



Probiotic-Enriched Biofloc Enhances Water Quality, Growth, Feed Utilisation, and Disease Resistance of Rohu (*Labeo rohita*) at Different Stocking Densities under Indoor Conditions

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Abstract

This study evaluated the effects of probiotic-enriched biofloc technology (BFT) on water quality, proximate composition of biofloc, growth performance, feed utilisation, and disease resistance of rohu (*Labeo rohita*) cultured at different stocking densities under indoor conditions. A 2 × 3 factorial design was employed with two culture systems—biofloc with probiotics (BP) and biofloc without probiotics (BWP), and three stocking densities (20, 23, and 26 fish m⁻²), each in triplicate, for 90 days using fish with an initial average weight of 3.06 ± 0.15 g. Proximate composition of biofloc revealed significantly higher crude protein (33.42 ± 0.94% vs. 31.38 ± 1.05%), crude fat (6.68 ± 0.47% vs. 5.34 ± 0.32%), and crude fibre (3.83 ± 0.05% vs. 2.89 ± 0.10%) in BP compared to BWP ($p < 0.05$). Water quality remained stable, with ammonia levels within safe limits (0.01–0.02 ± 0.01 mg L⁻¹) and higher dissolved oxygen concentrations (7.13–7.38 ± 0.31 mg L⁻¹) in BP groups. Growth performance was markedly enhanced in BP treatments, with final body weight (123.37 ± 3.39 g vs. 57.70 ± 2.26 g), specific growth rate (0.95–0.99 ± 0.02% d⁻¹ vs. 0.45–0.54 ± 0.01% d⁻¹), survival (73.33 ± 1.45% vs. 26.67 ± 1.76%), and feed conversion ratio (2.21–2.83 vs. 3.20–4.50) showing significant differences ($p < 0.05$).

Following intraperitoneal challenge with *Aeromonas hydrophila*, histopathological examination further confirmed the protective role of probiotics, with BP fish exhibiting preserved intestinal villi, reduced goblet cell hyperplasia, and limited renal tubular degeneration and inflammatory infiltration, whereas BWP fish displayed villus erosion, bacterial colonisation, tubular necrosis, and glomerular dilation. Overall, probiotic-enriched biofloc improved biofloc nutritive value, stabilised water quality, enhanced growth and feed utilisation, and provided effective protection against *A. hydrophila* challenge. These findings highlight probiotic biofloc as a sustainable and cost-effective strategy for intensifying freshwater aquaculture while improving fish health, renal integrity, and overall resilience.

Keywords: Biofloc technology, probiotics, *Labeo rohita*, growth performance, water quality, feed utilisation, histology

Introduction

Aquaculture continues to expand globally as a vital source of animal protein and income, with total fisheries and aquaculture production reaching over 223 million tonnes in 2022 (FAO, 2024). In India, freshwater aquaculture is dominated by Indian major carps (IMC), namely Catla (*Catla catla*), Rohu (*L. rohita*), and Mrigal (*Cirrhinus mrigala*), accounting for nearly 70–75% of inland aquaculture production (Department of Fisheries, 2024; FAO, 2024). Among these, rohu holds prominence, contributing approximately 35–40% of IMC output (FAO, 2004). However, intensification in carp farming presents chal-

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lenges such as deteriorating water quality, high feed costs, and frequent disease outbreaks, which impose significant economic burdens on farmers.

A major bottleneck in intensive aquaculture is the cost of high-protein feed, which typically constitutes the largest portion of input costs. In systems with high stocking density, water quality degrades rapidly, leading to stress and pathogen proliferation. Conventional mitigation via frequent water exchange and antimicrobial use is expensive, unsustainable, and environmentally detrimental. Thus, there is increasing interest in eco-friendly systems that economise feed use, improve water quality, and boost immune function of cultured species.

Biofloc technology (BFT) is an emerging, sustainable aquaculture approach that integrates the microbial processing of wastes with *in situ* production of natural food. In biofloc systems, organic wastes (uneaten feed, faeces) are assimilated by heterotrophic bacteria when a proper carbon-to-nitrogen (C:N) ratio is maintained, forming aggregates ("flocs") containing bacteria, algae, protozoa, and organic particles (Avnimelech, 1999; Crab, Defoirdt, Bossier, & Verstraete, 2012). These flocs act as both a biofilter, reducing nitrogenous waste, and as an additional feed source, thereby reducing reliance on high-cost external protein inputs (Hargreaves, 2006; Wasielesky, Atwood, Stokes, & Browdy, 2006). Recent reviews confirm that BFT can substantially improve water quality, enhance feed conversion, and lower water treatment costs (Khanjani, Sharifinia, Akhavan-Bahabadi, & Emerenciano, 2024; Matishov et al., 2025).

Beyond nutrient recycling, the microbial communities within bioflocs can positively influence the health of cultured animals. Bioflocs have been shown to provide probiotic-like benefits, modulating gut microbiota, improving immune responses, and enhancing disease resistance (Yang et al., 2012; Khanjani et al., 2024). Integration of BFT with other systems, such as biofilms, aquaponics, or recirculating aquaculture systems, has been proposed to overcome limitations (e.g., solid accumulation, oxygen demand) and improve scalability (Li, Ge, Dai, & Chen, 2025).

To further augment the benefits of BFT, incorporation of single- or multi-species probiotics has been explored. Probiotics can enhance digestive enzyme activity, antioxidant defense, and immune gene

expression in fish, thereby improving growth performance and resistance to pathogens (Sahu, Swarnakumar, Sivakumar, Thangaradjou, & Kannan, 2008; Amenyogbe et al., 2020; Mohammed & Pál, 2025). In fish, probiotic supplementation has been associated with higher weight gain, better feed conversion ratio, and stronger innate immune markers (Hoseinifar, Sun, Wang, & Zhou, 2018). Combined approaches, such as probiotic-enriched bioflocs, show promise in further boosting productivity and health of cultured species (Oliveira, Pilz, Furtado, Ballester, & Bicudo, 2021).

Rohu, being a column feeder and commonly stocked species, is susceptible to stress and disease in intensive culture systems. Enhancing its resilience is crucial in reducing economic losses and antibiotic use in Indian carp farming systems. Given water scarcity and environmental constraints, indoor biofloc systems with minimal water exchange represent a promising strategy. In view of this, the present study was designed to evaluate the growth, survival, feed efficiency, and histopathological responses following bacterial challenge of rohu cultured in indoor biofloc systems under varying stocking densities and probiotic interventions. The findings aim to shed light on the feasibility and effectiveness of biofloc-based approaches for sustainable rohu aquaculture in India.

Several previous studies have specifically evaluated the application of biofloc technology and probiotics in *L. rohita*. For instance, biofloc-based systems have been reported to improve growth performance, feed utilisation, and nitrogen metabolism in rohu culture systems (Kamilya et al., 2017; Das et al., 2024). Probiotic supplementation in carp species has also been shown to enhance digestive enzymes activity, gut health, and resistance to bacterial infections (Wang, 2011). Earlier foundational studies on biofloc technology (Avnimelech, 1999; Hargreaves, 2006) provide the basis for understanding microbial dynamics, while more recent studies demonstrate species-specific responses in Indian major carps. Therefore, integrating both classical and recent findings is essential for evaluating the effectiveness of probiotic-enriched biofloc systems in rohu culture.

Materials and Methods

The experiment was conducted under controlled indoor conditions at the Freshwater Fish Farm,

Eguvamitta, SPSR Nellore district, Andhra Pradesh, India. The study aimed to assess the effects of biofloc technology (BFT) with and without probiotic supplementation on growth, feed utilisation, proximate composition of biofloc, water quality, and histological responses of rohu (*L. rohita*) cultured at different stocking densities. A 2×3 factorial design was used with two culture systems—Biofloc with probiotic (BP) and Biofloc without probiotic (BWP)—and three stocking densities (20 fish m^{-2} , 23 fish m^{-2} , and 26 fish m^{-2}). Each treatment was maintained in triplicate, resulting in a total of 18 tanks (2 systems $\times$ 3 stocking densities $\times$ 3 replicates).

Rohu fingerlings with an average initial weight of 3.06 ± 0.15 g were stocked in 100 L circular indoor tanks. Continuous aeration was provided using air blowers to maintain dissolved oxygen and uniform suspension of bioflocs. The culture duration was 90 days.

Biofloc was developed by maintaining a carbon-to-nitrogen (C:N) ratio of 20:1–30:1 using molasses as a carbon source, following Avnimelech (1999). Aeration was applied continuously to prevent floc sedimentation. In probiotic treatments, a commercial blend containing *Bacillus subtilis*, *Lactobacillus* sp., and *Saccharomyces cerevisiae*, with a minimum concentration of 1×10^9 CFU g^{-1} (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used. The probiotic was applied at a dose of 1 g per 100 L of water on a weekly basis. The probiotic was dissolved in distilled water and evenly applied to culture tanks to ensure uniform distribution. Minimal water exchange was practiced, limited to replacing evaporative losses.

Fish were fed a pelleted diet containing 30–32% crude protein at 5% body weight per day, provided in two equal rations (09:00 h and 17:00 h). Feed amounts were adjusted fortnightly based on sampling. Biofloc samples were collected from each tank, pooled per treatment, and analysed for proximate composition (crude protein, crude fat, crude fibre, crude ash, and moisture) according to AOAC (2000).

Water quality parameters were monitored weekly. The pH, dissolved oxygen (DO), ammonia ($\text{NH}_3\text{-N}$), nitrite (NO_2^-), nitrate (NO_3^-), total alkalinity, and biochemical oxygen demand (BOD) were measured following APHA (2012).

Growth and feed utilisation were evaluated using

the following formulas:

- Weight Gain (WG, g) = Final weight – Initial weight
- Survival (%) = (Final number of fish / Initial number of fish) $\times$ 100
- Feed Conversion Ratio (FCR) = Feed intake (g) / Weight gain (g)
- Feed Efficiency Ratio (FER) = Weight gain (g) / Feed intake (g)
- Protein Efficiency Ratio (PER) = Weight gain (g) / Protein intake (g)
- Specific Growth Rate (SGR, % day^{-1}) = $[(\ln \text{Final weight} - \ln \text{Initial weight}) / \text{Days}] \times 100$

A challenge test was conducted using *A. hydrophila* at a dose of approximately 1×10^6 CFU fish $^{-1}$, based on previous pathogenicity studies (Austin & Austin, 2007). Fish were observed for 7 days post-injection, and samples were collected at the end of the observation period for histological analysis. Intestine and kidney tissues were excised, rinsed in physiological saline, and fixed in 10% neutral buffered formalin for 24–48 h. Samples were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were prepared using a rotary microtome (Leica RM 2255, India) and stained with hematoxylin and eosin (H&E) following Bancroft and Gamble (2008). Slides were examined under a compound microscope (Leica Microsystems, Germany), and photomicrographs were captured using a digital imaging system. Histological alterations were graded as mild, moderate, or severe according to standardised scoring protocols (Roberts, 2012).

All results were expressed as mean $\pm$ standard error of mean (SEM). Two-way ANOVA was used to determine the effects of culture system (BP vs. BWP), stocking density, and their interaction. Significant differences between means were identified using Duncan's multiple range test. Statistical significance was set at $p < 0.05$. Analyses were performed using SPSS v.22 (IBM Corp., Armonk, NY, USA).

Results and Discussion

The proximate composition of biofloc differed significantly between treatments (Table 1). Biofloc with probiotic supplementation showed higher

crude protein ($33.42 \pm 0.94\%$) compared with the biofloc without probiotic ($31.38 \pm 1.05\%$) ($p = 0.003$). Similarly, crude fat, crude fibre, crude ash, and moisture content were all significantly higher in probiotic-based biofloc than in the control system ($p < 0.05$).

In the present study, the proximate analysis of biofloc revealed that crude protein was 6.5% higher in biofloc with probiotic (BP) compared to biofloc without probiotic (BWP). Similarly, crude fat and crude fibre contents were enhanced by approximately 20–25% in BP systems. This indicates that the addition of probiotics played a central role in enriching the nutritive quality of biofloc. Probiotic strains such as *B. subtilis* and *Lactobacillus* spp. are known to accelerate the assimilation of nitrogen and organic carbon into microbial biomass, thereby increasing the protein content of the floc (Avnimelech, 1999; Das et al., 2024). Comparable improvements were reported in rohu fry, where daily probiotic application in biofloc systems resulted in significantly higher protein retention and growth (Das et al., 2024). Similarly, Hosain et al. (2025) observed a 12–15% increase in protein content in *Macrobrachium rosenbergii* post-larvae reared in higher biofloc volumes compared to control treatments.

The higher crude fibre content in BP treatments may be attributed to extracellular polymeric substances (EPS), cell wall polysaccharides from bacteria, and algal biomass embedded within mature flocs (Emerenciano, Martínez-Córdova, Martínez-Porchas, & Miranda-Baeza, 2017). Fibre fractions in biofloc play an important role in modulating gut health by stimulating mucosal protection and peristaltic activity. El-Haroun, Goda, and Chowdhury (2006) reported that Nile tilapia cultured in probiotic biofloc exhibited significantly longer intestinal villi (+18%) and greater goblet cell density, indicating

enhanced gut absorptive and protective capacity. This suggests that structural and functional improvements in the gastrointestinal tract may have improved the nutrient absorption and growth performance observed in BP systems. Thus, the enriched proximate composition of probiotic biofloc not only provides an additional protein source contributing up to 20–30% of dietary needs (Avnimelech, 2012) but also enhances digestibility and immunity, thereby reducing dependence on costly formulated feeds.

Water quality remained within acceptable ranges throughout the culture period (Table 2). Dissolved oxygen (DO) was significantly higher in biofloc with probiotic systems compared to without ($p = 0.035$). BOD values were also significantly affected by both culture system and stocking density ($p < 0.001$). No significant differences were observed in pH, ammonia (NH_3), nitrite ($\text{NO}_2\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), or alkalinity between treatments ($p > 0.05$).

Water quality parameters remained within the acceptable range for carp culture; however, probiotic-supplemented biofloc (BP) consistently maintained lower levels of toxic nitrogen metabolites compared to BWP. Although ammonia levels remained within safe limits and did not differ significantly between treatments, probiotic biofloc systems helped maintain stable nitrogen dynamics under intensive culture conditions (Kamilya et al., 2017). This reduction is primarily attributed to heterotrophic microbial assimilation, which converts inorganic nitrogen into microbial protein rather than relying solely on nitrification. Ghimire, Ghimire, Karki, Basyal, and Rai (2023) demonstrated that maintaining a C:N ratio of 20–25:1 in carp biofloc systems reduced total ammonia nitrogen (TAN) by 40–60% compared to control systems without carbon supplementation. The results of this study support those

Table 1. Proximate composition of biofloc with probiotic and without probiotic

Parameter	Biofloc with probiotic	Biofloc without probiotic	<i>p</i> -value
Crude Protein (%)	33.42 ± 0.94^a	31.38 ± 1.05^b	0.003
Crude Fat (%)	6.68 ± 0.47^a	5.34 ± 0.32^b	0.036
Crude Fibre (%)	3.83 ± 0.05^a	2.89 ± 0.10^b	0.003
Crude Ash (%)	0.62 ± 0.02^a	0.49 ± 0.04^b	0.030
Moisture (%)	12.19 ± 0.88^a	11.05 ± 0.82^b	0.025

Values were expressed as (mean $\pm$ SEM). Different superscripts in the same row indicate significant differences ($p < 0.05$).

findings, indicating that probiotics further stimulate microbial growth and nitrogen immobilisation.

Dissolved oxygen (DO) levels in BP tanks remained 0.5–0.7 mg L⁻¹ higher than BWP, which may be linked to better microbial turnover, lower organic sludge accumulation, and more efficient respiratory dynamics. Similar results were reported in *Clarias gariepinus*, where probiotic biofloc maintained DO at 6.8–7.4 mg L⁻¹, compared to 5.9–6.2 mg L⁻¹ in control tanks (Ademola & Batagarawa, 2024). Although biochemical oxygen demand (BOD) was slightly higher in BP groups (4.2–5.0 mg L⁻¹) relative to BWP (3.2–3.9 mg L⁻¹), this increase reflects enhanced microbial respiration due to greater floc biomass. Importantly, continuous aeration prevented any hypoxic stress. Xu, Pan, Sun, and Huang (2013) similarly reported that probiotic-supplemented biofloc systems maintained DO and kept nitrite-N below critical thresholds despite elevated BOD, highlighting the importance of strong aeration management. Therefore, by regulating nitrogen cycling and stabilising DO, probiotic biofloc systems not only sustain optimal environmental conditions but also reduce the risk of fish mortality due to toxic metabolite buildup, particularly under intensive stocking conditions.

Growth parameters were significantly higher in the biofloc with probiotic groups than in the without-probiotic group (Table 3). Final weight, weight gain,

survival, FCR, FER, PER, and SGR all showed significant improvements in BP treatments. Among treatments, higher stocking density combined with probiotic supplementation yielded the best performance, whereas BWP groups exhibited comparatively reduced growth and survival.

Survival rate was consistently higher in BP treatments (73–76%) compared to BWP (27–46%). Feed conversion ratio (FCR) was markedly improved in BP systems (2.21–2.83) compared to BWP (3.20–4.50). Specific growth rate (SGR) followed a similar trend, ranging from 0.95–0.99 % day⁻¹ in BP groups compared to 0.45–0.54 % day⁻¹ in BWP.

Growth performance of rohu in BP systems was significantly higher than in BWP across all stocking densities. Final body weight in BP-SD3 (26 fish m⁻²) reached 123.4 g, which was more than 2-fold higher than in BWP-SD3 (57.7 g). Specific growth rate (SGR) in BP treatments ranged from 0.95–0.99 % day⁻¹, while in BWP groups it was only 0.45–0.54 % day⁻¹, representing ~75–100% improvement. These gains are consistent with studies in Nile tilapia, where probiotic biofloc systems increased SGR by 0.3–0.4 % day⁻¹ compared to control systems (El-Haroun et al., 2006).

Feed conversion ratio (FCR) also demonstrated striking differences. FCR values in BP treatments

Table 2. Water quality parameters in biofloc with probiotic (BP) and biofloc without probiotic (BWP) systems at different stocking densities

Parameter	Experimental groups						Two-way ANOVA		
	BP-SD1 (20/m ²)	BP-SD2 (23/m ²)	BP-SD3 (26/m ²)	BWP-SD1 (20/m ²)	BWP-SD2 (23/m ²)	BWP-SD3 (26/m ²)	System	Stocking density	System × SD
pH	7.95 ± 0.06 ^{ab}	7.76 ± 0.06 ^{ab}	8.07 ± 0.37 ^a	7.67 ± 0.11 ^b	7.74 ± 0.14 ^{ab}	7.91 ± 0.20 ^{ab}	0.123	0.130	0.534
DO (mg L ⁻¹)	7.13 ± 0.24 ^a	7.38 ± 0.31 ^a	7.25 ± 0.56 ^a	7.25 ± 0.25 ^a	6.75 ± 0.56 ^{ab}	6.25 ± 0.14 ^b	0.035	0.257	0.131
NH ₃ -N (mg L ⁻¹)	0.02 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.00 ^a	0.493	0.619	0.619
NO ₃ -N (mg L ⁻¹)	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.00 ± 0.00 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.454	0.564	0.564
NO ₂ -N (mg L ⁻¹)	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.00 ± 0.00 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.454	0.564	0.564
Total alkalinity (mg L ⁻¹)	171.50 ± 5.84 ^a	169.00 ± 7.04 ^a	161.25 ± 6.68 ^a	179.50 ± 5.04 ^a	171.25 ± 7.18 ^a	168.25 ± 6.19 ^a	0.291	0.279	0.891
BOD (mg L ⁻¹)	5.00 ± 0.17 ^a	4.23 ± 0.36 ^b	4.18 ± 0.39 ^{bc}	3.60 ± 0.29 ^{cd}	3.24 ± 0.38 ^d	3.98 ± 0.19 ^{bc}	<0.001	0.033	0.023

Values were expressed as (mean ± SEM). Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 3. Growth performance and feed utilization of rohu cultured in biofloc with probiotic (BP) and without probiotic (BWP) systems at different stocking densities

Parameter	Experimental groups						Two-way ANOVA		
	BP-SD1 (20/m ²)	BP-SD2 (23/m ²)	BP-SD3 (26/m ²)	BWP-SD1 (20/m ²)	BWP-SD2 (23/m ²)	BWP-SD3 (26/m ²)	System	Stocking density	System × SD
Initial weight (g)	3.07 ± 0.11 ^a	3.07 ± 0.13 ^a	3.07 ± 0.10 ^a	3.06 ± 0.19 ^a	3.03 ± 0.43 ^a	3.06 ± 0.21 ^a	0.849	0.995	0.995
Final weight (g)	120.89 ± 2.78 ^a	116.69 ± 4.01 ^a	123.37 ± 3.39 ^a	68.40 ± 2.49 ^b	59.50 ± 2.96 ^{bc}	57.70 ± 2.26 ^c	<0.001	0.135	0.131
Weight gain (g)	117.82 ± 2.67 ^a	113.62 ± 3.88 ^a	120.30 ± 3.28 ^a	65.34 ± 2.38 ^b	56.47 ± 2.71 ^{bc}	54.65 ± 2.14 ^c	<0.001	0.116	0.112
Survival (%)	76.00 ± 0.57 ^a	73.00 ± 0.57 ^a	73.33 ± 1.45 ^a	45.67 ± 1.20 ^b	42.67 ± 2.33 ^b	26.67 ± 1.76 ^c	<0.001	<0.001	<0.001
FCR	2.83 ± 0.24 ^c	2.28 ± 0.28 ^c	2.21 ± 0.29 ^c	3.20 ± 0.45 ^b	3.80 ± 0.52 ^b	4.50 ± 0.60 ^a	<0.001	0.040	0.023
FER	0.36 ± 0.03 ^a	0.45 ± 0.05 ^a	0.47 ± 0.06 ^a	0.15 ± 0.02 ^b	0.11 ± 0.02 ^b	0.06 ± 0.02 ^b	<0.001	0.820	0.073
PER	1.07 ± 0.11 ^a	1.07 ± 0.01 ^a	0.85 ± 0.02 ^{ab}	0.63 ± 0.28 ^b	0.90 ± 0.01 ^{ab}	0.72 ± 0.09 ^{ab}	0.025	0.282	0.409
SGR (% day ⁻¹)	0.99 ± 0.01 ^a	0.95 ± 0.02 ^a	0.99 ± 0.02 ^a	0.54 ± 0.01 ^b	0.47 ± 0.02 ^c	0.45 ± 0.01 ^c	<0.001	0.008	0.023

Values were expressed as (mean ± SEM). Different superscripts in the same row indicate significant differences ($p < 0.05$).

were maintained between 2.2 and 2.8, whereas BWP systems exhibited poorer feed efficiency (3.20–4.50). This indicates that biofloc contributed substantially to protein recycling and feed utilisation efficiency. According to Avnimelech (2012), biofloc biomass can account for 20–25% of daily protein intake, which explains why fish in BP groups required less formulated feed to achieve higher growth. Amenogbe et al. (2020) emphasised that probiotics in aquaculture enhance digestive enzyme activity, gut microbiota balance, and nutrient absorption, all of which synergise with biofloc to improve feed utilisation. Survival rates were also considerably higher in BP groups (73–76%) compared to BWP (27–46%). This indicates that probiotic biofloc not only improves nutrition but also potentially improves health status and stress resistance, as supported by histological observations (Roberts, 2012; Panigrahi, Vinay, Patil, Nithin, & Diwan, 2023). For instance, Banu, Swain, Das, Velmani, and Kumari (2024) observed enhanced protease and amylase activities in biofloc polyculture, which correlated with improved survival and growth of *Puntius gonionotus*.

Stocking density had a significant impact: at higher

densities (26 fish m⁻²), survival in BWP groups dropped to 27%, while in BP it was maintained above 73%, showing that probiotics buffered the density-related stress. Similar density-dependent declines have been observed in catfish and tilapia biofloc systems, where probiotic supplementation improved tolerance to crowding stress by 30–40% compared with controls (Soliman, Alazwari, & Farouk, 2025). Collectively, these results confirm that probiotic biofloc systems can support higher stocking densities while ensuring superior growth, feed efficiency, and survival—critical factors for achieving economic sustainability in freshwater aquaculture.

Histological examination of the intestine of *L. rohita* revealed distinct differences among treatments following challenge with *A. hydrophila* (Fig. 1). Control fish showed normal intestinal morphology with intact villi and mucosal layers, without detectable abnormalities. In the probiotic biofloc (BP) groups, lesions were relatively mild. BP-SD1 fish exhibited mild to moderate infiltration of lymphocytes, macrophages, plasma cells, and fibroblasts in the lamina propria and submucosa. BP-SD2 fish showed localised fusion of intestinal villi, while

BP-SD3 demonstrated mild to moderate infiltration of mononuclear cells and fibroblasts, along with desquamation of villus epithelium and a slight increase in goblet cell density. In contrast, the biofloc without probiotics (BWP) groups displayed more severe intestinal damage. BWP-SD1 fish showed infiltration of inflammatory cells and increased goblet cells along villus epithelium. BWP-SD2 fish revealed infiltration of mononuclear and polymorphonuclear cells with marked goblet cell hyperplasia. BWP-SD3 presented severe infiltration of mononuclear cells within mucosa and submucosa, with abundant bacterial colonisation attached to villus surfaces. Overall, probiotic supplementation mitigated pathological changes by preserving villus integrity, reducing inflammatory infiltration, and

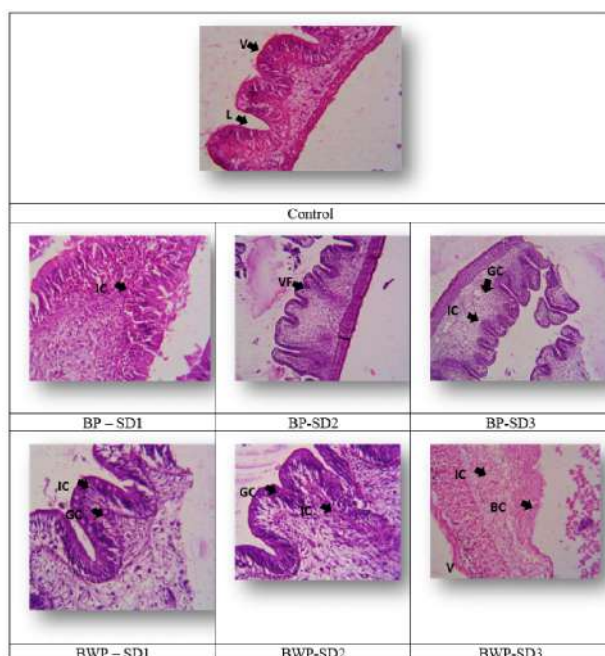


Fig. 1. Histopathological changes observed in the intestine of *Labeo rohita* reared in biofloc with probiotic and biofloc without probiotic at different stocking densities (SD1 = 20 fish m⁻², SD2 = 23 fish m⁻², SD3 = 26 fish m⁻²), following challenge with *A. hydrophila* (H&E stain; 400× magnification; scale bar = 50 µm)

Abbreviations: V = villi; L = lumen; E = epithelial layer; IC = inflammatory cells; GC = goblet cells; VF = villus fusion; ED = epithelial desquamation; BC = bacterial colonisation

moderating goblet cell proliferation compared to non-probiotic biofloc systems.

Kidney histopathology also showed significant

treatment effects (Fig. 2). The control fish displayed normal renal morphology with intact glomeruli and tubular structures. However, fish reared in biofloc systems exhibited varying degrees of renal alteration. In the BP groups, degenerative changes were evident but comparatively less severe than in the BWP treatments. BP-SD1 showed infiltration of inflammatory cells around peritubular and glomerular regions. BP-SD2 exhibited inflammatory foci with associated hemorrhage and tubular necrosis, while BP-SD3 displayed cystic spaces, glomerular atrophy, enlarged peritubular and glomerular spaces, and focal tubular degeneration with necrosis. In the BWP treatments, renal lesions were more pronounced. BWP-SD1 showed extensive inflammation, hemorrhage, and tubular necrosis. BWP-SD2 revealed widespread infiltration of inflammatory cells with enlargement of peritubular and glomerular spaces, whereas BWP-SD3 demonstrated the

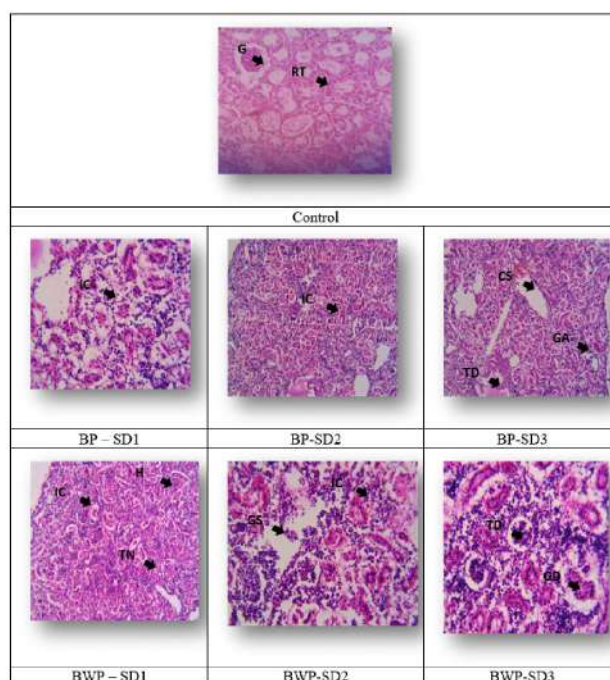


Fig. 2. Histopathological changes observed in the kidney of *Labeo rohita* reared in biofloc with probiotic and biofloc without probiotic at different stocking densities (SD1 = 20 fish m⁻², SD2 = 23 fish m⁻², SD3 = 26 fish m⁻²), following challenge with *A. hydrophila* (H&E stain; 400× magnification; scale bar = 50 µm)

Abbreviations: G = glomerulus; RT = renal tubules; IC = inflammatory cells; TN = tubular necrosis; TD = tubular degeneration; H = hemorrhage; CS = cystic spaces; GA = glomerular atrophy; GD = glomerular dilation; GS = glomerular space enlargement

most severe pathology, characterised by diffuse inflammatory infiltration, tubular degeneration, and marked glomerular dilation. These findings indicate that probiotic biofloc conferred partial renoprotective effects, reducing necrosis and inflammation relative to non-probiotic biofloc systems.

The present study demonstrated that probiotic biofloc (BP) markedly reduced the severity of *A. hydrophila*-induced histopathological alterations in the intestine and kidney of *L. rohita* compared with biofloc without probiotics (BWP). Intestinal lesions in BP groups were limited to mild leukocyte infiltration, partial villus fusion, and slight goblet cell hyperplasia, whereas BWP groups exhibited pronounced villus erosion, severe inflammatory cell infiltration, and bacterial colonisation on villus surfaces. Similarly, renal lesions were less extensive in BP fish, showing limited tubular degeneration and focal necrosis, while BWP fish displayed diffuse necrosis, hemorrhage, and glomerular dilation. These findings indicate that probiotics in biofloc systems exerted both local (intestinal) and systemic (renal) protective effects against *A. hydrophila*.

The preservation of villus morphology in BP groups is consistent with earlier studies reporting that probiotics enhance intestinal integrity, strengthen epithelial barrier function, and reduce pathogen adhesion (Cavalcante et al., 2020). The moderate increase in goblet cell density observed in BP fish likely reflects a beneficial mucogenic adaptation, as mucus secretion is a critical defense against bacterial invasion (El-Haroun et al., 2006). Comparable improvements in gut morphology were reported in Nile tilapia reared in probiotic biofloc systems, where villus height and goblet cell density were significantly improved relative to non-probiotic controls (Asha et al., 2024).

The reduction of renal lesions in BP groups suggests that probiotics contributed to systemic protection by modulating host immune and inflammatory responses. Probiotic supplementation in aquaculture has been shown to maintain renal tubular integrity, suppress oxidative damage, and alleviate inflammatory infiltration through immune modulation and improved metabolic efficiency (Al-Yahya et al., 2018; Ghimire et al., 2023). Recent studies in shrimp and common carp similarly reported that probiotic-amended biofloc systems enhanced kidney structure and function by regulating microbial composition, lowering ammonia toxicity, and reducing pathogen

load (Wang, 2011; Xu & Pan, 2014).

Stocking density also influenced histopathological severity, with higher densities (SD3) inducing greater intestinal and renal damage in both BP and BWP treatments. However, probiotic supplementation consistently alleviated these adverse effects, suggesting that probiotics improve host resilience under intensive rearing conditions. Similar density-probiotic interactions have been reported in catfish and tilapia, where probiotics mitigated but did not fully prevent density-related histopathological stress (Gomes, Andayani, & Yanuhar, 2023; Soliman, Abbass, Badry, Soliman, & Osman, 2025). Overall, the results confirm that probiotics integrated into biofloc systems offer dual advantages of improved water quality and enhanced disease resistance, thereby minimising histopathological damage in vital organs such as the intestine and kidney during bacterial challenge. These findings support the potential of probiotic biofloc as a sustainable approach to enhance fish health and resilience in intensive aquaculture. However, it is important to note that direct immune parameters such as lysozyme activity or respiratory burst were not measured in the present study. Therefore, conclusions regarding immune enhancement are based on histological and survival responses rather than quantitative immune assays.

This study demonstrated that probiotic-enriched biofloc systems significantly improved the proximate composition of biofloc, stabilised water quality, and enhanced growth performance and feed utilisation efficiency in *L. rohita* under indoor conditions. Probiotic supplementation increased the protein and fibre content of biofloc, maintained nitrogenous metabolites within safe limits, and improved survival, FCR, and SGR even under higher stocking densities. Notably, histopathological evaluation revealed that probiotic biofloc mitigated *A. hydrophila*-induced damage in the intestine and kidney by preserving villus structure, reducing goblet cell hyperplasia, and limiting tubular degeneration, necrosis, and inflammatory infiltration. These findings confirm the efficacy of probiotic biofloc technology as a sustainable, cost-effective approach to intensify freshwater aquaculture while simultaneously promoting fish health, renal integrity, and environmental sustainability. Probiotic-enriched biofloc systems improved growth performance, feed utilisation, and histopathological indicators of disease resistance in *L. rohita*.

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