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Effects of Multispecies Probiotics on Growth, Hematology, and Gut Health of Stinging Catfish (*Heteropneustes fossilis*) in Biofloc System

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Abstract: Probiotics are gaining popularity both empirically and scientifically as eco-friendly alternatives in aquaculture. The present research proposed to examine the influences of selective probiotics on the growth, hematology, microbes, and morphology of the intestine of stinging catfish (*Heteropneustes fossilis*) in a biofloc system. Additionally, this study evaluated the benefit–cost ratio (BCR) of specific probiotics used in the biofloc system. Stinging catfish fingerlings (average weight 0.86 ± 0.26 g) were equally distributed (250 fingerlings/400 L water-filled PVC tank) into three treatment groups and reared for 16 weeks. Two commercial probiotics (CP-1 and CP-2) and one laboratory-developed probiotic were used in the study. The results showed significantly higher growth (weight gain and specific growth rate), feed efficiency, improved intestinal microbiota, and enhanced morphology in fish reared with laboratory-developed probiotics in the biofloc system. Moreover, indicators of increased immune responses, such as enhancements in the intestinal mucosal fold, width of enterocytes and lamina propria, and abundance of goblet cells, were also observed in fish reared with laboratory-developed probiotics. The BCR, which determined business profitability, was also highest for the laboratory-developed probiotics in the biofloc system. Therefore, the results suggest that laboratory-developed probiotics are economically viable and environmentally friendly growth stimulators for stinging catfish culture in a biofloc system.

Keywords: aquaculture; hematology; intestinal condition; microbes; probiotics; sustainable aquaculture

1. Introduction

Aquaculture is recognized globally as a rapid means to enhance economic growth and livelihood security [1–3]. However, due to inadequate input quality and suboptimal culture technology, the aquaculture industry has yet to meet the expected demand [4]. Consequently, aquaculture production and productivity vary across different farms [5,6]. Two significant concerns arise in intensive aquaculture. Firstly, there is a decline in water quality due to excessive metabolites and, secondly, poor feed utilization results from frequent water exchanges [5]. Effective management, particularly in terms of water quality,

is crucial for maintaining an optimal growth environment in intensive aquaculture [6]. Approximately 20–30% of feed is absorbed into fish biomass; the remaining 70–80% of feed is deposited as uneaten feed and excreta in the water body [7,8]. The utilization of high-protein feed leads to a subsequent increase in toxic ammonia (NH_3) accumulation at the water body's bottom, posing a threat to aquatic animals [9–11]. In contrast, the biofloc system maintains the water column by using living microorganisms that remove ammonia through processes such as phytoplankton uptake, bacterial assimilation, and nitrification, where bacteria convert ammonia to non-toxic nitrite and then further into nitrate through oxidation [12–14]. Moreover, the protein component costs in commercial diets account for a significant portion of total production expenses in the aquaculture industry [15–17]. Therefore, implementing the biofloc system represents a potential method to reduce production costs in intensive aquaculture [18,19].

Biofloc technology has experienced a substantial surge in popularity in recent years as a relatively new approach in aquaculture. The fundamental principle of this technique involves converting aquaculture waste (ammonia) into microbial biomass, which serves as a valuable food source for cultured organisms [18–20]. Bacteria play a crucial role in this process by utilizing ammonia, leading to the formation of microbial biomass and simultaneous improvement in water quality [21]. The energy required for these operations is derived from the functioning of the "floc" system [22,23]. Additionally, the presence of nutrient-rich feed sources has the potential to reduce the cost and reliance on artificial feed inputs [5]. The utilization of biofloc technology offers several advantages, including enhanced biosecurity, efficient water consumption, improved feed conversion, and effective control over water quality through optimal land utilization and reduced light sensitivity [24]. Notably, biofloc technology ensures the continuous recycling and reused of nutrients, making it a cutting-edge, environmentally responsible, and reliable alternative solution [25,26]. In recent studies, researchers have combined biofloc technology with the incorporation of endogenous probiotic bacteria into the biofloc system, anticipating improved outcomes compared with using each technology independently [27]. Several investigations have reported that the addition of probiotics to the biofloc system resulted in enhanced immunity, and growth and survival rates of the aquatic animals, surpassing the benefits observed when biofloc was used alone [28–31].

Probiotics have emerged as one of the most environmentally friendly feed additives to increase fish production [32–34]. They directly influence water quality by playing a vital role in reducing levels of organic matter, pH, pathogenic bacteria, and hazardous nitrogenous compounds, including ammonia, nitrate, and nitrite. Furthermore, probiotics bring about changes in the microbial population of the water. Indirectly, probiotics contribute to increased growth and survival rates of farmed animals [16,35]. They have also shown promise in preventing several diseases in aquaculture species and improving immune responses [36–39]. Moreover, probiotics enhance the function of several digestive enzymes, thereby increasing nutrient availability and improving feed utilization [40–43]. Probiotics can be administered to fish in different ways, including ingestion, injection, or immersion by directly adding them to the water [44]. The interaction between bacteria in the aquatic environment and the gut microbiota is reciprocal, meaning that they influence each other's composition. Probiotics encompass live or dead microorganisms, microalgae, or yeast; they can be administered orally by mixing with feed or directly into the cultured system to improve growth performance, feed utilization, immunity, disease resistance, and stress responses [45,46].

The *Heteropneustes fossilis*, commonly known as "Singhi," is a stinging catfish [47] species native to the Indian subcontinent [48]. This fish is highly recommended in the diets of individuals who are sick or convalescing due to its high protein and iron content [49]. Additionally, it has gained global popularity due to its therapeutic potential, efficient protein digestibility, delicious taste, and low lipid content [47,50]. However, previous investigations have only focused on the influences of stocking density on *H. fossilis* in biofloc systems [51]; there have been no published reports on the influences of selective

probiotics on growth, health status, or economic viability of *H. fossilis* in biofloc systems. Thus, the current work was designed to examine the effects of selective probiotics on the growth, health status, and economic viability of *H. fossilis* cultured in a biofloc system.

2. Materials and Methods

2.1. Research Ethics Approval

The experimental procedures adhered to the guidelines approved by the Animal Care and Use Committee of Bangladesh Agricultural University, Mymensingh (Approval Number: BAU-FoF/2021/005).

2.2. Experimental Fish

Stinging catfish *H. fossilis* fingerlings were sourced from Sharnalata Agro Fisheries Ltd., Radhakanai, Fulbaria, Mymensingh, Bangladesh with mean initial weight and length 0.861 ± 0.26 g and 5.55 ± 0.48 cm, respectively. The fingerlings were transported to the Laboratory of Fish Ecophysiology, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. To allow them to acclimate to the tank environment, the fingerlings were placed in the tank with a polybag for 6 h. Prior to being released into the tank, the fingerlings underwent a 5 min treatment with salt water to prevent contamination.

2.3. Experimental Design

The experiment used PVC circular tanks with a capacity of 500 L, filled with 400 L of water, divided into three treatments with two replications, spanning a duration of 16 weeks. Three probiotic treatments were employed, consisting of two commercial probiotics (CP-1 and CP-2) and one laboratory-developed probiotic. Prior to usage, the tanks underwent treatment with potassium permanganate and were subsequently sundried for two days. Following this, the tanks were filled with water and limed at a rate of 100 g/1000 L. To maintain the dissolved substances in the tank, raw salts were added at a concentration of 0.5 g/L. One-inch L-shaped PVC pipes were placed at the bottom, forming rows to support the lift aeration system. An aeration system, comprising air stones and water hose pipes, was attached to a 0.50 horsepower (HP) aerator to ensure continuous aeration and optimal water quality for fish and floc preparation. To minimize nitrite and ammonia levels, a 10% water exchange was carried out on a weekly basis. Each tank was stocked with a total of 300 fingerlings; undersized or deformed fish were removed, resulting in a final stocking density of 250 fish in each of the six treated tanks. The fish were fed twice daily, with a feed amount equivalent to 4% of the total body weight. The feed used was a 0.8 mm floating pellet feed with a crude protein content of 38% (Quality Feeds Limited).

2.4. Floc Preparation from Three Selected Probiotics

Commercially available probiotics, namely CP-1 (consisting of *Bacillus licheniformis*, *B. subtillis*, *B. polymyxa*, *B. pumilus*, *B. amyloliquefaciens*, *B. megaterium*, *B. coagulans*, *Aspergillus niger*, and *A. oryzae*) and CP-2 (containing *B. licheniformis*, *B. subtillis*, *B. pumilus*, *B. megaterium*, *Rhodococcus* spp., *Rhodobacter* spp., Nitrosomonas, and Nitrobacter, along with enzymes such as amylase, protease, cellulose, xylanase, and lipase), were chosen for the experiment. In addition to the commercial probiotics, laboratory-developed probiotics consisting of *Bacillus* spp. (isolated from fish) at a concentration of 1×10^9 cfu/mL and *Lactobacillus* spp. (isolated from yogurt) at a concentration of 1×10^{11} cfu/mL were also included. These three probiotics were prepared using different methods, as illustrated in Figure 1. The recommended quantities of powdered and liquid probiotics were separately added to a bucket and carefully mixed with water to prevent cross-contamination.

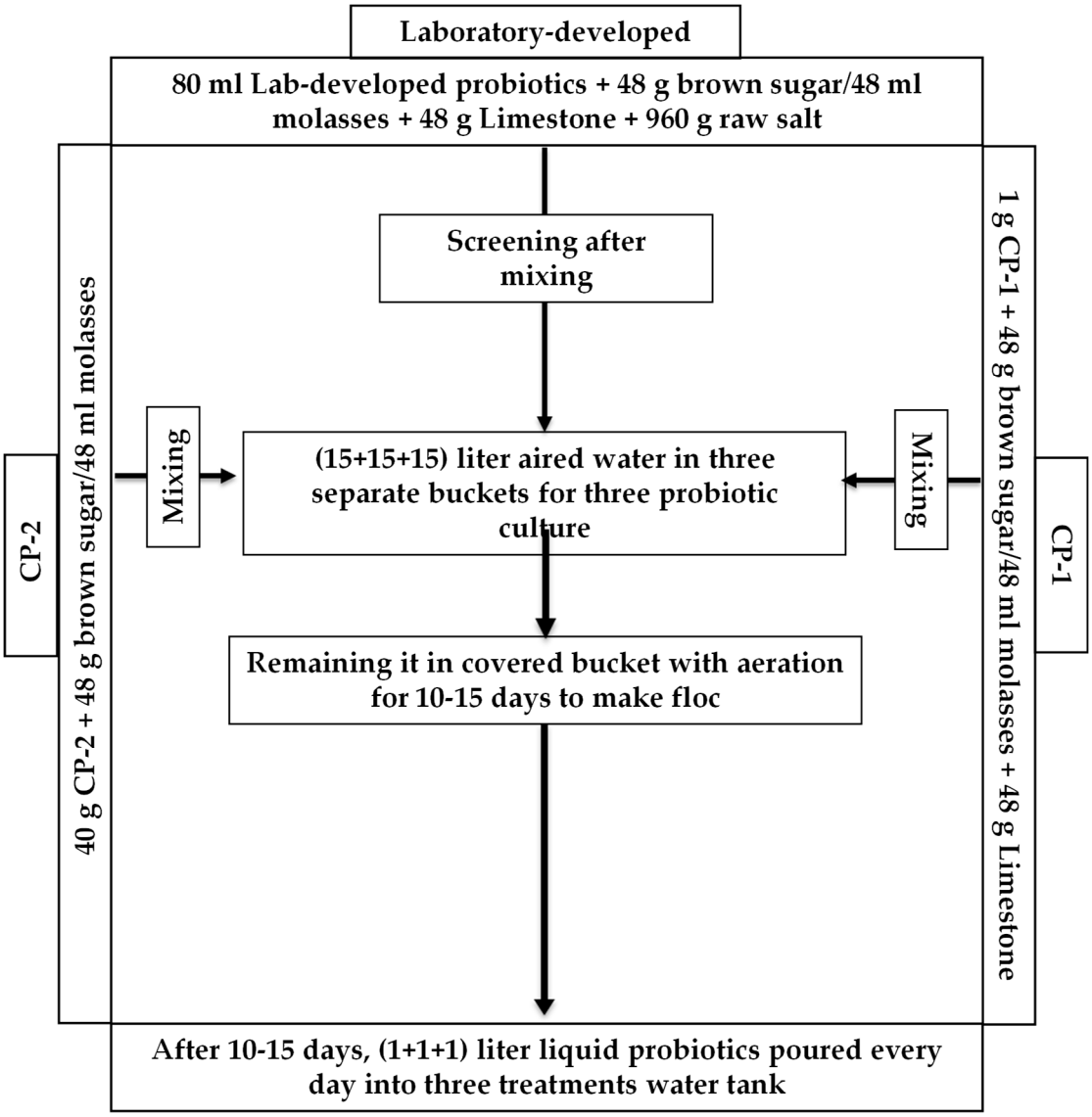

Figure 1. Floc preparation from three selected probiotics.

To measure the floc quantity, a 1 L cylinder-shaped glass bottle was filled with tank water and allowed to settle for 30 min, enabling the floc to settle below the marked scale. At 15-day intervals, the volume of the floc was measured; if it exceeded 30 mL, a 20% water exchange was conducted. In order to maintain floc volume and ammonia levels in the tanks, molasses were added daily as a source of carbon. Specifically, we used 0.125 mL of molasses per liter of water when the ammonia levels were at 2 ppm, 0.0625 mL/L for 1 ppm, 0.03125 mL/L for 0.5 ppm, and 0.0156 mL/L for 0.25 ppm. These amounts were determined through multiple tests conducted throughout the experiment to standardize the process.

2.5. Monitoring of Water Quality Parameters

A thermometer, portable dissolved oxygen (DO) meter (Lutron D5510, Taiwan), pH meter (Hanna 981,017, USA), titration, and ammonia testing kits (API Ammonia Test) were used regularly to monitor temperature, DO, pH, alkalinity, and ammonia, respectively.

2.6. Growth, Survival, and Feed Utilization

After 16 weeks of probiotic treatment, the total biomass, individual length, and weight of fish were determined from each treated tank. Growth parameters such as weight gain

(WG), percentage weight gain (%WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival were calculated using the following formulas:

i. Weight gain = Final body weight − Initial body weight.
ii. Specific growth rate, SGR (%/day) = (ln final weight-ln initial weight)/(Number of days reared) × 100.
iii. Feed conversion ratio, FCR = Dry feed fed (g)/Live weight gain (g).
iv. Survival (%) = (Number of fish harvested)/(Number of fish stocked) × 100.

2.7. Hematological Parameters

Six fish were sacrificed from each treatment at the end of the trials to gather the blood samples. A heparinized plastic syringe was utilized to obtain the blood samples from the caudal vein region to measure glucose (Glu; mg/dL) and hemoglobin (Hb; g/dL). Digital EasyTouch®GCHb (Model ET232, Glu/Hb double monitoring system, Bioptic Technology Inc. Taiwan 35,057) was utilized to determine Hb and Glu separately using hemoglobin and glucose strips.

2.8. Intestinal Microbiota Assessment

At the end of the experiment, six fish were chosen from each treatment to determine the total viable count (TVC) and the lactic acid bacteria (LAB) present in the intestine. This was performed using the single plate serial dilution spotting (SP-SDS) method following the procedures reported by Thomas et al. [52]. For the TVC and LAB count, plate count agar (Hi media, Thane, India), MRS agar (De Man, Rogosa, Bonnybridge, UK), and Sharpe (Hi media, Thane, India) were utilized. The results were expressed as colony-forming units per gram (cfu/g).

2.9. Histology of Intestine

After 16 weeks of rearing, six fish from each treatment were sampled for histological examination of the intestine, as described in previous studies [53,54]. Briefly, the preserved fixed intestinal tissues were subjected to a graded alcohol series and embedded in molten wax. Using a rotary microtome, the blocks were cut into sections with a thickness of 5 µm. The prepared sections were stained with hematoxylin–eosin and the intestinal morphology parameters were observed under a microscope (MCX100, Micros Austria, Gewerbezone, Austria).

2.10. Benefit–Cost Ratio (BCR)

The benefit–cost ratio analysis was performed by calculating the present value of benefits divided by the cost and investment of a system. The calculation of the cost profit for the culture system involved using the following formulas:

$$BCR = TR/TC$$

where TR is total revenue; TC is total cost.

$$TR = \text{Quantity of fish produced (Kg)} \times \text{Price/unit}$$

$$TC = \text{Fixed cost} + \text{Variable cost}$$

2.11. Statistical Analysis

The data collected throughout the experimental period were recorded, stored, and then analyzed using PASW statistical software (Version: 18.0; IBM SPSS Statistics, IBM, Chicago, IL, USA). The presented data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to determine the significant differences ($p < 0.05$) among the treatments. In addition, the Tukey test was employed to identify differences between treatments. The morphological analysis of the intestine was

carried out using an image processing analytical software program (Sigma Scan Pro5, SPSS INC), following the methodology described by Bullerwell et al. [55].

3. Results

3.1. Survival, Growth, and Feed Conversion Ratio

The growth performance data (including WG and SGR), FCR, and survival are presented in Table 1. The laboratory-developed probiotics exhibited the highest SGR and WG, while the CP-1 in the biofloc system showed the lowest SGR and WG. Significantly better feed efficiency was observed in the laboratory-developed probiotics ($p < 0.05$). When comparing the commercial probiotics (CP-1 and CP-2), CP-2 demonstrated better growth and feed utilization, which was noteworthy among all three probiotics. However, no significant differences were observed in survival rates among the probiotic-treated biofloc systems.

Table 1. Growth performance of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Parameters	Probiotics		
	CP-1	CP-2	Laboratory-Developed
Initial BW (g)	0.72 ± 0.10	0.72 ± 0.09	0.74 ± 0.08
Final BW (g)	22.13 ± 2.45 ^{b}	29.50 ± 5.01 ^{ab}	34.71 ± 4.30 ^{a}
Weight gain (g)	21.42 ± 2.40 ^{b}	28.77 ± 4.99 ^{ab}	33.97 ± 4.28 ^{a}
SGR (%/day)	1.72 ± 0.06 ^{b}	1.86 ± 0.09 ^{ab}	1.95 ± 0.07 ^{a}
FCR	1.15 ± 0.03 ^{b}	0.92 ± 0.07 ^{ab}	0.76 ± 0.06 ^{a}
Survival (%)	98.57 ± 0.29	99.12 ± 0.43	99.71 ± 0.15

Note(s): CP—commercial probiotics, BW—body weight, SGR—specific growth rate, FCR—feed conversion ratio. Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among probiotics. All values are expressed as mean ± SD ($n = 60$).

3.2. Hematological Parameters

The hematological parameters, i.e., Hb and Glu levels of stinging catfish (*H. fossilis*), are given in Figure 2. The highest and lowest values of Hb were noted in laboratory-developed probiotics and CP-1, respectively ($p < 0.05$). The glucose level was highest ($p < 0.05$) in CP-1.

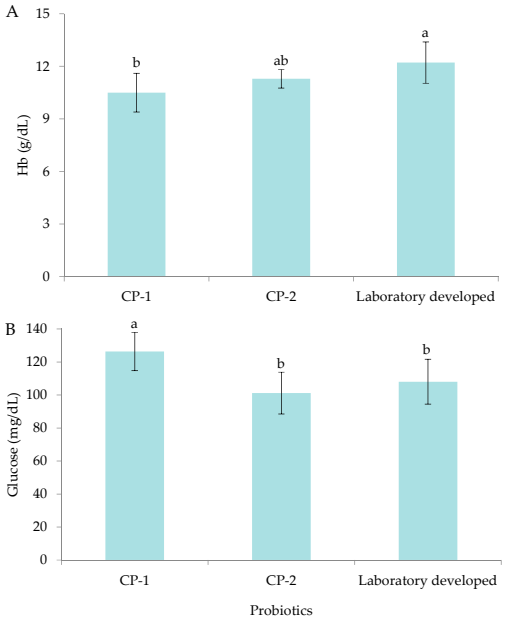

Figure 2. Changes in hemoglobin (**A**) and glucose (**B**) of stinging catfish *H. fossilis* reared in biofloc for 16 weeks. Bars with different superscripts differ significantly ($p < 0.05$) among probiotics. All values are expressed as mean ± SD (n = 6).

3.3. Changes in Intestinal Microbiota

TVC and LAB were determined to observe the probiotics' effects on the gut microbiota of *H. fossilis* (Table 2). Greater ($p < 0.01$) values of TVC and LAB were found in the intestines of fish treated with CP-2 and laboratory-developed probiotics compared with the fish treated with CP-1 probiotics in the biofloc system.

Table 2. Total viable count (TVC) and lactic acid bacteria (LAB) in the gut of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Parameters	Probiotics		
	CP-1	CP-2	Laboratory-Developed
TVC ($\times 10^7$ cfu/g of gut)	1.62 ± 0.38^{b}	171.50 ± 16.71^{a}	95.36 ± 2.93^{a}
LAB ($\times 10^3$ cfu/g of gut)	2.09 ± 0.33^{b}	19.20 ± 0.08^{a}	30.30 ± 11.22^{a}

Note(s): Values with different alphabetical superscripts in a row differ significantly ($p < 0.01$) among probiotics. All values are expressed as mean ± SD (n = 6).

3.4. Changes in Intestinal Histo-Morphology

Significantly higher ($p < 0.05$) values of the villus length, area, width, crypt depth, muscular thickness, and wall thickness of the intestine were noted in fish treated with laboratory-developed probiotics compared with fish treated with the other probiotics used in the biofloc system (Figure 3 and Table 3).

Table 3. Changes in gut morphology of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Parameters	Probiotics Used in Biofloc System		
	CP-1	CP-2	Laboratory-Developed
Villus length (μm)	188.75 ± 11.89^{b}	204.25 ± 4.81^{ab}	225.00 ± 4.61^{a}
Villus width (μm)	57.00 ± 9.4^{b}	47.00 ± 2.86^{ab}	72.75 ± 3.25^{a}
Villus area (mm^2)	10.77 ± 1.99^{b}	9.60 ± 0.65^{ab}	16.37 ± 0.74^{a}
Crypt depth (μm)	77.5 ± 10.35^{b}	91.75 ± 5.97^{ab}	105.75 ± 5.29^{a}
Thickness of wall (μm)	5.00 ± 1.48^{b}	9.00 ± 0.74^{ab}	13.50 ± 1.17^{a}
Thickness of muscular (μm)	9.75 ± 0.87^{b}	14.50 ± 1.17^{ab}	21.75 ± 2.01^{a}

Note(s): Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among probiotics. All values are expressed as mean ± SD (n = 6).

Additionally, alterations in numerous immune response indicators of the histological gut of *H. fossilis* reared in biofloc systems and treated with multispecies probiotics are presented in Figure 4 and Table 4. The indicators included mucosal fold, goblet cells, lamina propria width, and enterocyte width. Greater ($p < 0.05$) values of these immune response indicators were observed in the fish group reared with laboratory-developed probiotics in a biofloc system. However, concerning the intestinal morphological study and immune response indicators, acceptable performance was observed in fish treated with CP-2.

Table 4. Immune response indicators of the histological gut of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Parameters	Probiotics Used In The Biofloc System		
	CP-1	CP-2	Laboratory-Developed
Thickness of mucosal fold (μm)	10.25 ± 2.26^{b}	17.00 ± 1.65^{ab}	28.25 ± 2.01^{a}
Abundance of goblet cells (GB)	35.66 ± 4.42^{b}	44.33 ± 6.75^{ab}	80.92 ± 8.00^{a}
Lamina propria width (μm)	5.00 ± 1.28^{b}	7.50 ± 1.17^{ab}	11.50 ± 1.17^{a}
Enterocyte width (μm)	3.50 ± 0.52^{b}	4.75 ± 0.87^{ab}	7.25 ± 0.86^{a}

Note(s): Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among different probiotics. All values are expressed as mean ± SD (n = 6).

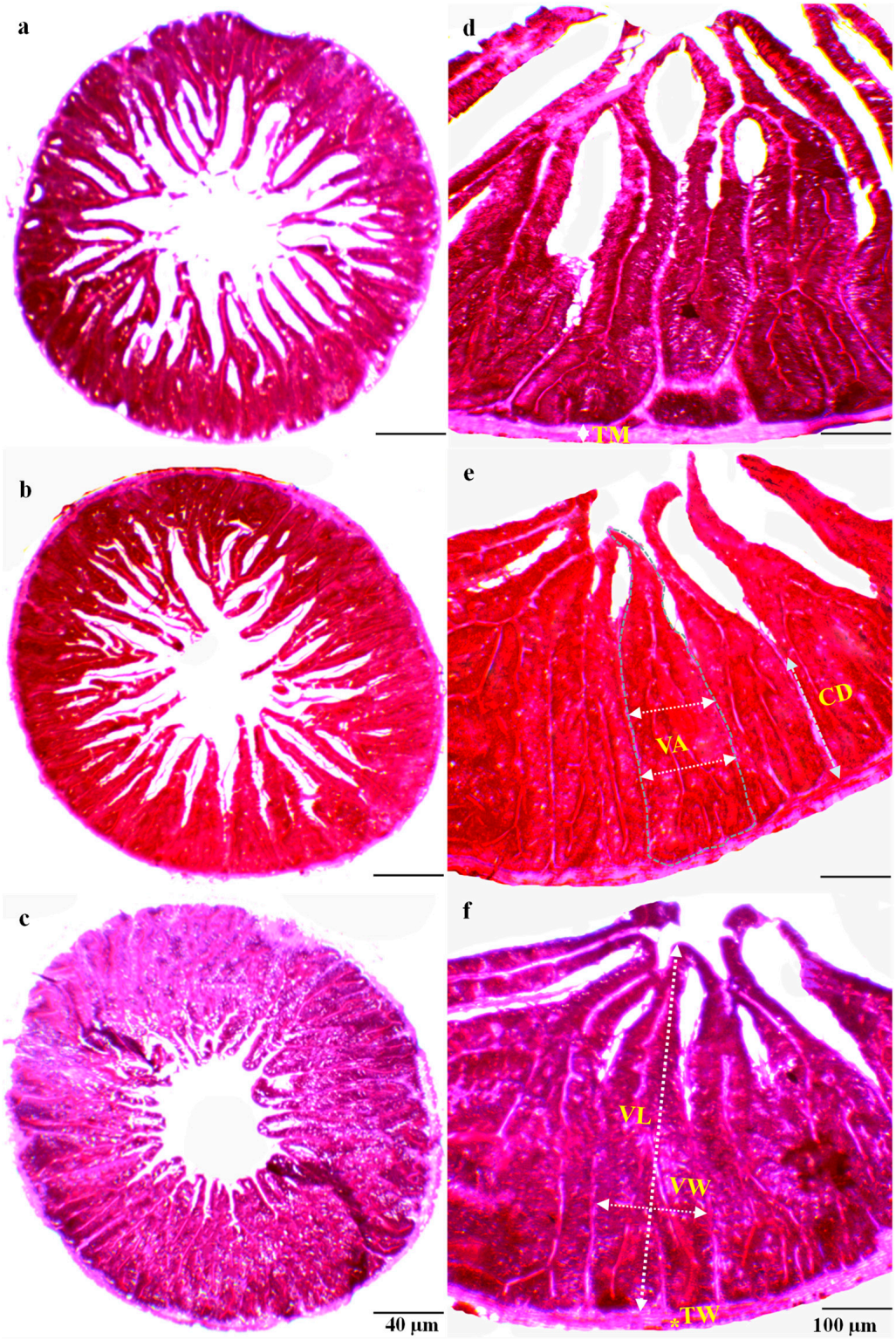

Figure 3. Histological modification of the intestine of stinging catfish *H. fossilis* reared in biofloc for 16 weeks; (**a**,**d**) CP-1, (**b**,**e**) CP-2, (**c**,**f**) laboratory-developed; TM—thickness of muscular, TW—thickness of wall, VW—villus width, VL—villus length, VA—villus area, CD—crypt depth.

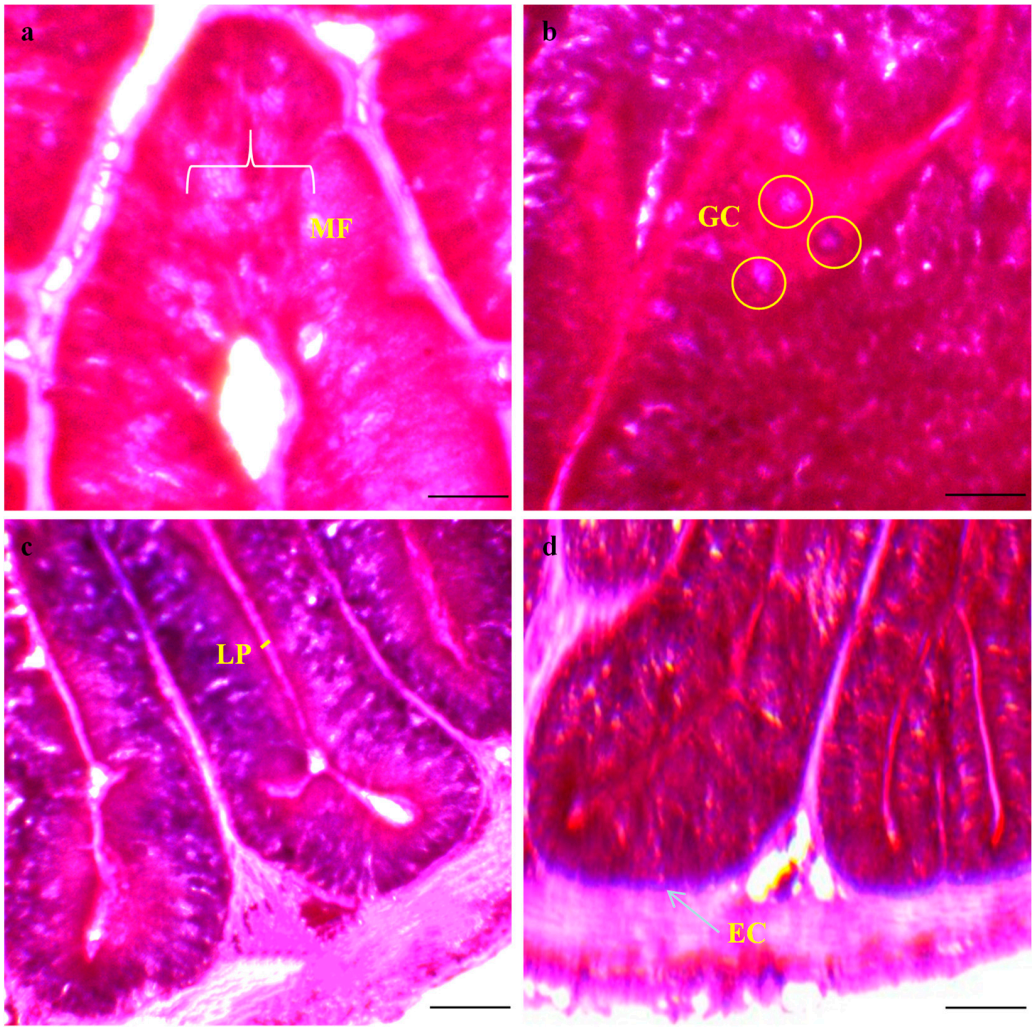

Figure 4. Immune response indicators of the histological gut of stinging catfish *H. fossilis* reared in biofloc for 16 weeks; (**a**) MF—mucosal folds, (**b**) GC—goblet cells, (**c**) LP—lamina propria, (**d**) EC—enterocyte. Scale bar 400 µm.

3.5. Water Quality Parameters and Floc Volume

The water quality parameters and their fluctuations are presented in Table 5. Parameters such as pH, DO, temperature, and alkalinity showed no significant differences ($p > 0.05$) across the various treatments. However, TDS and floc volume were remarkably ($p < 0.05$) higher in biofloc systems treated with laboratory-developed probiotics compared with the other treatments. It was noteworthy that higher TDS values were associated with increased floc volumes. The presence of ammonia did not demonstrate any significant ($p > 0.05$) differences among the different selective probiotics used in the biofloc system.

Table 5. Water quality parameters in biofloc of stinging catfish *H. fossilis* reared for 16 weeks.

Parameters	Probiotics Used in the Biofloc System		
	CP-1	CP-2	Laboratory-Developed
Temperature (°C)	28.86 ± 1.23 (25.00–31.00)	28.69 ± 1.30 (25.00–31.0)	28.67 ± 1.30 (24.50–31.00)
DO (mg/L)	5.96 ± 1.04 (2.40–8.00)	5.73 ± 1.14 (2.5–7.7)	5.45 ± 1.01 (2.3–7.7)
pH	7.30 ± 0.49 (6.1–8.6)	7.23 ± 0.50 (6.0–8.6)	7.27 ± 0.49 (6.0–8.5)

Table 5. *Cont.*

Parameters	Probiotics Used in the Biofloc System		
	CP-1	**CP-2**	**Laboratory-Developed**
NH_3	0.25 ± 0.45 (0.0–2.0)	0.22 ± 0.40 (0–2)	0.28 ± 0.59 (0–4)
TDS (mg/L)	455.47 ± 144.65 b (176–701)	457.61 ± 151.99 b (170– 744)	579.16 ± 94.11 a (283–721)
Floc volume (mg/L)	17.14 ± 4.52 b (10–25)	20.71 ± 4.16 ab (15–25)	30.00 ± 8.86 a (20–50)

Note(s): Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among probiotics. All values are expressed as mean ± SD (n = 6).

3.6. Cost and Return Analysis

Cost and return analysis was calculated based on fixed cost, variable cost, and growth data, as presented in Tables 6 and 7. The BCR showed that stinging catfish culture using laboratory-developed probiotics in the biofloc system was more profitable compared with other probiotics (as shown in Table 8). The cost appraisal showed that different probiotic-treated biofloc systems required a fixed cost of USD 96.54, with an additional annual depreciation cost of USD 10.78. Tank purchasing incurred the highest single cost, followed by aerator setting and testing kits (as shown in Table 6). Variable costs varied depending on the probiotics used, with the pond care probiotic-treated biofloc system having the lowest variable costs (as shown in Table 7). Variable costs constituted a significant proportion of overall fish farming expenses. Among the treatments, the CP-2-treated biofloc system had the highest cost at USD 39.80, followed by CP-1 (USD 34.44) and the laboratory-developed probiotics (USD 36.18). However, the laboratory-developed probiotic-treated biofloc system achieved the highest production of 13.46 kg after 16 weeks (as indicated in Table 8). After deducting all types of cost, the net return from the pond care probiotic-treated biofloc system was USD 3.37, followed by CP-2 (USD 7.36) and the laboratory-developed probiotics used in the biofloc system (USD 20.67). Notably, the laboratory-developed probiotic-treated biofloc system exhibited the highest BCR compared with other probiotics used in the biofloc system (as shown in Table 8).

Table 6. Investment cost and fixed cost of stinging catfish *H. fossilis* reared in biofloc for 16 weeks (USD). (USD 1.00 = BDT 103.50 according to 8 February 2023 (www.bb.org.bd, accessed on 6 July 2023)).

Cost Items	Unit	Price Per Unit (USD)	Total Price (USD)	Economic Life (Year)	Depreciation Cost (USD)
Tanks	6	28.99	173.91	30	5.80
Aerator (80 W)	1	57.97	57.97	5	11.59
Aerator tube	20	0.19	3.86	2	1.93
Aerator stone	15	0.19	2.90	2	1.45
Testing kits	1	48.31	48.31	5	9.66
Foam	5	0.14	0.72	2	0.36
Scoop nets	2	0.97	1.93	5	0.39
Plastic bucket	3	1.16	3.48	3	1.16
Total investment cost			289.61		
Total investment cost for each treatment			96.54		
Total investment depreciation for 3 treatments (per crop)					32.34
Depreciation for each treatment					**10.78**

Table 7. Variable cost and total cost (USD) of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Cost Items	Probiotics Used in the Biofloc System								
	Cp-1			CP-2			Laboratory-Developed		
	Unit	Unit Price	Total Cost	Unit	Unit Price	Total Cost	Unit	Unit Price	Total Cost
Fish seeds	300	0.01	2.90	300	0.01	2.90	300	0.01	2.90
Electricity (kWh)	70	0.05	3.38	70	0.05	3.38	70	0.05	3.38
Feed (kg)	11.76	1.13	13.29	11.76	1.13	13.29	11.76	1.13	13.29
Probiotics (kg)	0.042	23	0.97	0.28	23.19	6.49	0.56	4.83	2.71
Molasses (kg)	3.37	0.31	1.04	2.82	0.31	0.87	3.37	0.31	1.04
Calcium carbonate (kg)	0.17	11.59	1.93	0.17	11.59	1.93	0.17	11.59	1.93
Salt (kg)	0.5	0.29	0.14	0.5	0.29	0.14	0.5	0.29	0.14
Total variable cost (USD)			23.66			29.02			25.40
Fixed cost as depreciation (USD)			10.78			10.78			10.78
Total cost (TC) (USD)			**34.44**			**39.80**			**36.18**

Table 8. Revenue return and economic feasibility of stinging catfish *H. fossilis* reared in biofloc for 16 weeks.

Revenue and Cost–Benefit Analysis	Probiotics Used in the Biofloc System		
	CP-1	CP-2	Laboratory-Developed
After 2 months of harvesting (kg)	1.2	1.8	2.0
Unit price (USD/kg)	2.42	2.42	2.42
Revenue	2.90	4.35	4.83
After 4 months of harvesting (kg)	0.09	0.11	0.13
Unit price (USD/kg)	3.86	3.86	3.86
Revenue	34.91	42.81	52.02
Total revenue (TR)	37.81	47.16	56.85
Net benefit	3.37	7.36	20.67
Economic efficiency Benefit–Cost Ratio (BCR) = TR/TC	1.10	1.18	1.57

4. Discussion

Biofloc systems have the ability to maintain good water quality and promote bacterial growth, resulting in the production of short-chain fatty acids. These fatty acids play a protective role by shielding intestinal epithelial cells and preventing illnesses [25,56]. Moreover, biofloc serves as a source of probiotics, which can strengthen the immune system, control the spread of diseases, and enhance digestive enzyme activity. In this study, the use of various multispecies probiotics in the biofloc system for raising *H. fossilis* led to improved WG and SGR, as well as better FCR, particularly when laboratory-developed probiotics were utilized. It has been observed that probiotics have positive impacts on both fish growth and immunity [33,57,58]. Multispecies probiotics are known to enhance the growth performance of fish and shellfish by altering microbial community, excluding pathogens, boosting non-specific immune responses, and stimulating disease resistance [59–62]. Hence, it has been confirmed that locally produced or laboratory-developed probiotics are superior to commercial probiotics, as they are specifically isolated from the intended host and have shown comparable results in this study [63]. Although various commercial probiotics are now available, replacing the initially fed probiotic bacteria isolated from the gastrointestinal system of the host species, their viability and effectiveness vary depending on the strains and manufacturers [64]. Despite the existence of several imported probiotic preparations in the market, there is a lack of scientific data regarding their viability [65].

Earlier studies have reported the use of hemato-biochemical indices to determine the physiological status of fish [54,66–69]. Fish with higher levels of Hb in their blood were likely to have better oxygen transport to their tissues, resulting in improved growth [70]. Jahan et al. [53] suggested that increasing the amount of yeast probiotics in fish diets could raise Hb and Glu levels. In the current study, the use of laboratory-developed probiotics in the biofloc system significantly increased the Hb levels in stinging catfish, potentially due to improved dietary protein absorption. Jäger et al. [71] recommended the supplementation of probiotics to facilitate the absorption of essential amino acids. Similar results were observed by Abdel-Tawwab et al. [72] in Nile tilapia (*O. niloticus*), Sharma et al. [73] in mrigal (*Cirrhinus cirrhosis*), and Talpur and Ikhwanuddin [74] in Asian seabass (*Lates calcarifer*) when diets containing *S. cerevisiae* and probiotics were used. Consistent with the findings of the current study, Hossain et al. [75,76] noted that blood glucose levels did not significantly change after administering multispecies probiotics, indicating that fish raised with such probiotics remained in good health.

Digestion, metabolism, and nutrient absorption are known to be influenced by the gut microbiota [77–80]. The results of the present study showed that the LAB and TVC in the gut of *H. fossilis* increased significantly when laboratory-developed multispecies probiotics were used in the biofloc system. Previous research has suggested that probiotics can modify the structure and rate of cellular renewal in fish intestines, leading to improvements in histo-morphometric properties [75,76,81,82]. In this study, the use of laboratory-developed multispecies probiotics had a notable impact on intestinal morphology; it resulted in enhanced wall thickness, muscle layer, length, area, and width of the gut villus, as well as increased mucosal fold development and certain immune responses. These changes in intestinal morphology can be attributed to the combined influences of both *Lactobacillus* spp. and *Bacillus* spp. [83]. *Lactobacillus* and *Bacillus* probiotics promote the proliferation of beneficial bacteria in the intestines, inhibiting the growth of harmful bacteria [84]. Furthermore, probiotics compete with pathogenic bacteria for nutrients and adhesion sites, ultimately impeding their growth [85]. The effects of probiotics on the area of nutritional absorption, retention, villi length, enterocyte height, and goblet cell count of the intestines of various fish species have also been reported [41,53,66,86]. Enhancing the length, area, width, and thickness of intestinal villi indicated the formation of mucosal evaginations, which increased intestinal nutrient absorption and improved fish growth status and feed consumption [53,66,83,87,88].

Physicochemical properties of water play a crucial role in determining fish production [89]. The use of biofloc in aquaculture systems offers several advantages, one of which is the improvement in water quality, reducing or eradicating the need for water exchanges [90]. Green and McEntire [91] stated that the elevated ammonia levels in the culture system could be influenced by pH and temperature. In our study, the daily measurements of the water quality parameters, including DO, temperature, and pH, were within the acceptable range and aligned well with the published literature [5,51,92], indicating a favorable environment for the healthy growth of stinging catfish. Dauda et al. [90] showed similar results in their study. According to Avnimelech [5], fish had a limited capacity to grow at pH levels below 6.5 or above 9.0. The pH, DO, and temperature values that we noticed in our analyses (Table 5) were ideal [93]; hence, it was desirable for fish growth in the biofloc system. Additionally, El-Sayed [56] emphasized the importance of alkalinity (>100 mg $CaCO_3$ L^{-1}) for the formation of nitrifying bacteria in the biofloc system, stability of the biofloc, and optimal fish growth, all of which were maintained in our study. While surplus ammonia produced from biofloc systems has been utilized for floc development [25], occasionally, ammonia levels can rise due to interruptions or the absence of floc production [94]. Moreover, ammonia from biofloc can also increase due to the accumulation of fish waste and uneaten feed [51]. However, in our study, there were no discernible variations in ammonia content among the treatments since DO, temperature, and stocking density were maintained at optimal levels. The C:N ratio of 20:1—achieved by incorporating carbon sources such as molasses and wheat bran—proved to be an effec-

tive method for reducing and maintaining ideal inorganic N concentrations [95]. In this investigation, the floc volume was consistently kept at the required level, i.e., less than 50 mg/L, by supplying water and eliminating the settled material to prevent an increase in floc volume larger than 50 mg/L, similar to the findings of Shamsuddin et al. [51]. The TDS in the biofloc systems observed in our study was within the suggested threshold of <1000 mg/L [51]. TDS comprises various dissolved substances, including essential minerals and nutrients for the growth and development of biofloc organisms. Insufficient TDS levels can result in nutrient limitations, negatively impacting the growth and productivity of the biofloc community. Conversely, higher TDS levels can disrupt the osmotic balance, resulting in osmoregulatory stress on the organisms. This can lead to physiological imbalances, reduced growth rates, impaired immune function, and increased susceptibility to diseases.

In this study, the BCR analysis revealed that a significant portion of the investment was allocated to purchasing fish feed and fingerlings, which is supported by Alegbeleye et al. [96]. On the other hand, variable costs referred to the cost variations associated with the quantities of output, such as wages, seeds, feeds, and labor [51,97]. The ratio analysis in Table 8 demonstrates that the BCR was greater than 1.0. This ratio is commonly used in the discounting technique of project appraisal. According to the general rule, a company's BCR should be greater than one to indicate profit, equal to one for the breakeven point, or less than one to signify a loss [98]. This finding was in line with the study conducted by Emokaro et al. [99], which assessed the profitability of catfish farmers in utilizing resources. With a BCR of 1.57, it could be inferred that fish farming using laboratory-developed probiotics in biofloc systems was more profitable compared with other probiotics tested in this experiment.

5. Conclusions

Overall, the administration of laboratory-developed probiotics in the biofloc system resulted in increased growth, improved hematological parameters, and enhanced intestinal microbiota and morphology of stinging catfish. Additionally, it yielded the highest BCR. Furthermore, the implementation of a biofloc system reduced the need for water exchange by utilizing beneficial probiotics, thereby improving growth efficiency and maintaining water quality. The findings of this study highlighted the practicality and profitability of the biofloc system. It was also observed that CP-2 performed well in terms of growth, hematology, and gut health compared with CP-1. However, further research is necessary to elucidate the mechanisms via which probiotics in biofloc systems influence host species, including their impact on enzymatic activity and disease resistance.

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