trends, both parameters were higher in the biofloc treatments, suggesting an optimized oxygen transport mechanism and augmented physiological fitness. These results are consistent with previous findings that erythropoiesis is stimulated in fish reared in biofloc systems due to moderated stress and enriched nutrition (Ahmad et al., 2017; Sallam et al., 2024). The values of MCV, MCH, and MCHC indicated minor variations but remained within physiological levels, suggesting stable hematopoiesis. The lowest WBC counts were observed in T2, depicting minimal infection pressure and stress, as stable microbial communities in bioflocs tend to reduce the emergence of opportunistic pathogens (Kim et al., 2020). In the treated group, platelet levels exhibited a remarkable elevation in T2, signifying a more effective hemostatic balance and strengthened immune defense.

There was a considerable reduction in serum glucose levels in the biofloc groups, mainly in T1, reflecting reduced stress in these fish. Elevated levels of total protein and albumin in the biofloc-fed groups correspond with improved nutrient absorption and enhanced immune function, consistent with observations in other catfish species fed using biofloc technology (Zafar et al., 2022). The highest concentrations of cholesterol and triglycerides were found in the control group, demonstrating greater metabolic stress and lipid imbalance. Conversely, the lower levels observed in the biofloc groups suggest more efficient energy utilization and markedly lower fat storage.

The advantages of biofloc rearing were further validated by enzymatic biomarkers. The activities of ALT and AST, which are markers of hepatic function, stayed within normal limits; however, AST levels were elevated in T1 at 90 days. ALP activity was somewhat improved in the biofloc treatments, indicating active metabolism and mineralization of bones. Notably, urea levels were moderately increased in T1 but normalized in T2, whereas creatinine and bilirubin were not significantly different among the different groups, signifying no kidney dysfunction (Yamagata et al., 2019). These biochemical results reveal enhanced metabolic efficiency, lowered stress, and balanced organ function under biofloc conditions.

The cumulative impact on hematological and biochemical parameters suggests that biofloc technology stimulates homeostasis by reducing stress, improving oxygen transport, enhancing immune functions, and maintaining liver and kidney health. This effect is probably due to bioflocs serving both as a means of water-quality stabilization and as a consistent source of naturally balanced protein, vitamins, and bioactive compounds (Ekasari et al., 2014; Liu et al., 2025).

CONCLUSION

This research demonstrates that biofloc technology has significant positive effects on the growth, survival, hematological health, and biochemical stability of *Pangasius pangasius*. Fish reared in biofloc tanks displayed significantly higher growth rates and survival compared to controls. Additionally, they exhibited increased levels of

hemoglobin and platelets, along with lower white blood cell counts. Serum protein and albumin concentrations were also extensively improved. Reduced glucose and lipid levels indicated lower stress and enhanced energy metabolism. Liver enzymes and renal markers remained within normal physiological ranges, reflecting metabolic stability. In general, biofloc systems create a better physiological environment that boosts immunity, oxygen transport, and nutrient utilization in *P. pangasius*. These findings support the use of biofloc technology as a sustainable method of aquaculture, effectively minimizing feed costs while improving fish health and productivity. Further studies should explore the potential for large-scale application of biofloc technology in *P. pangasius* under fluctuating environmental conditions, as well as its impact on product quality and profitability.

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Table 2: Mean ± SE values and statistical analysis of hematological parameters in *Pangasius pangasius*.

Parameters	Controlled Pond	Mean±SE of Sampling 1 (45 days)		Significance Level		Mean±SE of Sampling 2 (90 days)		Significance Level	
		T1	T2	T1	T2	T1	T2	T1	T2
RBC	2.23±0.05	2.35±0.02	2.44±0.04	(P>0.05)	(P<0.05)	2.48±0.13	2.26±3.22	(P<0.05)	(P>0.05)
Hb	13.74±0.22	14.04±0.17	34.5±0.74	(P>0.05)		13.24±0.54	11.72±0.17	(P<0.05)	
HCT	33.94±1.25	32.7±0.46	34.5±0.74	(P>0.05)		38.98±2.04	35.66±0.48	(P>0.05)	
MCV	154.28±1.11	138.44±0.51	141.58±1.15	(P<0.05)		155.48±0.31	151.26±4.62	(P>0.05)	
MCH	60.52±3.76	60.7±1.40	58.06±1.51	(P<0.05)		52.46±0.83	58.06±1.51	(P>0.05)	
MCHC	39.84±2.41	43.28±1.27	40.96±1.35	(P>0.05)		33.78±0.40	33.46±0.47	(P>0.05)	
WBC	220±1.01	219.3±1.31	212.16±0.82	(P>0.05)	(P<0.05)	205.24±5.94	191.18±3.22	(P<0.05)	(P<0.05)
PLT	8.8±2.26	23±0.44	18.2±0.37	(P<0.05)		16.8±1.15	30.4±6.38	(P<0.05)	

RBC, Red Blood Cells; Hb, Hemoglobin; HCT, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; WBC, White Blood Cells; PLT, Platelets.

*T1= Treatment 1, *T2= Treatment 2, *(P>0.05) = Non-Significant, *(P<0.05) = Significant.

Table 3: Mean ± SE values and statistical analysis of serum parameters in *Pangasius pangasius*.

Parameters	Controlled Pond	Mean±SE of Sampling 1 (45 days)		Significance Level		Mean±SE of Sampling 2 (90 days)		Significance Level	
		T1	T2	T1	T2	T1	T2	T1	T2
Glucose	129.8±2.2	41.6±10.1	85.6±5.67	(P<0.05)		42.4±10.2	86.6±5.53	(P<0.05)	
Total Protein	4.92±0.03	5.2±0.14	5.32±0.12	(P>0.05)	(P<0.05)	4.4±0.07	3.88±0.08	(P<0.05)	
Albumin	1.82±0.03	2.58±0.05	2.62±0.05	(P<0.05)		1.14±0.05	1.04±0.02	(P<0.05)	
Cholesterol	235.8±4.14	225.6±10.94	213.6±3.37	(P>0.05)	(P<0.05)	293.2±2.85	202.4±6.78	(P<0.05)	
Triglycerides	1104±9.85	611±1.73	647.4±4.65	(P<0.05)		508.8±49.94	1066.6±74.06	(P<0.05)	(P>0.05)
ALT	15±0.44	13±1.87	11.4±0.92	(P>0.05)	(P<0.05)	14.6±1.63	12±0.70	(P>0.05)	(P<0.05)
AST	195.6±16.07	230.2±3.07	202±13.87	(P>0.05)		595.8±63.49	159.6±9.05	(P<0.05)	(P>0.05)
ALP	68.2±1.42	83.6±1.43	116.8±2.08	(P<0.05)		86±1.41	5.32±0.12	(P<0.05)	
Urea	14±0.70	15.8±0.37	13±0.70	(P<0.05)		17±0.44	14.8±0.58	(P<0.05)	(P>0.05)
Creatinine	0.4±0.07	0.4±0.1	0.32±0.03	(P>0.05)		0.5±0.04	0.42±0.03	(P>0.05)	
Bilirubin	0.34±0.05	0.32±0.03	0.4±0.07	(P>0.05)		0.42±0.03	0.5±0.07	(P>0.05)	

ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase

*T1= Treatment 1, *T2= Treatment 2, *(P>0.05) = Non-Significant, *(P<0.05) = Significant